Review

Fish skin as a biomaterial for halal collagen and gelatin

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ABSTRACT

Around 40% of the total catch weight of fish is regarded as byproducts, consisting of skin, fins, bones, scales, viscera, etc. The utilization of these byproducts is important to increase their commercial values as well as to prevent environmental pollution. Meanwhile, nowadays, it is getting a global trend to provide foods and other industrial materials which have been accredited as halal products for Moslem communities. As a way of processing fish byproducts to meet the halal criteria, preparation of collagen and gelatin would be useful to fulfill the market demand. As a result of screening studies on fishery byproducts, fish skin has been found to be the good source for halal collagen and gelatin, which show satisfactory quality compared with those from bovine sources which could cause bovine spongiform encephalopathy (BSE).

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1. Introduction

Fish are widely known as excellent protein sources for human. Fish are classified based on their habitats, namely, freshwater, brackish water, and sea water fish. The edible part of fish is mostly meat (muscle), thus, fish processing industries produce large quantities of inedible parts or wastes such as skin, fins, bones, scales, and viscera. They are considered as byproducts for which the utilization has still been limited. However, these byproducts can be good sources of protein, fat, minerals, biofunctional substances, etc. (Nam et al., 2020).

Nowadays, the demands for ‘halal’ foods as well as non-food industries tend to increase globally. The term ‘halal’, which comes from Moslem communities, literally means ‘permissible in an Islamic way’ (Ahmed et al., 2020). Halal has strong correlation not only with food but also with non-food stuffs. On the other hand, the source of collagen and gelatin industries generally comes from bone as well as skin of mammalian (mainly, porcine and bovine) materials. It has been reported that more than 70% of collagen and gelatin are from porcine stuff in the world, followed by bovine one. It is widely known that porcine products and the derivatives are absolutely prohibited for Moslem communities. Bovine products are allowed as long as the slaughtering process follows the prescribed Islamic way (Boran and Regenstein, 2010). In addition, bovine spongiform encephalopathy (BSE) or mad cow diseases are a serious concern for its production development. The demand of collagen and gelatin tend to increase. Thus, the materials from fish have high potentials for fulfilling Moslem requirements in terms of halal.

Fish products including capture and aquaculture tend to increase year by year. The increase in production would result in increase in the amount of byproducts. Above all, fish skin, bone, and swim bladders are rich in collagen. Fish skin, which occupies about 8–10% of the total weight of fish, is one of the major byproducts from fish filleting industry. Generally, the utilization of fish skin has been mainly for animal feeding. On the other hand, fish skin is rich in collagen, and thus, preparation of collagen and gelatin from this part will be promising for the value addition to this byproduct (Benjakul et al., 2009; Alfaro et al., 2013; Amiza et al., 2015; Nurilmala et al., 2020a; Ge et al., 2020).

Collagen can be further converted to gelatin by thermal or acidic/ base treatments. The term ‘gelatin’ comes from a Latin word “gelare”, which means ‘frozen’. The insoluble fibrous structure of collagen becomes water-soluble gelatin when treated with acid, alkali, proteolytic enzymes, or heat. Gelatin is a compound that never occurs naturally (Glicksman, 1969). Gelatin is swollen in water and thus softened, gradually absorbing water up to five to ten times of the weight. Since gradual heating of collagen breaks the structure into tropocollagen of several chains, it is easily dissolved in hot water and forms a gel when cooled down. The conversion of collagen to gelatin is usually based on the extraction temperature. The high temperature denatures the protein, causing the reduction of functional properties such as gel strength and viscosity. Thus, gradual temperature raising is applied to prevent such damages to this protein. The extraction temperature range used is generally between 50 and 100 °C. Gelatin is widely used for food as well non-food purposes such as for medicines, cosmetics, and photographic applications. Here, the preparation procedures and properties of collagen and gelatin mainly from fish skins will be described.

Generally, commercial collagens and gelatins are obtained from the skins, hides, and bones of cattle, with the largest single amount coming from pork skin. The potential presence of prions causative of BSE, commonly known as mad cow disease, is a concern for the gelatin from bovine sources. Both Muslim and Jewish dietary laws prohibit all the pork products, and many consumers from those communities also reject bovine gelatins that were not obtained from religiously slaughtered animals (Boran and Regenstein, 2010; Ahmed et al., 2020). Some Hindus would also reject such gelatin. Fish skin can be a perfect source of collagen for Moslem communities unlike the mammalian counterparts, and is also free from the concern of BSE infection. With the increase of fish filleting services in many countries, the new by-products are becoming available for exploitation. Collagens from fish sources thus have been widely studied for those from the skin and bones of big eye snapper (Pristipomoides cynclus) (Kittiphattanabawon et al., 2005), the cartilage of sharks (Chiloscyllium punctatum and Carcharhinus limbatus) (Kittiphattanabawon et al., 2010), abalone (Haliotis discus hannai) (Dong et al., 2012), and rainbow trout (Onchorhynchus mykiss) (Tabarestani et al., 2010).

2. Fish skin for collagen and gelatin sources

2.1. Characteristics and structures of fish skin

Fish skins have been characterized by histological techniques (Kiernan, 1990; Rieppo et al., 2019). Masson’s trichrome is one of the methods used to locate collagen in a given tissue based on selective staining of collagen with aniline blue which dyes collagen in blue. The light microscopy of the dorsal and ventral skins from four fish species revealed the localization of collagen in these skins (Figs. 1 and 2, respectively). Figs. 1a and 2a show the tissue structures of catfish dorsal and ventral skins. The skins have a plenty of fat cells (refer to 2 in the figure). It has been reported that fish skin contains layers of epidermis and dermis containing collagen. The dermis layer is a very thick binding tissue which contains a number of collagen fibers (Drelich et al., 2018). In the inside of stratum compactum are located several fat layers, where the layers are the boundaries containing collagen. Stratum compactum of tilapia ventral skin (Fig. 2b) apparently contains a larger amount of collagen than the dorsal skin, which is recognized by its darker blue color (Fig. 1b). Previous research on catfish Pangasius hypophthalmus skin showed that the connective tissue in larger fish had thicker stratum compactum and thinner of stratum spongiosum (Hidayati et al., 2021). In the inside part of both dorsal and ventral skins, stratum compactum faces the fat layers with each layer being separated by septa with collagen (3 in the figure), but the membranes of fat cells do not contain collagen at all. However, the membrane in the ventral part shows blue color, demonstrating the presence of collagen. Epimysium in the dorsal skin contains collagen and fat cells.

The stratum compactum (1) of the ventral (Fig. 2c) and dorsal skins (Fig. 1c) of red snapper consisted of wavy long fibers along the skin surface. The wavy form of the dorsal stratum compactum was more oblique than the ventral counterpart. The stratum compactum is very tightly arranged (Arumugam et al., 2018). The ventral epimysium which is close to the skin shows deeper blueeness and thickness than the dorsal one. The deeper the meat part from the skin, the thinner the blueeness of the epimysium, suggesting that collagen content is lower in the deeper part.
The fibers of dorsal skin collagen tissue appeared to be thicker and tighter in parrotfish (Fig. 1d). The collagen fibers in both the dorsal and ventral skins of this fish are wavy and declivous (Fig. 2d). The fat layers are rarely found in the parrotfish skin below the stratum compactum into the inner part of the meat. Presence of the thick collagen layer in the parrotfish skin could have resulted in higher gelatin yield compared with the other types of fish skins.

2.2. Proximate composition of fish skin

Generally, proximate composition analysis is conducted for provisional research on characterization of raw materials. Accordingly, the water, protein, fat, ash, and carbohydrate contents in the fish skin before extraction were determined. Table 1 shows the proximate composition of catfish, red tilapia, red snapper, and parrotfish skins, all of which are the excellent candidates for collagen and gelatin production. The red tilapia skin had the highest water content. The highest protein and ash content were found in the red snapper skin. The fat content of the pangasius skin is 8.29%, higher than any other fish skins. Protein contents from marine fishes tend to show higher values than those of freshwater fishes. Protein content in the red tilapia skin was lower than the value reported by Jamilah et al. (2011), but higher than the value for the blackspotted croaker (Protonibea diacanthus) skin (Jakhar et al., 2012). The composition could have been affected by differences in species, habitats, genetics, and food preference of fishes (Koli et al., 2014).

Protein content in the fish skins can affect the yield of gelatin. Therefore, the amount of resulting gelatin could be estimated based on the protein content in the raw material, even though it is not a direct parameter (Jamilah et al., 2011). Gelatin yield can also be measured based on hydroxyproline (Hyp) content in the product (Nalinanon et al., 2008).

3. Collagen and gelatin

3.1. Properties of collagen

Collagen is a fibrous and insoluble protein, representing about 30% of the total animal protein from vertebrates and invertebrates (Boran and Regenstein, 2010). Collagen is the main protein found in connective tissue of animals and is present in the skin, bones, meat, cartilage (hyalin), ligaments, blood vessels, teeth, cornea, intervertebral, and placenta (Rao et al., 2012). This protein consists of tropocollagen as the basic unit, and the main amino acids are glycine (Gly), proline (Pro), and hydroxyproline (Hyp), which is a specific marker for the presence of collagen and gelatin (Boran and Regenstein, 2010). Among them, Gly content is the highest, and occupies more than 30% of the total amino acids (Motowidlo et al., 2008). The contents of the imino acids (Hyp, Pro) in fish collagens reach 40–48%, close to those of mammals (45%). The amino acid compositions of collagens are species-specific and would be affected by habitat environment and especially temperature. Fish
Collagens show lower thermal stability than the mammalian counterparts, because of the lower imino acid contents. The contents of the imino acids, which are stabilized by the aid of hydrogen bonds, greatly affect the thermal stability of collagens. It has been reported Pro and Hyp contents in the skin were higher than in the bone in case of big eye snapper (*P. tayemus*) (Kittiphattanabawon et al., 2005). Collagen molecule has a macromolecular structure including its unique triple helix, which is formed by three identical $\alpha$ polypeptide chains. Each $\alpha$ chain shows a triple repetitive sequence $(\text{Gly-X-Y})_n$, where X and Y are often occupied by Pro and Hyp (Zahrani, 2012). Pyrrolidine rings in Pro and Hyp lead to restrictions on the adjustment of the polypeptide chains and enforce the triple helix of collagen molecule (Singh et al., 2011). Most of the fish skin collagen investigated so far is type I collagen, which is characterized as a natural scaffold for tissue engineering and wound recovery (Mocan et al., 2011), and also is extensively used for biomaterial applications (Hayashi et al., 2012). Type I collagen from fish is unique in that it shows high solubility in acids than the counterparts from birds and mammals. Type I collagens from fish also consist of $\alpha_1$ and $\alpha_2$ subunits (Hayashi et al., 2012).

The yellowfin tuna (*Thunnus albacares*) skin collagen was prepared by the pretreatment with 0.1 M NaOH and 0.5 M acetic acid and subsequent papain digestion at 7000 U/mg/g skin (dry weight), which could produce the collagen of highest solubility as well as the highest yield by dry weight basis (Nurilmala et al., 2019). This method meets the halal requirement, since papain is a plant-derived enzyme.

### Table 1
Proximate compositions of fish skins (%).

| Species         | Water | Protein | Ash  | Fat  |
|-----------------|-------|---------|------|------|
| Pangasius       | 63.92 | 26.73   | 0.39 | 8.29 |
| Red tilapia     | 71.19 | 23.93   | 0.42 | 2.30 |
| Red snapper     | 65.47 | 29.72   | 0.45 | 2.37 |
| Parrotfish      | 68.37 | 27.17   | 0.43 | 2.47 |
| Red tilapia     | 70.43 | 29.07   | 0.51 | –    |
| Ganglomo        | 75.80 | 20.63   | 1.06 | 2.48 |

Notes:

[1] Nurilmala et al. (2020b).
[2] Jamilah et al. (2011).
[3] Jakhar et al. (2012).
3.2. Properties of gelatin

The amino acid composition of gelatin is almost similar to that of collagen, where Gly is the main amino acid (Charley, 1982). It has been known that gelatin is a linear polymer composed of repeated units of Gly-Pro or Gly-Hyp (Fig. 3).

Unlike collagen, gelatin can be used as a multifunctional ingredient and thus be applicable to various industries. The use of gelatin in food processing is due to the unique functionality based on the physical and chemical properties. In the food industries, gelatin plays important roles as a gelling agent, stabilizer, thickener, water binder, coating and emulsifier. Gelatin as a colloidal protector can also be useful in the photography and metal coating in the electropainting industry. Other unique properties of gelatin are the ability to change reversibly from sol to gel and vice versa as well as to be swollen even in cold water. Those properties of gelatin make it preferable to the other ingredients such as xanthan gum, carrageenan and pectin.

Gelatin is classified into two types based on the different processing conditions, namely, types A and B. Type A is obtained by soaking and treating in acid solution, while type B is obtained by alkaline treatment (the alkaline process). Usually, the gelatin produced from fish is type A. Acid treatment, which requires a shorter time, is preferred to alkaline one. The differences in properties between both types are listed in Table 2 (Tourtellote, 1980).

Generally, main sources of gelatin production are porcine and bovine skins and bones. Gelatin derived from pork skin reaches more than 50% of the total commercially available gelatin in the global market. The alternative raw materials for collagen and gelatin are definitively fish skin, bone, and swim bladder. The intrinsic characteristics of the final products are species-specific (Gomez-Guillen et al., 2011). Recently, this topic has become a widespread matter of interest among the scientists on wound healing, neoplasms, cell growth, etc. (Ge et al., 2020). The bioactive peptides from collagen and gelatin are being replaced for the synthetic agents (Ngo et al., 2014; Zhang et al., 2014).

3.3. Preparation of gelatin

There are three main stages in gelatin production, namely 1) preparation of raw materials, 2) conversion of collagen to gelatin, 3) purification and drying gelatin. The extraction temperature of the skin ranges between 50 °C and 100 °C or lower (Hinterwaldner, 1977). The fresh fish skin can be directly extracted or stored frozen before extraction, namely at −20 °C. Preparation is initiated by washing skins as raw materials. Fish skins are generally chopped into smaller sizes (approx. 0.5 × 0.5 cm) to facilitate the extraction process. For the production of type A gelatin, raw materials are subjected to acid treatment (acidic process). Weak acids such as acetic acid and citric acid are used for the extraction of fish skin or swim bladder. While strong acids such as hydrochloric acid are also used to extract gelatin from bones. On the other hand, alkaline treatment is used for the production of type B gelatin (alkaline process), where sodium hydroxide is used. The conversion of collagen into gelatin is carried out by denaturing collagen through breaking hydrogen bonds. The denaturation process is carried out by heating collagen starting from 40 °C or by adding hydrogen breaking compounds at room temperature or lower, in three steps, namely, lateral hydrolysis, hydrolysis of peptide bonds, and destruction of the helical structure of collagen. The hydrolysis can be carried out under acidic conditions (pH 4.0–4.4 or lower), but requires rapid handling to prevent further degradation, followed by extraction at 50–100 °C for several hours. Gelatin structure will then be broken into one, two or three polypeptide chains randomly.

One of the extraction procedures of gelatin from fish skins using citric acid are as follows (Nurilmala et al., 2017). Chopped skins are washed with tap water, and then soaked in squeezed lime juice (20% w/v) with the ratio of water and skin 1:1 w/v for 10 min. Subsequently, the skins are washed again with water to remove residual lime. The skins are then hydrolyzed by soaking in 1% citric acid at a ratio of 1:3 (w/v) for 12 h at room temperature. Neutralization with water is then carried out until the pH reaches 6. The treated skins are subsequently extracted with distilled water at 65 °C for 6 h with a ratio of water and the skin 1:1 (w/v) after swelling. The extract is filtered through a layer of calico fabrics to obtain solubilized gelatin, followed by drying process using an evaporator at 60 °C for 1 h and mashing up into dried powder ready for analysis of proximate composition, yield, acidity (pH), color, viscosity, gel strength, molecular weight range estimation, and amino acid composition.

The physical, chemical and functional properties of gelatin determine the quality of gelatin. The parameters in determining the quality include gel strength, viscosity, and yield. Gel strength is influenced by pH, the presence of electrolytes and non-electrolytes, and additives. On the other hand, the viscosity is affected by the hydrodynamic interaction between gelatin molecules, temperature, pH and concentration.

3.4. Proximate composition of gelatin

Table 3 shows the proximate compositions of fish gelatin. The water content was the highest in the gelatin from catfish. The water contents of gelatins from catfish, red tilapia, red snapper, and parrotfish matched that of the gelatin standard (18% maximum) (GMIA, 2019), and also that of the laboratory standard (10.04%) (Pranoto et al., 2011).

The protein content ranged from 87.2 to 88.48%, higher than the reported values for catfish (P. pangasius) gelatin (87.10%) (Ratnasari and Firlianty, 2016) and cobia (Rachycentron canadum) gelatin (89.7%) (Amiza et al., 2015). Protein content of gelatin is related to the gel strength. Gelatin of high protein content has a lot of residual amino acids, possibly because the polypeptide chains are still long, and the hydrogen bonds between protein molecules are also strong, resulting in higher water binding capacity (Amiza et al., 2015). The formed gel is stronger and flexible and cannot be broken easily, as suggested by high gel strength. When dissolved in water, such gelatin can trap a lot more water molecules. High protein content of gelatin thus guarantees its high quality.

Table 2

| Properties of gelatin. | Type A | Type B |
|-----------------------|-------|-------|
| Gel strength (bloom)  | 75 – 300 | 75 – 275 |
| Viscosity (Cp)        | 2.0 – 7.5 | 2.0 – 7.5 |
| Ash content (%)       | 0.3 – 2.0 | 0.05 – 2.0 |
| pH                    | 3.8–6.0 | 5.0–7.1 |
| Isoelectric point     | 9.0–9.2 | 4.8–5.0 |

Fig. 3. Partial structure of collagen (Poppe, 1992).
Ash content could be affected by washing process, and the lower ash content indicates that most of minerals have been washed off. Table 3 shows that the ash contents in the gelatins from red snapper and parrotfish had higher values than those from red tilapia and catfish. Since the high fat content was caused by the improper process of skin washing, there were still plenty of fat remaining during the extraction process. The fat content would affect the shelf-life of gelatin. Fat content is also linked with the quality of gelatin, because fat oxidation could lower the nutritional value and cause deteriorative odor.

3.5. Yield

Yield is one of the important parameters in gelatin production. A high yield indicates that the treatment given in the gelatin conversion was efficient and effective. Yield values could vary depending on the raw skin materials. The yields from the skins of four fish species are shown in Fig. 4. The yield ranged from 20.00 to 24.65%. The highest yield was found for marine parrotfish (Scarus ghobban). The parrotfish skin has compact stratum compactum and thus contains a lot of collagen (Figs. 1d and 2d). The differences in the constituent or structure of the skin main layer result in different yields (Rawdkeun et al., 2013). The high gelatin yield is expected from thick skins, i.e., that of parrotfish containing more collagen than the other species. The yield from freshwater catfish (P. pangasius) skin is 21.93% (Ratnasari and Firlianty, 2016), while that from black tilapia (Oreochromis mossambicus) skin is 8.49% (Koli et al., 2014), grouper (3.68%), and mackerel (Rastreiliger kanagurta) (2.04%) (Irwandi et al., 2009).

The differences in yields resulted from different types of fish may have been affected by several factors. Different habitats could cause different structures and physical properties of gelatins. The characteristics of gelatins are determined based on the intrinsic properties of skins and the collagen content, the water-dissolved part, and the loss of collagen through crosslinking and breakdown during swelling process of skins though washing or incomplete hydrolysis (Kittiphattanabawon et al., 2010; Gomez-Guillen et al., 2001). The conversion level of collagen into gelatin depends on preparation methods of raw materials, processing steps, extraction time, temperature, pH, pretreatment conditions, and raw material characteristics. Another factor is acid treatment which could remove the acid-soluble protein components, fat, undesired compounds, and break the cross-links in collagen molecules. As a result, the skin is swollen and the extraction of collagen can become efficient (Ahmad and Benjakul, 2011).

3.6. Acidity

Degree of acidity could affect physical properties such as viscosity and gel strength, also affecting the gelatin application in the product form. The final pH of gelatin is affected by the overall chemical solutions given to the pre-treatment process. The pH value is thought to be affected only by the acid or alkali concentration for the extraction, the time of washing, and pretreatment before the immersion process.

The pH values of gelatins so far reported are 3.05 for red tilapia gelatin (Jamilah and Harvinder, 2002), and 4.29, 4.34, and 4.17 for tuna, shark, and rohu gelatins, respectively (Shyni et al., 2014). Octopus (Octopus aroeatus) skin gelatin gave pH 10 (Shinduja and Monharaj, 2016), while the values were 5.2 for gourami (TavakoliPour, 2011), and 4.66 for red tilapia (Alfaro et al., 2013). The pH values of gelatin from the four fish species are shown in Fig. 5.

### Table 3

| Species          | Water (%) | Ash (%) | Protein (%) | Fat (%) |
|------------------|-----------|---------|-------------|---------|
| Pangasius sp     | 9.59      | 0.49    | 87.52       | 0.72    |
| Red tilapia      | 8.72      | 0.48    | 87.54       | 0.56    |
| Red snapper      | 9.28      | 0.96    | 88.41       | 0.56    |
| Parrotfish       | 8.42      | 0.86    | 88.48       | 0.49    |
| Catfish          | 2.08      | 0.05    | 87.10       | 0.002   |
| Cobia            | 7.01      | 0.71    | 89.70       | 2.58    |
| Red snapper [4]  | 9.91      | 4.02    | 71.11       | 1.58    |

Notes:
[1] Nurilmala et al. (2020b).
[2] Ratnasari and Firlianty (2016).
[3] Amiza et al. (2015).
[4] Pranoto et al. (2011).

Fig. 4. The yields of gelatin from fish skins (Nurilmala et al., 2017). Small letters indicate significant differences (p < 0.05).

Fig. 5. The pH values of prepared gelatin from fish skins.
Color values of gelatin from fish skins.

| Parameter | Pangasius | Red tilapia | Red snapper | Parrotfish |
|-----------|-----------|-------------|-------------|------------|
| L*        | 83.18 ± 4.34a | 78.39 ± 3.89b | 60.42 ± 6.37c | 30.04 ± 2.38a |
| a*        | −1.16 ± 0.19a | −0.36 ± 0.95a | −1.33 ± 0.78a | −1.43 ± 1.90a |
| b*        | 24.31 ± 1.81a | 30.04 ± 1.11c | 20.05 ± 3.97c | 12.96 ± 0.73c |

Notes: Different numbers followed by superscripts indicate significant differences (p < 0.05).

Fig. 5 (Nurilmala et al., 2017). The highest pH value of Pangasius gelatin is still in the range of food grade and standard of edible gelatin applications (pH 3–4.5). The resulted gelatin can also be applied to soft and hard capsules, and tablets that are in the pH standard of 3.8–4.5, and the food industries of gum and wafers, but is not applicable to photography (pH 5.65–5.85) (GMIA, 2019).

The washing process is an important step for removing acidic remnants of fish skin. Optimal washing process will reduce the acid content trapped in the skin, so the final pH value will be closer to neutral. A low pH value is suspected, when the washing process is not optimal, and thus citric acid used is still remaining during the extraction process. Soaking the skin in acid makes the skin to swell and the acid remains trapped in the tissue. During the extraction procedure, the acid comes out from the fibrils and reduces pH value.

3.7. Color

Color, one of the aesthetic factors, can be used as a parameter in the quality evaluation of gelatin. The color scale consists of L* value (lightness), a* value (green-redness), and b* value (blue-yellowness). The color values of the fish gelatins are shown in Table 4. L values of the gelatins ranges from 30.04 to 83.18. The color of gelatin derived from the skin of catfish shows high lightness value compared with those from the other fish species. The L* value of red snapper gelatin was in line with the previous report (63.57) (Pranoto et al., 2011). The L* values of gelatins from kuma-kuma (Brachyplatystoma filamentosum) and red tilapia are 61.8 (Silva et al., 2017), and 89.25 (Alfaro et al., 2013), respectively. The color of skin gelatin from the four fish species are shown in Table 4 (Nurilmala et al., 2017).

The L value of gelatins varied depending on the fish species. The differences in the lightness of gelatins could have been caused by the pigments contained in the skin. The gelatin from catfish skin showed the highest lightness of 83.18. The a* values ranged from −1.43 to −0.36. These values were different from the values in the previous study for the gelatin from kuma-kuma (B. filamentosum) skin (2.24, slightly red) (Silva et al., 2017), but similar to the value of red tilapia gelatin (−0.44, green) (Alfaro et al., 2013). The b* values of gelatins ranged from 12.96 to 30.04. The gelatin from red tilapia skin gave the highest yellowness (30.04). The reported values for the gelatins from the other sources are 9.72 for kuma-kuma skin (Silva et al., 2017), 2.48 for red tilapia (Alfaro et al., 2013), and 30.50 for red snapper (Pranoto et al., 2011).

The appearance of fish gelatin is generally bright and shiny. However, the color of commercial gelatins from bovine and porcine sources is usually pale yellow to dark brown. The difference in the color of fish gelatin can be caused by several factors, namely, the differences in raw materials (Ratnasari et al., 2013), skin pigments such as melanin (Jamilah et al., 2011), extraction temperature (Chanchareem et al., 2016), and Maillard reaction products and non-enzymatic browning (Wang et al., 2011). The color of gelatin does not affect the functional properties of gelatin, but bright colors are desirable for various types of foods without the need for additional dyes (Shyni et al., 2014). Turbidity due to a non-enzymatic browning reaction would occur at higher extraction temperature (Chanchareem et al., 2016).

3.8. Gel strength

The gel strength of fish skin gelatin is shown in Fig. 6. Gel strength ranges from 54.0 g to 118.4 g (bloom). The highest value was obtained for the parrotfish skin gelatin. The gel strength values of fish gelatins so far reported are as follows: 3.9 g for commercial cold water fish gelatin (See et al., 2010), 2.8–187 g for fivelined threadfin bream (Nemipterus tumboloides) (Pranoto et al., 2016), 62.6 g for bigeye snapper (P. tayenus) (Sukkwai et al., 2011), 56–111 g for bamboo shark (Chiloscyllium punctatum), 10–17 g for blacktip shark (Carcharhinus limbatus) (Kittipattanabawon et al., 2010), 84 g for carp (Hypophthalmichthys molitrix) (Tavakolipour, 2011), 219 g for red snapper (Lutjanus altilifornalis) (Pranoto et al., 2011), 232 g for cobia (R. canadum) (Silva et al., 2014), 244 g for kuma-kuma (Silva et al., 2017), and 300 g for red tilapia (O. nilotica) (Jamilah et al., 2011). Gel strength is also an important parameter for gelatin properties. One of the important properties of gelatin is the ability to turn from a sol state to a reversible gel. It is known that fish from tropical waters can provide gelatins of higher gel strength compared with cold water species, which give gelatins of low gel strength (<50 g). The yield of gelatin affects the gel strength of the products. The gelatin from the parrotfish skin shows significantly higher gel strength compared with the counterparts from the other species.

Generally, gel strength of fish skin gelatins are lower than those of the commercially available gelatins from bovine and porcine sources. However, fish skin gelatins are still in the range of food grade applications (50–300 g) and tablets (75–500 g) (GMIA, 2019). Several factors such as the extraction process using acid treatment could be associated with the low gel strength of fish gelatins. The acid treatment can inhibit the gel formation, because the hydrogen bonds between the polypeptides forming collagen are weakened resulting in very short and damaged monomers. This is in accordance with the other study that compared the effects of acid and alkaline treatments on the gelatin from carp skin (84 g and 176 g after each treatment, respectively) (Tavakolipour, 2011). Acid treatment would result in higher yield of gelatin compared with alkaline treatment, but the physical quality of alkaline treated gelatin is better (Tavakolipour, 2011).
Other causes of low gel strength are related to the polypeptide chain length. Longer chains favor high gel strength. The difference in gel strength is caused by the polypeptide length, the composition of amino acids, and habitat temperature (Jongjareonrak et al., 2010). Gel strength is also influenced by the molecular weight of gelatin, which is influenced by the presence of α chain (Karim and Bhat, 2009). Gelatin with many α chains shows good functional properties including gel strength (Nagarajan et al., 2012). Gel strength depends on the proportion of fractions, the molecular weight of which being around 100,000 Da (Schrieber and Garies, 2007).

### 3.9. Viscosity

Viscosity is the ability to withstand flowing, and thus the viscosity of gelatin is to be determined by measuring the thickness of gelatin solution at certain concentration and temperature. Viscosity is the second most important parameter for the gel strength (CMIA, 2019). The viscosity values of fish gelatins are shown in Fig. 7. The viscosity of fish gelatins ranges from 16.0 to 22.0 cP (Nurilmala et al., 2017), in contrast to the value of standard gelatin (1.5–7.5 cP) (CMIA, 2019). The highest viscosity value is obtained for the gelatin from the parrotfish skin. These values are higher than those reported previously, namely, 4.91 cP for cobia (R. canadum) (Silva et al., 2017) and 7.07 cP for red snapper (Pranoto et al., 2011). The viscosity was found to be species-specific. This is in accordance with the previous study on the species-specific differences in viscosity (Shyni et al., 2014).

Viscosity is the flow process of a liquid (Schrieber and Garies, 2007), and is also related to the molecular distribution of gelatin (Stansby, 1977), while molecular weight is directly related to the peptide length. It means that the longer the amino acid chains, the higher the viscosity value, and if the viscosity is low, intensive hydrolysis is considered to have proceeded, resulting in the decrease of molecular weight. The viscosity of a given protein solution depends on the intrinsic elements of biopolymers such as molecular mass and volume, size, shape, surface charge, amino acid content, and environmental elements including pH, temperature, ionic strength of solvent (Masuelli and Sansone, 2012).

Acid treatment can also affect the viscosity. At higher acid concentration, the structures of collagen and gelatin become more loosen, causing the breakdown of polypeptide chains so that shorter chains will be produced resulting in lower values of viscosity (Stansby, 1977). In addition, the high viscosity value is influenced by the distribution of gelatin molecules in solution and molecular weight of gelatin. The higher the molecular weight range of the gelatin, the lower the distribution rate of the gelatin molecules in the solution, causing high viscosity value. Viscosity, gel strength, and melting point of gelatin are influenced by the presence of α1/α2 chains and molecular weight distribution (Gomez-Guillen et al., 2002). Viscosity is also influenced by the existence of a β chain (Muyonga et al., 2004). Another factor causing low viscosity values is the content of mineral ions, which bind to gelatins. Binding of mineral ions to gelatin causes the decrease in the numbers of hydrogen bonds in the gelatin, so that the distribution rate of the gelatin becomes faster and the viscosity value drops (Wulandari et al., 2013).

### 3.10. Amino acid composition

Since gelatin is derived from collagen, the amino acid composition is basically the same as that of collagen. Amino acids are important parameters to determine the properties of gelatin, especially to estimate the gel strength and viscosity. High amino acid contents are related with the high viscosity and gel strength. The amino acid compositions of fish skin gelatins are shown in Table 5 (Nurilmala et al., 2017). The total amino acid is the highest in the parrotfish skin gelatin. The total amino acids are higher when compared with those of pangasius and red tilapia counterparts, which amounted to 754 and 655 mg/g, respectively (Ratnasari et al., 2013). Pro and Hyp are the most abundant amino acids in gelatin, ranging from 15 to 23% of the total amino acids (Karim and Bhat, 2009). The total amounts of amino acids in the gelatins from warm-water species (16–20%) are slightly higher compared with those of cold-water species (14–17%). The Gly and Pro contents in the parrotfish skin gelatin are 286 and 120 mg/g, respectively.

| Amino acid     | Species        | Red tilapia | Red snapper | Parrotfish |
|----------------|----------------|-------------|-------------|------------|
| Alanine        | Pangasius      | 84.39 ± 0.50 | 73.33 ± 0.35 | 87.74 ± 0.53 | 92.55 ± 0.80 |
| Arginine       | Pangasius      | 101.05 ± 0.00 | 95.13 ± 0.55 | 95.46 ± 0.10 | 110.37 ± 0.55 |
| Aspartic acid  | Pangasius      | 39.83 ± 0.21 | 34.90 ± 0.24 | 37.62 ± 0.10 | 37.78 ± 0.24  |
| Glutamic acid  | Pangasius      | 77.57 ± 0.06 | 69.21 ± 0.71 | 75.36 ± 0.17 | 75.98 ± 0.54  |
| Glycine        | Pangasius      | 260.25 ± 1.13 | 239.11 ± 0.82 | 247.04 ± 0.15 | 285.59 ± 1.86 |
| Histidine      | Pangasius      | 11.96 ± 0.04 | 8.97 ± 0.00  | 10.27 ± 0.00 | 11.28 ± 0.12  |
| Isoleucine     | Pangasius      | 13.15 ± 0.07 | 11.02 ± 0.00 | 7.34 ± 0.06  | 9.30 ± 0.12   |
| Leucine        | Pangasius      | 27.58 ± 0.07 | 25.27 ± 0.13 | 22.30 ± 0.29 | 24.05 ± 0.32  |
| Lysine         | Pangasius      | 27.44 ± 0.14 | 24.37 ± 0.43 | 28.02 ± 0.18 | 27.40 ± 0.38  |
| Phenylnalaine  | Pangasius      | 36.48 ± 0.05 | 31.64 ± 0.22 | 32.31 ± 0.42 | 38.55 ± 0.58  |
| Proline        | Pangasius      | 111.07 ± 0.90 | 104.12 ± 0.59 | 103.24 ± 0.64 | 119.63 ± 0.58 |
| Threonine      | Pangasius      | 30.37 ± 0.40 | 28.56 ± 0.21 | 33.42 ± 0.73 | 29.43 ± 0.18  |
| Tyrosine       | Pangasius      | 10.83 ± 0.01 | 9.23 ± 0.05  | 8.91 ± 0.10  | 10.85 ± 0.11  |
| Histidine      | Pangasius      | 11.96 ± 0.04 | 8.9 ± 0.00   | 10.27 ± 0.00 | 11.28 ± 0.12  |
| Total          |                | 894.31       | 815.91       | 840.98      | 925.59       |
The contents of these amino acids in the patin gelatin are reported to be 167 mg/g and 117 mg/g, respectively (Ratnasari et al., 2013), and those of red tilapia are 197 mg/g and 124 mg/g, respectively (Jamilah et al., 2011). Gly, Pro, and Hyp ratios in the cobia gelatin are 21%, 10%, and 7.4%, respectively (Amiza et al., 2015), while Gly content in the red snapper gelatin was 229 mg/g (Pranoto et al., 2011). Gly contents in cobia and croaker gelatins are 307 and 322 mg/g, respectively (Silva et al., 2014), while Gly content in the belida Chitala lopis gelatin was reported to be 334 mg/g (Kittiphattanamabwon et al., 2016).

The differences in amino acid contents depend on the purity of gelatin. Gelatins of lower amino acid contents will result in lower viscosity and gel strength (Amiza et al., 2015; Silva et al., 2017). This is in line with the results for the catfish and tilapia gelatins of lower gel strength. Each species has its own intrinsic amount of amino acids (Giménez et al., 2005). Amino acid content of gelatin is influenced by molecular weight distribution, habitat temperature, species and sex as well as extraction processes (Jongjareonrak et al., 2010). Incidentally, differences in the habitats of salmon, either sea water or freshwater, did not affect amino acid compositions of gelatins (Lee et al., 2016).

3.11. Molecular weight distribution

The molecular weight distribution can be examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to estimate the type of gelatin, because the physical and chemical properties of gelatin depend on the molecular weight distribution of collagen and the subunit ratio of $\alpha_1/\alpha_2$ (Gomez-Guillen et al., 2002). The SDS-PAGE patterns of gelatins from the four fish species are shown in Fig. 8. Conversion of collagen to gelatin causes inter- and intra-molecular bonds of collagen and hydrolyzed peptides so that gelatin consists of the fragments with the molecular weights ranging from 80 to 250 kDa. Three typical subunits are detected in the red snapper and parrotfish skin gelatins, namely, the $\beta$, $\alpha_1$, and $\alpha_2$ chains. The bands detected for catfish and red tilapia gelatins are very weak, suggesting that the $\beta$ chains of the red snapper and parrotfish skin gelatins are more tolerant to the hydrolysis than those of the former. The parrotfish skin gelatin consisted of $\beta$ subunit (255 kDa), $\alpha_1$ subunit (156 kDa), $\alpha_2$ subunit (128 kDa), while the red snapper skin gelatin consisted of $\beta$ subunit (252 kDa), $\alpha_1$ subunit (155 kDa), and $\alpha_2$ subunit (127 kDa). The red tilapia skin gelatin consisted of $\beta$ subunit (200 kDa), $\alpha_1$ subunit (120 kDa), and $\alpha_2$ subunit (113 kDa), while the pangasius counterpart consisted of $\beta$ subunit (210 kDa), $\alpha_1$ subunit (125 kDa), and $\alpha_2$ subunit (118 kDa) (Nurilmala et al., 2017). Gelatins of high $\alpha$ chain content show better functionalities including gel strength (Nagarajan et al., 2012).

The cold water species, cobia and croaker, have collagen of type $\alpha_1$ (100 kDa) and $\beta$ chain (200 kDa) (Silva et al., 2011), while subunit compositions of shark, rohu, and tuna skin gelatins are $\beta$ and $\alpha$ chains, but the former has been found only in sharks (Shyni et al., 2014). In the rabbitfish (Chimaera monstrosa) gelatin, the presence of $\alpha$ and $\beta$ chains is not recognized (Sotelo et al., 2017), while the white snapper (Lates calcarifer) gelatin consisted of $\beta$ and $\gamma$ chains (Sae-Leaw et al., 2016).

The process of gelatin preparation by using citric acid might have affected the molecular weight distribution and caused the breakdown of the higher structures of collagen. It is suspected that citric acid broke the peptide bonds and thus the molecular weight was reduced (Niu et al., 2013), though citric acid effectively removes phospholipids, and also plays an important role in binding amino acid residues in collagen (Benjakul et al., 2009).

3.12. Potency of bioactive peptides from collagen and gelatin

Collagen and gelatin can be converted to bioactive peptides, which will be produced during gastrointestinal digestion or by controlled enzymatic hydrolysis. Such bioactive peptides show positive biological functions for human health (Gomez-Guillen et al., 2011). The proteases such as Alcalase, pepsin, and trypsin can hydrolyze collagen and gelatin to active peptides. Over the last decade, not a few researchers have investigated enzymatic hydrolysis of collagen and gelatin for the production of bioactive peptides. Collagen peptides have been reported to show anti-aging potential supported by their antioxidant activity (Ngo et al., 2014; Czech and Seagarden, 2016; Nurilmala et al., 2020a). It has also been reported that the antioxidant activity of gelatin increased through the hydrolysis process (Gomez-Guillen et al., 2011; Aleman et al., 2011). The tuna skin collagen hydrolysate using Alcalase from has been reported to show higher anticancer activity than those from the other fish species (Han et al., 2011). Antioxidant activity of tuna skin collagen without hydrolysis shows lower activity compared with those of its hydrolysate. Recently, bioactive peptides with antioxidant activities have been prepared from various enzymatically hydrolyzed proteins in the field of pharmaceutical, health care, and food processing industries. The antioxidant activity of gelatin peptides is useful to prevent oxidative stress without any adverse effect. In addition, the peptides with strong antioxidant activity are widely used in anti-aging studies (Sonani et al., 2015).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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