The physico-chemical properties of pangas catfish (Pangasius pangasius) skin gelatin

K A Pradarameswari¹, K Zaelani¹, E Waluyo¹ R Nurdiani¹,²

¹ Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia
² BIOSEAFOOD Research Group (Aquatic Biotech Exploration Sustainable), Brawijaya University, Malang, Indonesia
E-mail: rahmi_nurdiani@ub.ac.id

Abstract. Gelatin can be used as emulsifier and stabilizer in food products. Until now, the most widely used raw materials for the production of gelatin industry are cow bone, cow skin and pig skin. Fish gelatin has been highlighted as a better alternative to replace mammals gelatin based on ethical and religious perspective. Fish gelatin was extracted from Pangas catfish skin to determine its physico-chemical properties. Different temperatures (45 °C, 50 °C, 55 °C) were employed during gelatin extraction. Higher temperature increased the yield and fat contents of Pangas catfish skin gelatin. In contrary, higher water, protein, ash contents were observed during lower temperature. Temperature significantly (p < 0.05) affected the gel strength, viscosity, melting point, and gelling point of fish skin gelatin. Based on the FTIR spectrum catfish skin gelatin functional groups can be identified as N-H, O-H, C = H, C-O and C-H.

1. Introduction
Gelatin is a hydrocolloid product obtained by hydrolyzing collagen proteins found in the skin, bone, and connective tissue. Sobral [1] explained that gelatin is a denatured protein derived from collagen and is an important function of biopolymers with a very wide application in various industrial fields. Its functional properties depend on the processing conditions and raw materials. The quality of gelatin depends on its chemical physics, rheological properties and manufacturing methods. Gelatin has been applied in food as gel-forming agents, thickeners, emulsifiers, and is widely used in the pharmaceuticals, healthcare, cosmetics and photography industries because of its unique functioning [2].

Until recently, the most widely used raw materials for the production of gelatin are cattle bones, cattle skin and pig skin. The utilization of gelatin from mammals is still experiencing many obstacles such as the beliefs held by consumers, such as Hindus who are prohibited from consuming products derived from cows. Some people are also worried about consuming cattle byproducts due to mad cow disease, foot and mouth disease, and Bovine Spongiform Encephalopathy (BSE). In addition, ingredients derived from pigs cannot be consumed by Muslims [3].

Strong competition occurs between producers for the procurement of pig skin or other mammal sources, which has created an increased demand and raised costs. Researchers have not only continued to look for an alternative to gelatin but also to discover new sources of gelatin. In the last decade, there has been an increased interest in the market for gelatin derived from fish and poultry. The meat, skin,
and bone of poultry and fish are expected to produce gelatin in the near future, but commercial production is currently limited due to low yields [4].

With this in mind, fish gelatin has been highlighted as a better alternative to mammalian gelatin from an ethical and religious perspective. However, the quality is at lower melting point, so the faster solubility in the mouth and the absence of residual 'supple' feelings have affected commercial applications. Therefore, the production of fish gelatin is still in its infancy, contributing to only about 1% of the world’s annual gelatin production [5]. 78% of total fish catch has been utilized as human food, leaving about 21% for non-food purposes [6]. Processing leads to the generation of a large biomass (50-75% of total catch) of fish waste (eg, skin, bone, and fins), which are generally discarded [7, 8].

Freshwater fish are seldom used as a source of raw material for gelatin extraction. Several studies, however, have ensured that certain freshwater fish have a large amount of by-product after the removal of edible parts, and high gelatin yields can be expected from Tilapia [9] Nile perch [10], striped catfish (Pangasius sutchi fowler).

In a study conducted by Ratnasari et al. [11] on gelatin extraction and the characterization of different freshwater fish as alternative sources of gelatin, the gelatin obtained from catfish (Pangasius pangasius) had the highest rheological properties compared to the others. It has the following properties: gel strength of 273.58 g, 36.5 cP viscosity, melting point at 32 °C, gelling temperature at 12 °C, melting temperature at 29 °C and a total amino acid content of 754.47 mg / g.

2. Methodology
2.1. Method of research
The method used was the experimental method. The analysis of data used in this research was through Completely Randomized Design (RAL) with three replications. There are three extraction temperatures employed i.e 45 °C, 50 °C, and 55 °C. The dependent variables are the proximate analysis of gelatin product was also conducted, yield, viscosity, strength gel, melting point, gel point and pH.

2.2. Procedure of research
The procedure of gelatin production from catfish skin includes the process of obtaining fresh skin and storing it in ice as described by Sae-Law and Soottawat [12], with some modifications. To remove the non-collagen protein, the skin was immersed in 0.05 M NaOH with a 1: 6 (w / v) skin / solution ratio and stirred for 2 hours at room temperature (± 26-28°C), alkaline solution was replaced every hour. The immersion process of the NaOH solution resulted in swelling that can remove unwanted material (fat and non-collagen proteins). The alkaline skin is then washed with running water until neutral pH was reached. Furthermore, the skin was immersed in 0.05 M acetic acid with a 1 : 6 (w / v) skin / solution ratio for 1 hour with gentle stirring until the collagen swelled in a skin matrix. The treated skin was thoroughly stirred with acid as described previously. After swelling, the swollen skin is immersed in distilled water with a 1 : 6 (w / v) skin / water ratio in a waterbath shaker with a temperature of 45 °C, 50 °C and 55 °C to extract the gelatin from the skin. The extract was filtered and dried using an oven at 55 °C for ± 48 hours. The dried gelatin sheets are then put through a grinder.

3. Result and discussion
The results of the proximate test and physico-chemical properties of catfish skin gelatin with the extraction temperatures of 45 °C, 50 °C and 55 °C and compared to commercial gelatin, SNI standard for gelatin and GMIA standard for gelatin is presented in table 1.
The results showed that the higher extraction temperature used, the higher yield obtained. The increase of temperature causes higher yields by increasing the breakdown of collagen fiber structure so that it binds with water to form more gelatin. This is in accordance with the results of the study by Mayangsari et al. [13]. The optimum temperature for extraction were 50 °C, 60 °C and 70 °C. If temperature is too low it will result in low yields, while temperatures that are too high will result in low gelatin quality. Increased long ripening (extraction time) or heating in water increases the solubility of collagen so that the yield of the gelatin will increase [14].

### 3.2. Water content
From the results obtained, there is a tendency that when a higher extraction temperature was used, the value water content was lower. This was due to higher extraction temperatures that result in weak water holding capacity in gelatin and will cause the water to evaporate at the time of drying. According to Astawan and Aviana [15], the decrease in moisture content is due to an increasingly open collagen structure with a weak stroke, resulting in gelatin with a weak structure, and hence its water holding capacity is also weaker. The weak water holding capacity of the gelatin will make the water volatile at the time of drying.

### 3.3. Ash content
During the extraction process at high temperature, there was a decrease in ash content due to the decomposition of mineral elements. According to Wijaya [16], the low ash content is caused by the release of mineral components such as carbon, calcium, phosphate from the collagen at the time of washing after immersion, as well as the elements dissolving upon extraction by heating so that there is a decrease in ash content.

### 3.4. Protein content
From the research, the tendency seen is that the higher the temperature of extraction, the lower the value of the resulting protein. Gelatin can be obtained by the heat denaturation of collagen. The heating of the collagen will gradually cause the structure to break down and chains to be separated. The molecular weight, shape, and conformation of the collagen solution are sensitive to temperature...
changes that can destroy its macromolecules. Furthermore, according to Lehninger [14], the protein will be damaged and denatured by not only heat, but by the influence of pH, which is a change in the main structure of peptide chains in proteins. If the denatured protein of the polypeptide chain bond arrangement is disrupted, the open protein molecule becomes randomized and subsequently coagulated, resulting in lower amounts of collagen being extracted.

3.5. Fat content
Fat content is influenced by the use of different temperatures during the extraction process at the temperatures of 45°C, 50°C and 55°C for 6 hours, so there are differences in the erosion of fat on the catfish skin. Another factor that affects the fat content of catfish skin gelatin is the process of immersing NaOH for 2 hours. According to Tazwir and Rosmawaty [17], caustic soda which is called NaOH (Sodium hydroxide) in chemistry is a kind of strong metal base. In the medical world, it is known as a substance that dissolves fat tissue.

3.6. Gel strength
Higher temperatures will lead to more amino acid chain breaks. This is due to the continued hydrolysis of collagen that has become gelatin and causes a short-chain of amino acid so that the gel strength is low. Short-chain amino acids cause lower interactions with water molecules and is unable to form into gel [18].

3.7. Viscosity
The higher the extraction temperature, the more open the structure of the amino acid chain will be and will cause the chain to become shorter and viscosity to decrease. The higher extraction temperature causes the triple helical structure of the collagen to transform into a single chain structure. Changes in the collagen chain structure leads to a decrease in the molecular weight of gelatin. Purnomo [19] explains that the high temperature will break the bonds between the joined molecules and form smaller units that will facilitate the fluid to flow.

3.8. pH
The different extraction temperatures in the production of catfish skin gelatin had no effect on the pH value. Basically the pH is not affected by heat or high temperature but will be affected by the decomposed medium at high temperatures that produce acids or bases. It is this acid or base that will affect the pH. According to Amiruldin [20], during immersion (curing), the skin collagen fibers undergo a swelling process and result in the decreased internal cohesion of the skin fibers. When swelling occurs, the amino acid bonding structure of the collagen molecule is exposed and the curing material is trapped within the bonding structure and is insoluble during the neutralization process.

3.9. Melting point
The higher the extraction temperature, the lower the melting point value of the catfish skin. It is suspected that high temperatures will cause the hydrolysis of proteins to break the amino acid chain so that it will weaken the ability to bind protein and water. According to Astawan and Aviana [15], another factor that affects the melting point is the initial state of gel formation. When the gel forms quickly, the resulting gel is less stable and melts faster. In addition, the gelatin that experiences drying at higher temperatures generally indicates a higher melting point as well.

3.10. Gelling point
The gelatin gel point of the research is thought to be caused by the increase in protein content along with the increase of the extraction temperature of the treatment. The protein content of the gelatin determines the amount of amino acid content of hydroxyproline in it. According to Amiruldin [20] who had conducted a study on the amino acid of gelatin derived from from tuna bones, the gel point was influenced by the amount of amino acid hydroxyprolin, the gel point would be lower if the amount
of amino acid hydroxiprolin was also low and the low hydroxiprolin would cause the hydrogen bond in the gelatin to be weaker.

3.11. Analysis of FT-IR
The result of the catfish skin gelatin spectra is presented in figure 1.

![Figure 1. Spectrum FTIR of Gelatin Skin Catfish](image)

Overall the FTIR spectra curve for patin skin gelatin extracted at the temperatures of 45 °C, 50 °C, and 55 °C has an intensity from amide A to larger amide II. The peaks in amide III are almost invisible. This is in accordance with the theory that collagen has been successfully denatured into gelatin. Based on FTIR analysis, patin skin gelatin extracted at a temperature of 55 °C is the most prominent uptake of a typical gelatin-functional group having a complete absorption area.
Table 2. Peak Position and functional spectrum FTIR of patin fish skin gelatin.

| Absorbansi area | Absorption peak (cm\(^{-1}\)) | References | Notes |
|-----------------|-------------------------------|------------|-------|
|                 | 45\(^{0}\) 50\(^{0}\) 55\(^{0}\) |            |       |
| Amida A         | 3630,03 3435,22 3001,24 3650-3580 | Free NH    | NH stretching of an amide group associated with a hydrogen bond, OH group |
|                 | 2922,16 2924,09 2924,09 3600-2300 | CH\(_2\) stretching symmetrical/ C-H alkana/ aldehide |
|                 | 2852,72 2852,72 2852,72           | CH\(_2\) stretching asymmetrical /C-H alkana/ aldehide |
| Amida I         | 1743,65 1701,22 1701,22 1685,79 1700-1600 | Stretching C=O ; OH group pair with COO' |
|                 | 1550,77 1454,33 1544,98            |           |       |
| Amida II        | 1458,18 1419,61 1377,17 1560-1335 | NH bending pair with CN stretching CH\(_2\) bending |
| Amida III       | 1242,16 1170,79 1278,81 1246,0 1240,23 1240-670 | NH bending C – O |
|                 | 1012,63 920,05 813,96              |            |       |

4. Conclusion
Gelatin is a denaturated of protein derived from collagen and is an important function of biopolymers with a very wide application in various fields of industry. Based on this research, the best treatment was found at the yield of 24.65 % at 55 °C; the yield of water content was 9.4 % at the temperature of 50 °C. The result of ash content was at 0.83 % at the temperature of 55 °C; protein content was 89.61 % at 55 °C; while fat content was at 1.47 % at 45 °C. Meanwhile, the gel yield strength was of 105.30 blooms at the temperature of 45 °C; the viscosity was 7 cP at 45 °C; the yield of the melting point was 28 °C at 45 °C; gel point result was 11 °C at 45 °C; and the FTIR results produced NH, CH, OH, and CO at 55 °C.

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