IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 12, Issue 4 Ser. I (April 2019), PP 51-54
www.iosrjournals.org

Amino Acid Composition of Gelatin from *Ephinephelus* sp.

Ghea Regita Maharani Siregar[1], Eddy Suprayitno[2]
[1](Departement of Fisheries and Marine Science, Brawijaya University, Indonesia)
[2](Departement of Fisheries and Marine Science, Brawijaya University, Indonesia)
Corresponding Author: Ghea Regita Maharani Siregar

Abstract**:** *gelatin source can be obtained from fish skin,scale or bone. Fish gelatin can be an alternative to mammalian gelatin for use in food, photography and pharmaceutical industries. The objective of this study is to know amino acid content of gelatin from Ephinephelus sp. (Carp). Gelatin extracted by citrid acid with concentration 0,5%; 1% and 1,5%, with physco-chemical characteristic were studied. Gelatin with 1% concentration has the best results based on phsyco-chemical characteristic which yield, proximate analysis, pH, viscosity, gel strength and amino acid content. Glycine and prolin being the most dominant component that are important for gelatin for gel strength as physical properties.*
Keywords: *Amino Acid, Collagen, Ephinephelus sp., Fish skin, Gelatin*

Date of Submission: 06-04-2019 Date of acceptance: 22-04-2019

I. Introduction

Gelatin is a biopolymers obtained from collagen hydrolysis from skin, muscle tissue and mammals bones. Gelatin obtained from pre-treatment in acid (type A gelatin) with an isoelectric point between pH 4,7-5,4 or alkaline (type B gelatin) with an isoelectric point between pH 7-9. Gelatin from skin generally obtained from acid treatment wherefore collagen tissue in skin more soft. Gelatin can be made from source that contain collagen, such as skin and scale. Skin has the highest collagen content than bones and scale. Skins have a lot advantages as a gelatin source which can be faster when soaking in acid. While, using solid sources like bones and scales will take more time when soaking in alkaline solution. Molecular weight from gelatin which between 90-300 kD (**1**). In general, amino acid structure is an C atom binding four clusters: amine (NH2), carboxyl (COOH), hydrogen atom (H) and residual clusters (R) (**2**). Amino acid content of fish gelatin and mammals gelatin have major different. Amino acid in mammals gelatin can give a stable structure and great viscoelastic than fish gelatin.

II. Materials and Methods

2.1 Sample Preparation

Fish skin samples *Ephinephelus* sp. (Carp) were purchased from PT. Alam Jaya in Surabaya, East Java Indonesia. The skin soaked in 60^0C water for 30 minutes to sparate meat and skin. After soaked, fish skin were dried and ready for soaked in acid.

2.2 Curing preparation

Curing solution using acetic acid in concentration 0,5%; 1% and 1,5%. Solution was made from 0,5g, 1g and 1,5g (w/v) acetic acid dissolved into a measuring flask 100ml which already contains 100ml aquadest.

2.3 Gelatin Extraction

The fish skin soaked in 0,5%; 1%, and 1,5% (w/v) acetic acid for 24 hours. After 24 hours, fish skin were washed with running tap water until neutral pH (6-7,5). The next step, fish skin were soaked completely in aquadest for next extraction in waterbath. Extraction of gelatin takes 6 hours in 70^0C. after extraction, residual water were filtered and ovened for 48 hours in 60^0C. The dried gelatin is smoothed by mixer and ready for next analisys.

2.4 Proximate analysis

For protein analysis, using Kjeldhal method. Fat analisys using soxhlet method. Water analysis using thermogravymetri method and ash analysis using furnace method.

2.5 Determination of Gel Strength

15 ml of gelatin samples was taken and incubated in 10^0C for 17 hours. Gel strength measured by CT3 Texture Analyzer.

2.6 Determination of Viscosity
15 ml of gelatin sampels was taken and measured in 60^0C with Viscometer 60 rpm

2.7 Determination of pH
pH was detrmined using pH pen calibrated to an neutral pH range of 6

2.8 Amino acid Analysis
amino acid analysis were using UPLC (*Ultra-high Performance Liquid Chromatography*).

III. Results and Discussion

3.1 Gelatin Extraction

Average yield of gelatin from *Ephinephelus* sp. Are tabulated on (TABLE 1). The highest yield obtained from 1% soaked skin. The lower yield could be do because too low or too high concentration of acetid acid. The low concentration could not change the collagen tissue into collagen which not change triple helix collagen into single helix. Too high concentration could damaging protein structure leads to denaturation. Protein can be damage not just because high temperature, but also low pH (acid) will change main chain structure of peptide in protein (**4**).

Table 1: Yield of gelatin in percentage

Parameter	**Concentration**		
	0,5%	**1%**	**1,5%**
Yield average (%)	17,3	21,75	19,45

3.2 Proximate Analysis

Average of proximate analysis are tabulated on (TABLE 2). Highest protein obtained from 1% citric acid soaked skin. Protein content were influenced by soaked and extraction process. Immersion process breaks hydrogen bonds and opened collagen structure and when extraction in right temperature and time, protein will be extracted a lot. High protein content show that gelatin has the best quality. The higher the protein content, the stronger the strength of the gel (**5**).

Table 2: Proximate analysis of gelatin in percentage

Parameter	**Concentration**		
	0,5%	**1%**	**1,5%**
Protein (%)	76,05	84,99	82,66
Fat (%)	0,335	0,395	0,398
Water (%)	1,14	1,63	1,68
Ash (%)	0,40	0,33	0,34

The best fat content in gelatin were obtained from 0,5% citric acid soaked skin. Fat content in gelatin influenced by processing from start until the end of process. Best treatment from every process will reduce fat content in gelatin. High or low fat content in gelatin also influenced by sample source (Yenti *et al*.,2016). Fat content in gelatin is very important because being thedetermination of storage. Gelatin with lower fat content have the longest storage period (**6**).

The best water content in gelatin were obtained from 0,5% citric acid soaked skin. Water content take major role for gelatin quality in texture, tase and storage period. The difference of water content between each concentration could be because how much collagen were made, it cause hydrogen bound from non collagen will bound with water molecule, so that when dried process water will evaporate and reduce water content in gelatin.

The best ash content were obtained from 1% citric acid soaked skin. Ash content used to see quality and level of success from gelatin extraction process. Ash content caused by minerals in raw materials. The higher the minerals content in raw material, the higher the minerals content will be masured in gelatin (**7**).

3.3 determination of gel strength

Average of gel strength analysis are tabulated on (TABLE 3). The best gel strength were obtained from 1% citric acid soaked skin. Gel strength known as the ability of gelatin to forming a thermoreversible gel in water. Gel strength influenced by gelatin concentration, intrinsic factor, pH, temperature and additives.

Table 3: Gel strength of gelatin in newtown

Parameter	**Concentration**		
	0,5%	**1%**	**1,5%**
Gel Strength average (N)	0,83	12,12	10,80

3.4 determination of viscosity

Average of viscosity analysis are tabulated on (TABLE 4). The best viscosity were obtained from 1% citric acid soaked skin. Viscosity is influenced by water content in gelatin. The lower water content in gelatin, hence the ability to binding water (gelling) is high. The more amount of water bound by the gelatin, the solution will be thicker which results in a high value of viscosity.

Table 4: Viscosity of gelatin in centiPoise (cP)

Parameter	**Concentration**		
	0,5%	**1%**	**1,5%**
Viscosity average (cP)	2,0	6,5	5,0

3.5 determination of pH

Average of pH analysis are tabulated on (TABLE 5). All of the pH in all of concentration has met the standard from Gelatin Manufacturers Institute of America and British Standard (1975). High or low in pH values could be caused by extraction process, swelling of collagen or imperfect washing to make a neutral pH after soaked in acid. Based on GMIA pH gelatin for acid process were 3,8-5,5 for food application such as soft capsule for pharmacies.

Table 5: pH of gelatin

Parameter	**Concentration**		
	0,5%	**1%**	**1,5%**
pH average	6,0	4,8	4,5

3.6 Amino Acid Component

Amino acid component are tabulated on (TABLE 6). Amino acids are the main components of protein constituents and are divided into two components namely essential amino acids and non essential amino acids. Essential amino acids cannot be produced in the body so they must be added in the form of food intake, while non-essential amino acids can be produced in the body. Amino acids are generally in powder form and are easily soluble in water, but are not soluble in non-polar organic solvents (**8)**

Table 6: Amino Acid Component

Parameter	**Unit**	**Result**
L-Serin	%	2.63
L-Asam Glutamat	%	7.57
L-Fenilalanin	%	2.12
L-Isoleusin	%	0.79
L-Valin	%	1.67
L-Alanin	%	8.6
L-Arginin	%	8.06
Glisin	%	21.07
L-Lisin	%	2.58
L-Asam Aspartat	%	4.11
L-Leusin	%	1.92
L-Tirosin	%	0.42
L-Prolin	%	10.23
L-Threonin	%	2.37
L-Histidin	%	0.71

The amount of amino acids contained in gelatin is influenced by the type of solution used. Acid curing material is known to have a stronger performance in breaking down the molecular bond of amino acid chains, thus allowing certain chains of amino acids to undergo denaturation and dissolution processes until they are permanently damaged and result in changes in the composition of the amino acids themselves. During the washing and neutralization process, it is possible that a number of dissolved collagen proteins are wasted along with the rest of the washing which will ultimately affect the composition of amino acids contained in a gelatin. Amino acids are a major component of collagen protein that is sensitive to acids, where contaminated acids directly affect the structure of collagen proteins.

The largest amino acid content in gelatin is the amino acid glycine. The gelatin polypeptide chain is composed of repetitions of the amino acid glycine-proline-proline or glycine-hydroxyproline-proline. Another amino acid content which is quite large after glycine is proline. Gelatin with high amino acid glycine and proline content will also have high gel strength values. The amino acid content that is important in influencing the characteristics of gelatin is the amino acid proline (**9**).

The results of the study by Santoso et al. (2013), showed that gelatin which has high amino acid glycine and proline also has high gel strength. When gelatin is applied in making marshmallows, the

composition of amino acids proline and glycine play a role in gel formation. From the best treatment with citric acid immersion concentration of 1%, total glycine in the amino acid test on the gelatin of grouper skin (Ephinephelus sp.) Was 21.07% while the proline was 10.23%. When compared with a study by Suryanti et al., (2017), glycine produced from tilapia skin gelatin (Oreochromis niloticus) was 14.58% while proline was 3.84%. Whereas according to research by Saputra et al., (2015), glycine produced from gelatin of catfish skin (Pangasius pangasius) was 21.08% and lysine produced was 3.64%.

The level of amino acid gelatin in an acidic way is lower than that of a base, this difference can occur due to the loss of amino acids during the hydrolysis of collagen in an acidic manner. Glycine and proline are the two main amino acids, which are almost a quarter of the total amino acid Gelatin made from the partial hydrolysis of collagen. The alpha chain in collagen generally has repeated sequences of glycine-X-Y. Proline often occurs at position X so that the amino acids are most abundant in gelatin. This combination is preferred for steric and electrostatic reasons (**10**)

Glycine is the most found amino acid in gelatin. This type of amino acid accounts for 23% of the total amino acids. It is known that thermal stability is influenced by the number of amino acids. In addition, the amount of amino acids in gelatin derived from fish depends on fish habitat. The amino acid content of gelatin from cold water fish tends to be lower than the number of amino acids from gelatin extracted from warm water fish species (**11**).

IV. Conclusion

Gelatin is a soluble polypeptide derived from collagen which is the main constituent of animal skin, bones and connective tissue. Gelatin is carried out through partial hydrolysis of collagen when collagen is treated with acid or base and followed by heat. The fibrous structure of collagen is broken irreversible and produces gelatin. Gelatin is a type of protein that is extracted from collagen tissue of skin, bones or animal ligaments. Gelatin is widely used in the fields of food, pharmaceutical, cosmetics and photography. The use of gelatin in the industry to increase flower power, texture and stability, for example in the food industry, namely meat products, gelatin is used to increase the binding capacity of water. In the pharmaceutical industry, gelatin is used for capsule hard manufacturing. In the cosmetics industry, gelatin is used as an emulsifier and softening agent and is used in cream and lotion products and is the main ingredient in "protein" for shampoo products and "protein" hair conditioners. The photographic film industry, gelatin is used as a binding medium and protective colloid for image forming materials.

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