Histological structure of striped catfish (Pangasius Hypophthalmus) skin from different body size (age) and its relation to the quality of gelatin based on the melting point

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Abstract. Gelatin is a protein derived from collagen denaturation. Currently, fish gelatin has been developed as an alternative to halal gelatin. Fish gelatin can be extracted from the skin of several fish including striped catfish (Pangasius hypophthalmus), because the skin's connective tissue contains a lot of collagen. In industrial applications, melting point is a very important characteristic of gelatin. The biological factors of raw materials such as the size or age of fish are thought to influence the content of collagen, thereby affecting the melting point of gelatin. The aim of this study was to compare the histological structure of the striped catfish skin at different body sizes, especially the observation of collagen in the skin’s connective tissues and to determine its relationship with the quality of its gelatin product based on melting point. The gelatin pretreatment was performed using acid-base treatment, extraction with distilled water (55°C) were conducted prior to melting point measurement. The results of histological analysis showed that the connective tissue in the skin of larger fish contained thicker stratum compactum and thinner of stratum spongiosum. The melting point of large catfish skin gelatin has a higher melting point (25.5°C) than the smaller size (24°C).

1. Introduction
Gelatin is a protein compound derived from collagen denaturation [1]. Gelatin raw material sources are generally from mammals, especially skin of porcine, beef bones, poultry and fish [2,3]. Porcine gelatin and other mammals that are not slaughtered properly, has problems in the aspect of religion [4]. Currently, fish gelatin has been developed as an alternative to halal gelatin. Fish skin is a by-product of the fishery and fish fillet industry which have low economic value [5]. Therefore, it can be a source of alternative raw materials in the manufacture of gelatin. In industrial applications, gel strength, viscosity and melting point are very important characteristics of gelatin. These properties are influenced by several factors in the extraction process including the concentration of the gelatin solution, gel heating temperature, and pH [6] and the biological factor of raw materials, such as the size or age of the fish [7]. Several studies reported that the age of fish affects the characteristics of gelatin bonds and gelatin color are thought to affect the color of the gelatin. [7,8], we investigated the histological structure of the striped catfish skin at different body sizes, especially the observation of
collagen in the skin's connective tissues. We also determined the relationship the skin histological structure with the quality of gelatin product based on melting point.

2. Material and methods

2.1. Histological Analysis

The skin samples of striped catfish which is used as a source of raw material for making gelatin has differences in age that represented by the size of the fish. The samples of small and large fishes were represented by fishes with average body length 38.9 ± 0.5 cm (sub adult size) and 48.6 ± 1.9 cm (adult size) respectively. Firstly, the skin samples that obtained from the ventral body part were immersed in 10% neutral buffered formalin (NBF 10%) for overnight, followed by 20% nitric acid for three days until the tissues were soft prior to processed using standard histological processing technique. The tissue were processed using paraffin method with sectioned in thickness of 3-5 μm and stained using Masson’s Trichrome [9,10]. The skin histological observation, primarily collagen in the connective tissue, were analyzed under light microscopy that connected with digital photomicroscope using magnifications of 40x and 100x.

2.2. Gelatin extraction

Skin were scrapped for removing the non-target tissues such as muscles and fat. Then, the scrapped skin were washed with distilled water, placed in a cold water and preserved at temperature of -20°C. Prior to extraction, pretreatment of the catfish skin was carried out by immersing the catfish in acid and alkaline solution. Each 10 grams of catfish skin is wrapped first with a filter cloth and soaked in 0.12 mol CH₃COOH with a ratio of skin weight to a solution of 1:10 (w/v) and stirred for 60 minutes at room temperature. Then the skin is rinsed under running water to a neutral pH (6.0-7.0). Then the skin was soaked using 0.2 mol of NaOH with a ratio of skin weight to a solution of 1:10 (w/v) and stirred for 60 minutes at room temperature. Then the skin is rinsed under running water to a neutral pH (6.0-7.0). After pretreatment, the extraction process is carried out. The pretreated skin was put in distilled water with a skin weight ratio of 1:10 and heated using a water bath with temperatures of 55°C. The results of the gelatin extraction are then removed and put in a refrigerator with a temperature of 4°C for ±2 hours to form a gel. The gel is then cooled in the freezer until frozen. Furthermore, the frozen gel was dried using a Freeze dryer with a temperature of -35°C and a vacuum of ±0.3 mbar until it was completely dry. The dry gelatin is then stored at room temperature in a dry and tightly covered containers [7,11].

2.3. Melting Point Measurement

The dry gelatins were dissolved in distilled water to a concentration of 6.67%, melted at 60-65°C [12] and homogenized with a magnetic stirrer. Samples were stored at ±10°C for 17 ± 2 hours. Melting point were measured by heating the sample in a water bath. A buckshot ball was placed on the surface of the gelatin sample. The melting point temperature was determined by transition of gel-sol gelatin that observed by the movement of the buckshot ball fell to the bottom of the sample [13].

3. Result and discussion

The cross-sectional of large (adult) and small (sub adult) striped catfish skin histology are presented in Figure 1. Referring to Elliot [14], the skin structure of catfish consists of the epidermis, the dermis which is mainly composed of connective tissue of the stratum spongiosum (blue and white transparent) and the stratum compactum (predominantly blue) and the hypodermis which is continually composed of fat tissue (white colored) and muscles (colored red). The white transparent color of the stratum spongiosum and stratum compactum shows the space between cells that contains a matrix. While the blue color indicated the structure of collagen. The histological analysis exhibits the structural differences in the stratum spongiosum (SS) and stratum compactum (SC) in small (Fig. 1a and Fig. 1b) and large fish (Fig. 1c and Fig. 1d). The connective tissue in the skin of larger fish contained thicker SC hence the more dominant of thread-like blue color because the SC rich of
collagen structure. Meanwhile, the SS part of the skin of small fish is larger hence it more transparent since it more predominantly matrix rather than collagen. This histological features are similar with the previous study that reported the collagen will look blue with a special dye Masson trichrome [15]. Structurally, collagen has a triple helical structure to maintain its stability, so that in the preparation it looks like a thread that is arranged in layers. Collagen in the skin is collagen type III which is composed of three α1 chains [16].

![Cross-sectional tissue of catfish skin](image)

**Figure 1.** Cross-sectional tissue of catfish skin: the small size using magnification of 40x (1a) and 100x (1b); the large size using magnification of 40x (1c) and 100x (1d) magnification. SS = stratum spongiosum; SC = stratum compactum; F= Fat; M=Muscle tissue

The melting point value of skin gelatin from large catfish size (adult size) was in average of 25.5°C while the melting point of skin from smaller fish (sub adult size) was in average of 24°C. Meanwhile, the pH of all gelatin extracts were in neutral pH (7.0-7.7) since they were prepared using acid-based treatment. The highest melting point of gelatin from larger fish value indicated that the adult fishes skin have high content of the amino acids glycine and hydroxyproline [6]. The content of the amino acid hydroxyproline is thought to correlate with age, which in this study was correlated with body size. The increasing age in vertebrates the higher the amino acid content of hydroxyproline [17]. This result is consistence with the histological analysis that showed the larger (adult) fish contain higher collagen due to the thicker of stratum compactum.

In general, the melting point of striped catfish gelatin in this current research were in range of 24 – 25.5°C were in the range of fish melting point 11-28°C [3]. In addition to the melting point of the gelatin is important in chemical properties because it can affect the properties of gelatin to determine the quality and subsequent application [18]. Based on the melting point of gelatin in current research and several references [3,19,20], it can be recommend several application of striped catfish gelatin as presented at Table 1.
Table 1. Several application of striped catfish gelatin

| Sample Group | Application based on Melting Point (MP) |
|--------------|----------------------------------------|
|              | MP:18-31°C | MP: 20-31°C | MP:25-31°C |
|              | Food products | Pharmaceutical uses | Medical uses | Technical use |
| Larger size (adult) average MP 25.5°C | ✓ | ✓ | ✓ | ✓ |
| Smaller size (Sub-adult) average MP 24 °C | ✓ | ✓ | - | - |

The large (adult) fish skin that have melting point value of 25.5°C and neutral pH can be applied in more fields than smaller one, including for food products (gel formers, stabilizers and emulsifiers), pharmaceutical uses (tablets, soft capsules and hard capsules), the medical uses (tissue engineering, wound healing, bone formation, genetic therapy, antioxidant applications, anti-adhesion and antimicrobial applications) and the technical uses (photography, paper mills, colloid application protectors, abrasive protectors, match factory, cosmetics) [3] [19].

4. Conclusion
The results of histological analysis showed that the connective tissue in the skin of larger (adult) fish contained thicker stratum compactum and thinner of stratum spongiosum than the smaller (sub adult) fish. The thicker stratum compactum of large catfish skin gelatin is correlated with higher number of collagens and higher value of the melting point has a higher melting point

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