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Effect of different acetic acid concentration on physicochemical characteristics of gelatin from starry trigger fish skin (*Abalistes stellaris*)

H Muyasyaroh[1*] and A A Jaziri[1,2]

[1] Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Universitas Brawijaya, Jl. Veteran Malang 65145, East Java, Indonesia
[2] Bio-Seafood Research Group, Universitas Brawijaya, Malang 65145, Indonesia

*Corresponding author: hidayatun@student.ub.ac.id

Abstract. Starry trigger fish skin (*Abalistes stellaris*) is generally considered as waste from fish processing industry, especially in the traditional smoked fish industry. The purpose of this study was to determine the effect of different acid concentrations, used in the immersion process, on the physicochemical characteristics of starry trigger fish skin gelatin. Various concentration of acetic acid (0.2 M; 0.4 M; and 0.6 M) was applied during the gelatin soaking process. The experiment was done in triplicate. The extraction process was carried out for 4 hours at temperature of 55ºC. The results showed that different concentration of acetic acid used in the soaking process had a significant effect ($p<0.05$) on the water, protein, and ash contents, yield, viscosity, gel strength, gelling point, and melting point. The results showed that the higher concentration of acetic acid used during the soaking process would affect the physical and chemical characteristics of gelatin from starry trigger fish skin (*Abalistes stellaris*).

1. Introduction

Fishery processing wastes are residues from fishery production process that comes from industry or domestic. Fisheries waste generally consists of two types, namely solid and liquid waste. Solid waste are usually in the form of skin, bones, fins, head and stomach contents, while liquid waste in the form of blood and water used for production. More than 60% of these by products, including skin, head, fins and bones are considered as waste [1]. The amount of fishery waste, calculated based on fishery production and capture fisheries production data, was 4.65 million tons [2]. Smoked fish industry is one of contributor of fishery waste.

Traditional seafood processing activities are less able to utilize waste properly, as the wastes are just thrown away. During smoked fish production, each production house may produce fish skin waste of up to 20-30 kg/day. A significant amount of protein still remains in fish skin waste. Fish skin, in particular, is a rich source of collagen and gelatin [3].

Gelatin is a high molecular weight protein, a heterogenous mixture of the water-soluble molecule and derived from collagen by thermal denaturation [4]. Gelatin, a collagen derivative product, has many benefits and is widely applied in the pharmaceutical products and food industries [5,6,7]. It has

several functions in food industry, such as creating chewiness, creamy mouth feel, texture, emulsification and water-binding [8]. Gelatin has special properties, such as: a) being able to expand in cold water, b) can form films, c) can affect the viscosity of a material, and d) can protect colloidal systems [9].

The quality of gelatin depends on several factors, such as physicochemical properties, processing methods and parameters. In this research, gelatin was made using different concentrations of acetic acid during the soaking process. The purpose of this study was to determine the effect of different acid concentrations used in the immersion process on the physicochemical characteristics of gelatin from starry trigger fish skin (*Abalistes stellaris*).

2. Material and method

2.1. Preparation of gelatin from starry trigger fish skin (Abalistes stellaris)

Gelatin was extracted from the waste of starry trigger fish skin (*Abalistes stellaris*) using modified method by Mehraj and Soottawat [10]. The frozen fish skin waste was thawed and reduced by 1x1 cm^2 size. Before the extraction process, a pre-treatment process was carried out to help the process in opening the collagen structure on the skin of the fish. Fish skin is immersed in 0.1 M NaOH solution with a ratio of 1: 5 (w/v) for 2 hours with continuous stirring. The purpose of this process was to remove non-collagen protein and pigment. Alkaline pretreated skins were washed with tap water until the pH of wash water was 7 [11,12]. The skin was then soaked in acetic acid solution (CH_3COOH) with three different concentrations i.e.: 0.2 M, 0.4 M and 0.6 M using a ratio of 1: 5 (w/v) for 2 hours. At the time of immersion, stirring was carried out slowly and continuously. Stirring was aimed to accelerate the process of opening the skin matrix in collagen tissue.

The extraction process was carried out in water bath for 4 hours at 55ºC, initiated by adding distilled water to the treated fish skin at a ratio of 1: 3 (w/v). After that, solubilized gelatin was separated from residual skin fragments by filtration through a nylon filter. The extracted gelatin was concentrated at 65ºC for 48 hours in the oven. Gelatin sheets were milled and packaged in vacuum plastic and stored in a desiccator for subsequent process.

2.2. Gelatin yield and proximate composition

The yield of gelatin from waste starry trigger fish skin (*Abalistes stellaris*) was calculated on basis of the weight of fresh skins [13]:

$$\% \text{ yield} = \frac{\text{Dry weight of gelatin}}{\text{Wet weight of skin}} \; x \; 100\% \quad (1)$$

Moisture, ash, fat and protein contents of gelatin from starry trigger fish skin (*Abalistes stellaris*), waste were determined according to standard methods from *Official Methods of Analysis of The Association of Official Analytical Chemist* (AOAC) [13]. A conversion factor of 5.55 was used to calculate protein from total nitrogen [14].

2.3. Physical measurement of gelatin: pH, gel strength, viscosity, gelling point, and melting point

The pH values of gelatins were determined as follows: 1% (*w/v*) solutions of gelatins were prepared by diluting 0.2 gr sample in 20 ml of distilled water at 80ºC. The sample was homogeneous with a magnetic stirrer. The degree of acidity (pH) was measured at room temperature with a pH meter [15].

The gelatin gel strength was determined according to the method of Gaspar [16]. The measurement of gel strength was carried out using the Rheoner RE 3305 with a size of 400 x 0.01 mm, with a speed of 0.5 mm/s, sensitivity of 0.2 v and a cylinder probe of 5 mm.

The determination of viscosity was done according to the method described by British Standart 757 [15]. Gelatin viscosity was measured using a *Brookfield Syncro- Lectric Viscometer*. Before measuring, gelatin samples with a concentration of 6.67% (w/w) were prepared with distilled water (7 g of gelatin in 105 ml of distilled water) then the solution was measured for viscosity. The measurement process was carried out at a temperature of 60ºC with a shear rate of 60 rpm using a spindle.

The determination of gelling point and melting point was carried out on method described by Suryaningrum and Utomo [17]. Gelling point and melting point measurements were carried out using a gelatin solution with a concentration of 6.67% (w/w) prepared with distilled water. The sample was incubated at 10 ºC for 17 ± 2 hours. Melting point measurements were carried out by heating gelatin gel in a water bath. The melting point measurement was carried out in a test tube connected to a digital thermometer. Ice was given to the outer circumference of the reaction tube.

3. **Results and discussions**

3.1. Physical and chemical parameters of gelatin from starry trigger fish skin (Abalistes stellaris)

The physical and chemical parameters of gelatin from starry trigger fish skin (*Abalistes stellaris*) shown in Table 1, Table 2.

Table 1. Chemical parameters of gelatin from starry trigger fish skin (*Abalistes stellaris*).

Acetic Acid	Chemical Parameters				
	Protein	Moisture	Fat	Ash	pH
0.2 M	85.70 ± 0.76^{a}	9.45 ± 0.53^{a}	0.35 ± 0.12^{a}	1.21 ± 0.21^{a}	5.91 ± 0.71^{a}
0.4 M	86.62 ± 0.63^{b}	9.24 ± 0.77^{b}	0.37 ± 0.11^{a}	1.33 ± 0.20^{b}	5.73 ± 0.84^{a}
0.6 M	88.87 ± 0.44^{c}	8.10 ± 0.63^{b}	0.31 ± 0.09^{a}	1.55 ± 0.30^{b}	5.40 ± 0.89^{a}

Table 2. Physical parameters of gelatin from starry trigger fish skin (*Abalistes stellaris*).

Acetic Acid	Physical Parameters				
	Yield	Viscosity	Gel Strength	Gelling Point	Melting Point
0.2 M	10.45 ± 0.73^{a}	4 ± 0.89^{a}	11.36 ± 0.82^{a}	11.71 ± 0.81^{a}	17.65 ± 0.68^{a}
0.4 M	12.56 ± 0.84^{b}	5 ± 0.63^{b}	13.23 ± 0.71^{b}	10.75 ± 0.75^{b}	23.21 ± 0.64^{b}
0.6 M	13.84 ± 0.86^{c}	6.16 ± 0.75^{c}	14.68 ± 0.75^{c}	10.22 ± 0.77^{c}	23.95 ± 0.66^{c}

Analysis of variance showed that different concentration of acetic acid used in the soaking process had a significant effect ($p<0.05$) on the water, protein and ash contents, yield, viscosity, gel strength, gelling point, and melting point. The results showed that higher concentration of acetic acid used during the soaking process would affect the physical and chemical characteristics of gelatin from starry trigger fish skin (*Abalistes stellaris*).

3.2. Proximate composition of gelatin from starry trigger fish skin (Abalistes stellaris)

Different acid concentration in soaking process affected the proximate composition of gelatin from starry trigger fish skin. Based on Table 1, 0.6 M acetic acid produces the best protein (88.87%), water content (8.10%), and fat content (0.31).

Increased protein levels are associated with changes in the amount of amino acid binding structure that makes up collagen proteins. Increased concentration caused the amino acid bonds to

break, so that more protein is dissolved during the extraction process. Increasing the concentration of the solution will increase the number of dissolved collagen. Continuous heating in the extraction process after the curing process makes the collagen sustain a process of dissolution or solubilization [18].

Additionally, the ash and fat contents of these fish skins were lower than those of other fish species such as Nile perch (5.0-6.8% fat and 3.7-6.0% ash) [19], skipjack tuna (18% fat and 4.4% ash), rohu (2.9% fat and 2.0% ash) [20], cobia (7.4% fat and 2.6% ash), and croaker (3.9% fat and 1.9% ash) [21]. The skin's low lipid and ash contents suggested that lipid-removal and demineralization prior to collagen extraction should not be necessary [11].

3.3. Yield

Results showed that the different concentrations of acetic acid given during the immersion process significantly affected the yield of the resulting gelatin ($P<0.05$) (Tabel 2). The higher concentration of acetic acid given would increase the amount of yield produced.

The yield tends to increase with increasing acid concentration [22]. This increase was related to the large amount of collagen that was converted and undergoes transformation into gelatin under the influence of acid. The increase in acid concentration triggered an increase of H^+ ions during the immersion process so that it helped accelerate the process of hydrolysis. Faster hydrolysis rate will increase the number of collagen molecules that are converted into gelatin, which consequently increases the yield. Increasing the amount of H^+ ions can accelerate the rate of collagen hydrolysis. Faster hydrolysis rate means more triple helix breaks into chains α, ß and γ so that the collagen that is converted into gelatin will be more abundant [23].

3.4. pH

Analysis of variance showed that the treatment of different concentrations of acetic acid given during the immersion process had no effect on the pH value of the resulting gelatin ($P>0.05$) (Table 1). The low pH value was caused by an increase in the amount of acetic acid used in the immersion process. It is suspected that there are still remnants of acetic acid used when the demineralization process is still carried away during the extraction process, which will affect the acidity of the resulting gelatin. According to Nurilmala [24], the higher the concentration and type of acid used would affect the acidity of the resulting gelatin.

3.5. Gelling point

The treatment of different concentrations of acetic acid given during the immersion process significantly affected gelling point of gelatin produced ($P<0.05$) (Table 2). The results showed that the higher concentration of acetic acid given would accelerate the process of gel formation in gelatin. The result is in line with the statement of Nurilmala [25], the gelling point of gelatin gel increases due to increased protein levels along with the increasing concentration of acid used for the immersion process.

3.6. Melting point

The treatment of different concentrations of acetic acid given during the immersion process significantly affected the melting point of the resulting gelatin ($P<0.05$) (Table 2). The results showed that the higher concentration of acetic acid given would slow down the melting process in gelatin gel. The results were similar to the melting point of Tiger toothed croaker (20.36^{o}C) and Pink perch skins (19.23^{o}C) [26]. The melting points of cold water fishes such as Cod fish (13.8^{o}C), Hake (14^{o}C) [27] and Hoki (16.6^{o}C) [28] was known to be lower than that of warm water species Black tilapia (*Oreochromis mousambica*) (28.9^{o}C) [29]. Fish gelatin has a lower melting temperature so it is useful in product development to control the texture and flavor release during mastication [26].

3.7. Viscosity

Different concentrations of acetic acid given during the immersion process significantly affected the viscosity of the resulting gelatin ($P<0.05$) (Table 2). The results showed that the higher concentration of acetic acid given in the immersion process increased the viscosity of resulting gelatin product. This was due to the bonding between polypeptide chains so that the molecular weight becomes greater, thus the value of viscosity also increases [30]. Based on previous research, it was found that the viscosity value of catfish, stingray and red snapper skin were 2.03 - 4.13 cP [9], 3.0 - 4.92 cP [31] and 6.7 cP [32], respectively .

3.8.Gel strength

Different concentrations of acetic acid used during the immersion process significantly affected the strength value of the resulting gelatin gel ($P<0.05$) (Table 2). Based on research conducted by Yenti et al. [33], the gel strength of gelatin from fish ranged from 0.67 to 1.46 N. Cow gelatin has a gel strength of 3.22 N, while fish gelatin strength was 1.81 N [34].

Increasing the acid concentration used in the process soaking is thought to increase the strength value of the gel. According to Said [35], an acid solution works in breaking the amino acid polymer chains at the right and optimum limits, so that in the end it gives an improvement effect in the process of gel formation. The amino acid monomer chains combine with one another to form a continuous triple helix structure and bind water to form a compact gel structure. The strength of the gel is highly dependent on the hydrogen bonding between the water molecules and the free hydroxyl group of the amino acid group, the size of the protein chain, the concentration and molecular weight distribution [25,36]. Amino acid composition of fish gelatin from starry trigger fish skin (*Abalistes stellaris*) can see in Table 3.

Table 3. Amino acid composition of fish gelatin from starry trigger fish skin (*Abalistes stellaris*).

Parameter	Gelatin from starry trigger fish skin (%)	Commercial gelatin (%) [37]
Aspartic acid	5.50	4.93
Glutamic acid	9.22	9.43
Serine	3.18	2.18
Histidine	0.86	0.03
Glycine	24.01	23.01
Threonine	2.53	2.87
Arginine	8.23	8.95
Alanine	10.17	10.24
Tyrosine	0.68	0.15
Methionine	1.58	0.55
Valine	2.64	1.60
Phenylalanine	1.85	1.92
I-leucine	0.96	1.13
Proline	12.53	12.34
Leucine	2.28	-
Lysine	4.18	2.86

Based on the amino acid composition, gelatin from starry trigger fish skin contained higher glycine and proline compared to other amino acids, where these amino acid are the main component of

gelatin. Amino acid composition of gelatin is almost the same as collagen, where glycine as the main amino acid (2/3 of all amino acids that make gelatin) besides proline and hydroxyproline [38].

4. Conclusions

The difference in the concentration of acetic acid used in the immersion process significantly affected the yield, protein content, moisture content, ash content, viscosity, gel strength, melting point and gelling point of gelatin from starry trigger fish skin. On the other hand, the effect of different concentrations of acetic acid on the immersion process did not significantly influence the results of the value of fat content and pH.

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