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# Functional properties of gelatin recovered from scales of farmed sea bass (*Dicentrarchus labrax*)

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**Abstract:** In the current study gelatin was recovered from scales of farmed sea bass (*Dicentrarchus labrax*). The first part of the study was planned to determine the yield of acid-soluble collagen and pepsin-soluble collagen from the scales. Both collagens were characterized as type I collagen, containing α-1 and α-2 chains. In the second part, gelatin was extracted using acetic acid and functional properties were determined in this product. Gelatin protein value was found to be 96% and the yield was found to be 18.49%. Proline and hydroxyproline values in the amino acid were found to be high. The gel strength of gelatin was 305 g and viscosity of the gelatin was 33 cP. The gelatin was identified as type A gelatin due to its properties. Fourier transform infrared spectroscopic analysis showed the characteristic similarities of gelatin produced from sea bass scales and calf skin.

**Key words:** Collagen, gelatin, gel strength, sea bass scales, imino acids

## 1. Introduction

The recent improvements in fish processing technology enable development in the utilization of fish processing byproducts. In the fishmeal industry, the interest in the utilization of byproducts to improve the protein and polyunsaturated fatty acids contents in the fishmeal has increased due to high competition in aquaculture in the last decade. Therefore, converting fish processing byproducts into new value-added products or biomolecules has become a commercially important research area. It was estimated that 25% of the global fish processing production is discarded as waste or processed into fish oil, fishmeal, or pet food (1).

Collagen can be extracted from byproducts of fish. Collagen was previously produced from the skin and scale of carp (*Cyprinus carpio*) (2) and bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*) (3). However, no information about gelatin recovery from sea bass scales (*Dicentrarchus labrax*) has been reported previously. The aim of this study was to characterize the proteins of sea bass scale collagen and to assess the functional properties of gelatin prepared from these scales.

Collagen type I has a triple helical structure and is commercially used in the food, cosmetic, pharmaceutical, and biomedical industries, and in particular in tissue engineering due to its excellent biocompatibility and biodegradability properties (4). The utilization of aquatic resources accounts for just 1% of total gelatin production (5). Almost all the gelatin produced from aquatic resources originates from the fish intestine and fish skin (6). The extraction of gelatin requires an alkaline or an acid treatment to break the cleavage of collagen crosslinks, followed by extraction with warm water. Heat treatment is necessary to destabilize the triple helix configuration of collagen to convert its helical conformation into a coiled conformation, resulting in a gelatinous state when the matter is cooled. This extraction method accounts for 60% of total gelatin production in the world. In particular, Islamic countries prefer gelatin produced from aquatic sources (1%) and animals like cows and calves (28%), which tends to be more expensive compared to other countries that produce gelatin from swine.

In 2012, the Turkish Statistical Organization (7) estimated that 26.80% of the total fishery production was supported by the aquaculture industry in Turkey. Farmed sea bass (*Dicentrarchus labrax*) production amounts to 24.90% (47,013 t) of the total aquaculture production. Generally, fish are processed in two stages. First, they are bled and gutted, and then their scales are removed. The scales are separated from the rest of the byproducts, which are collected for rendering. Many fish processors render the scales due to the risk of clogging the drainage and filtration system of the plant. Typically, the scales of sea bass constitute nearly 5% of the edible part of the fish.

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In other words, for each specimen, 6.5 g of fish scale can be recovered. In Turkey, the skin recovery from sea bass may output approximately 1280 t of fish scales available for commercial purposes. Despite the lack of commercial efforts for gelatin recovery in Turkey, gelatin from fish skin is widely commercialized in the rest of the world and primarily in China, which creates an advantage for seafood processors in seafood business and marketing.

## 2. Materials and methods

### 2.1. Fish scale preparation

Scales of sea bass (*Dicentrarchus labrax*) with an average body weight of 200–300 g were collected at Uğurlu Ltd. Company in Didim, Aydın. The scales of sea bass were removed using a scaling machine (AGK Nr. 300 scaling machine, AGK Kronawitter GmbH, Wallersdorf, Germany), packaged in polyethylene bags, iced, and quickly transported to the laboratory. Consequently, scales were divided into two groups: the first group for collagen extraction and SDS-PAGE (100 g) and the second group for gelatin extraction (1200 g dry scale). The first group was kept at –80 °C until use for acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) experiments. The second group was treated with chilled water, washing it twice to clean the scales before being dried.

### 2.2. Gelatin extraction

The chosen method was used to extract type 1 collagens based on collecting collagen proteins from the scales by removing noncollagenous proteins, removing lipids, and removing mineral contents. Dried scales (1200 g) were initially stirred with 5% NaCl solution (1/10, w/v) for 30 min at room temperature. This step was repeated twice and the second step was completed by stirring with 0.4% NaOH (1/10, w/v) for 60 min to remove the noncollagenous proteins from the scales. Alkali solution was changed every 30 min and the third step proceeded using 10% isobutyl alcohol (1/4, w/v) to remove lipids from the scales. This step was repeated three times for 30 min in a digital linear shaker (Dragon Lab SK-330 model, Beijing, China). The final step was demineralization with 0.5 N EDTA solution at an inherent pH of 7.66 for four different time periods of shaking: 12 h, 2 h, 2 h, and 1 h (Dragon Lab SK-330 model). In each step, scales were recovered by filtering through a sieve and washed with distilled water to remove any residual matter. Recovered scales were soaked in 0.05 M acetic acid solution for 3 h. After filtering, 1/3 (w/v) water was added and heated at 60 °C overnight in an oven. This was filtered and the filtrate (dried thin films) was dried in plastic trays at room temperature using air conditioning overnight (set at 18 °C, flow temperature was 10 ± 2 °C). Dried thin films were ground using a coffee grinder. The yield of gelatin from the fish scales was calculated on a dry weight basis and expressed as a percentage.

#### 2.2.1. Extraction of acid-soluble collagen

The extraction of ASC was performed at 4 °C following the method of Matmaroh et al. (8). Fish scales (100 g) were ground prior to collagen extraction. Small pieces of fish scale were then suspended in 0.1 N NaOH for 6 h at the ratio of 1:10 (w/v) with continuous stirring to remove noncollagenous proteins and pigment. The solution was changed every 3 h. Treated scales were washed with chilled water to achieve a neutral pH. Demineralization of fish scales was carried out using 0.5 M EDTA-2Na solution (pH 7. 4) for 48 h at the ratio of 1:10 (w/v) and the solution was changed every 12 h. Demineralized fish scales were then subjected to ASC extraction with 0.5 M acetic acid for 48 h. The mixture was filtered through two layers of cheese cloth. The supernatant was subjected to salting out by adding NaCl to obtain the concentration of 2.5 M in the presence of 0.05 M Tris(hydroxymethyl) amino methane (pH 7.0). The precipitated matter was collected by centrifugation at 20,000 × *g* for 1 h. The pellet was dissolved in a minimum volume of 0.5 M acetic acid at the ratio of 1:9 (w/v) and subsequently dialyzed in 20 volumes of 0.1 M acetic acid, followed by distilled water. All processes were carried out at 4 °C. Collagen was lyophilized using a freeze-dryer (Alpha 1-2 LD Plus freeze dryer; Martin Christ GmbH, Osterode, Germany) in –50 °C for 24 h. The collagen was stored at –80 °C until use. The collagen thus obtained was referred to as ASC.

#### 2.2.2. Extraction of pepsin soluble collagen

Undissolved matter from ASC extraction was further extracted in 0.5 M acetic acid containing 1% pepsin (w/w) at a ratio of 1:10 (w/v) for 48 h at 4 °C. After extraction, the supernatant was obtained in the same manner as ASC preparation. The precipitation, dialysis, and lyophilization were carried out in the same manner as for ASC. The collagen obtained with the aid of pepsin was referred to as PSC.

### 2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Protein patterns of ASC and PSC were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Matmaroh et al. (8). The purification and precipitation part of the analysis was done in Turkey and identification by using SDS-PAGE was done at the Max Rubner Institute, Hamburg, Germany. The samples were dissolved in 5% SDS and the mixtures were incubated at 85 °C for 1 h in Thermoblock. The mixtures were centrifuged at 8500 × *g* for 5 min at room temperature to remove undissolved debris. Solubilized samples were mixed at a ratio of 1:1 (v/v) with the sample buffer (0. 5 M Tris-HCl, pH 6.8, containing 4% SDS and 20% glycerol) in the presence of 10% 3-MPD and 500

mL of bromphenol blue (1%) and were then incubated at 95 °C and allowed to boil for 3 min. Samples (10 µL of protein) were loaded onto polyacrylamide gels consisting of a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis at an initial current of 20 mA, with the current of samples entering into the running gel at 25 mA/gel using a Maxigel Biometra unit (Biometra GmbH, Göttingen, Germany). After electrophoresis, the gel was fixed with 50% (v/v) methanol and 5% acetic acid for 30 min. The gel was then stained for 3 h with 0. 05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid. The gel was finally destained with 30% (v/v) methanol and 10% (v/v) acetic acid. SDS-PAGE protein markers (Serva Electrophoresis GmbH, Heidelberg, Germany) were used to estimate the molecular weight of proteins. The band intensities were quantified with the Quantity One software package for imaging and analyzing electrophoresis gels (Bio-Rad Laboratories, Hercules, CA, USA).

**2.4. Proximate composition**

The moisture content (oven-drying procedure), ash content, crude protein, and fat contents of sea bass scales and gelatin were evaluated using AOAC official methods 934.01, 942.01, 954.01, and 991.36, respectively (9).

**2.5. Determination of gel strength**

The Bloom strength of gelatin was determined using the standard method (10). Samples were weighed into the Bloom bottles and dissolved in distilled water to a final concentration of 6.67% (w/v). After placing samples in the refrigerator for 16 h at 5 °C, Bloom strength was determined using a TAXT Plus (Stable Micro Systems, Godalming, UK) with a 25-kg load cell, equipped with a GL 4/P 05S probe of 1.27 cm in diameter. The maximum force (in grams) when the probe had penetrated 4 mm into the gelatin gel's surface was recorded.

**2.6. Determination of turbidity**

The turbidity of FSG solution (6.67% w/v) at different pH levels (3–10) was determined using the preferred method (11). The samples were dissolved in distilled water at 60 °C and the pH solution was adjusted with either 6 N NaOH or HCl. The turbidity was determined by measuring the absorbance at 360 nm using a PerkinElmer Lambda 25 UV-Vis spectrophotometer (Waltham, MA, USA).

**2.7. Determination of viscosity**

Gelatin was dissolved with distilled water (6.67% w/v) swirled at 60 °C for 30 min in a magnetic stirrer until completely dissolved. Viscosity was determined by Brookfield DV + II Pro viscometer (Middleboro, MA, USA) at 60 rpm with using an HA-4 spindle and helipad stand at 25 °C with 100 mL of gelatin solution. This methodology was based on the method used by Zhou and Regenstein (12).

**2.8. Texture profile analysis**

Samples were weighed into Bloom bottles and dissolved in distilled water to a final concentration of 6.67% (w/v). Next, 100 mL of solution was put inside a rectangle plastic mold (200 mL) for gel formation at 5 °C for 16 h. After gel maturation the gel block was taken and cut into square pieces of 2 × 2 cm. The samples were compressed twice at a crosshead speed of 0.80 mm/s to 65% of their original height using a cylindrical probe of 5 cm in diameter. From the resulting force/deformation curves, the mechanical properties of hardness, cohesiveness, springiness, adhesiveness, resilience, and chewiness were evaluated using the modified method of Yang et el. (13).

**2.9. Amino acid analysis**

A 5-mg aliquot from gelatin sample and scale sample was dissolved in 3 mL of HCl, dried, and hydrolyzed in vacuum-sealed glass tubes at 110 °C for 24 h using a dry bath (dry bath incubator, Fisher Scientific Co., Hampton, NH, USA) After hydrolysis, samples were vacuum-dried, dissolved in citric acid buffer (pH 2.2, Sigma Chemical Co., St. Louis, MO, USA), and injected into an amino acid auto analyzer (Amino acid analyzer S-433H, Sycam, Fürstenfeldbruck, Germany).

**2.10. Fourier transform infrared spectroscopy**

Fourier transform infrared (FT-IR) spectroscopy of gelatin powder was performed using an FT-IR spectrophotometer (Bio-Rad FTS 3000, Excalibur). Briefly, samples consisting of 2 mg of gelatin powder were mixed with 100 mg of potassium bromide (14) and placed on the crystal cell of the FT-IR spectrophotometer. Measurement was performed at 4000–500 $cm^{-1}$ at room temperature and automatic signals were collected from 32 scans at a resolution of 4 $cm^{-1}$.

**2.11. Statistical analyses**

Analysis of variance (ANOVA) was performed and means comparison was done using Duncan's multiple range tests for amino acid comparison between scales and gelatin data. For comparison, the t-test was used. Analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

## 3. Results

**3.1. Proximate composition of scales and gelatin**

Scales of sea bass (*Dicentrarchus labrax*) contained 0.10 ± 0.02% fat, 40.95 ± 2.34% moisture, 27.86 ± 0.05% ash, 0.80 ± 0.02% carbohydrate, and 28.47 ± 1.21% protein. After the extraction, the proximate composition of sea bass scales gelatin contained (in dried form) 3.20 ± 0.02% moisture, 0.01 ± 0.01% ash, 0.04 ± 0.00% carbohydrate, 96.00 ± 2.0% protein, and no fat. After summing the three filtrates together, total yield of gelatin was found to be 18.49% (221.84 g of dried gelatin from 1200 g dry scales).

### 3.2. Yield of ASC and PSC and protein patterns

The yield of ASC was found higher in sea bass scale (ASC 0.87% and PSC 0.75%). Protein patterns of ASC and PSC from sea bass analyzed by SDS-PAGE are shown in the Figure for α-1 and α-2 chains of ASC with molecular weight of 117 and 107 kDa, respectively. Both α-1 and α-2 chains of PSC had slightly higher molecular weight (118 and 108 kDa) when compared with ASC. The ratio of ASC to PSC was determined to be approximately 2:1 and that of α-1 and α-2 chains in ASC and PSC was determined to be approximately 1:1.

### 3.3. Determination of gel strength, turbidity, and pH of gelatin

The fish scale gelatin produced using an acidic technique gave us high Bloom values (Table 1). Based on the pH and turbidity values, type A gelatin was identified (Table 2).

### 3.4. Texture profile analysis (TPA) results

Mechanical compression was used to characterize the gelatin gels. Texture profile data obtained from sea bass scale gelatin were as follows: hardness, 3956.64 ± 798.21; chewiness, 3075.03 ± 667.19; springiness, 0.95 ± 0.02; and resilience, 0.66 ± 0.04. All TPA results can be seen in Table 3.

### 3.5. FT-IR spectroscopy results

The FT-IR spectrum was used to compare the structure of extracted sea bass scale fish gelatin using 0.05 N acetic acid solution and the calf skin gelatin from a database. Three major peak regions were marked as 1 (3600–2700 $cm^{-1}$), 2 (1900–900 $cm^{-1}$), and 3 (900–400 $cm^{-1}$) and assigned to the following respective bonds: amide A and B; amide I, II, and III; and amide IV, V, and VI.

Both samples showed the three major peak regions, 1 (3600–2700 $cm^{-1}$), 2 (1900–900 $cm^{-1}$), and 3 (900–400 $cm^{-1}$). Ten peaks were identified by the software and 8 peaks for amide bonds were identified based on their wave number. Peak shape , wave number at peak, intensity, and the whole range of intensity wave number were used to characterize the differences in the structures of the materials and revealed that the samples had similar protein structures.

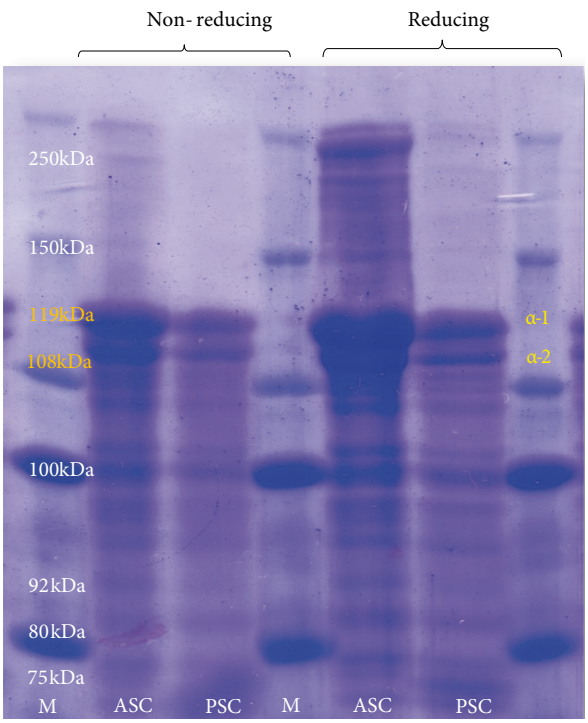


**Figure.** SDS-PAGE patterns of collagen from the scales of sea bass. M: Molecular weight marker; ASC: acid-soluble collagen; PSC: pepsin-soluble collagen.

**Table 1.** Gel strength, pH, and viscosity of gelatin.

| Gel strength (g) | pH | Viscosity (cP) | Torque (%) |
|---|---|---|---|
| 305.34 ± 8.10 | 5.52 ± 0.05 | 32.92 ± 8.60 | 0.49 ± 0.13 |

Arithmetic means and standard deviations. Gel strength, n = 20; pH, n = 3, viscosity, n = 20.

### 3.6. Amino acid composition

Amino acid composition of sea bass scale and produced gelatin is given in Table 4.

## 4. Discussion

The amount of protein in gelatin was determined as 96% in the current study. This result is higher in comparison to Amur sturgeon skin gelatin (90.4%) and Nile tilapia skin gelatin (88.5%) (15). The proximate composition values of the sea bass scales gave us high ash content and high protein content. In the literature, both higher and similar values in different types of fish scales were determined. Wang et al. (16) reported 39.4% ash content in scales of deep-sea red fish and lower protein content in silver carp scale (37.91%). Matmaroh et al. (8) reported 43.43%

**Table 2.** The turbidity of gelatin solutions (6.67% (w/v)) at different pH levels.

| pH | Absorbance (360 nm) |
|---|---|
| 3 | 0.1534 ± 0.00 |
| 4 | 0.1562 ± 0.00 |
| 5 | 0.1654 ± 0.00 |
| 6 | 0.1668 ± 0.01 |
| 7 | 0.1765 ± 0.00 |
| 8 | 0.1770 ± 0.00 |
| 9 | 0.1785 ± 0.00 |

Arithmetic means and standard deviations, n = 3.

**Table 3.** TPA results.

| Hardness (g) | Adhesiveness | Springiness | Cohesiveness | Chewiness (g) | Resilience |
|---|---|---|---|---|---|
| 3956.64 ± 798.21 | –35.56 ± 5.2 | 0.95 ± 0.02 | 0.83 ± 0.02 | 3075.03 ± 667.19 | 0.66 ± 0.04 |

Arithmetic means and standard deviations, n = 20.

protein in common carp scale and 34.46% protein in golden goatfish. As mentioned, the changes in proximate composition values depend on the species.

In the extraction process, yield of ASC and PSC contents was determined. ASC yield was found higher than PSC yield in the current study in sea bass scales. This was not in agreement with the previous studies of Matmaroh et al. (8) and Zhang et al. (17). Matmaroh et al. (8) found ASC and PSC as respectively 0.46% and 1.20%, and Zhang et al. (17) determined ASC and PSC as respectively 0.86 and 2.32%. This result may again be due to using different aquacultured fish species.

The difference in efficiency of pepsin in extracting collagen might be governed by fish species (8). On the other hand, the origin of the used pepsin enzyme was the stomach of porcine species, which means that it is not possible to use it in Islamic countries. The importation of porcine products is not allowed by law in Turkey. That is the why the structure and production of gelatin using the ASC technique was examined in this study. The results showed that acids and enzymes affect the extraction of collagen in nearly the same pattern in sea bass scales. Before the gelatin extraction period it was necessary to identify the type of collagen in sea bass scales. Protein patterns were determined using SDS-PAGE. The results showed that collagens in sea bass scales were type I collagens. ASC was also classified as type I collagen in previous studies in a different species, spotted golden goat fish, by Matmaroh et al. (8). In their study, they suggested that pepsin more likely cleaved the telopeptide region, in which γ-chains and β-chains were cleaved and α-chains were released to greater extent as evidenced by the coincidental increased band intensity of the α-chains in PSC. However, in the current study, strong decrease in γ- and β-chains and the near disappearance of high-molecular-weight fragments could be seen in both samples.

Gel strength is among the important data used to determine the quality of gelatin. It is exactly in relation to the contents of imino acids (proline and hydroxyproline). The stability of the triple helical structure in denatured gelatin is proportional to the total contents of imino acids (18). Hydroxyproline and its hydrogen-bonding properties have a major role in the stabilization of the triple helical structure of collagen (19). This means that gelatin has better viscosity and better gel strength with high hydroxyproline content. The Bloom value is the weight in grams that is required for a specified plunger to depress the surface of a standard, thermostated gel to a defined depth under standard conditions. The gelling strength of commercial gelatins ranges from 100 to 300 g, but gelatins with Bloom values of 250–260 g are the most desirable (10). Due to the Gelatin Manufacturers Institute of America (GMIA) standards (10), commercial gelatin Bloom strength varies from 100 to 300 g in the sector. By that scale, gelatin with Bloom value above 250 g is defined as high-Bloom gelatin. Fish gelatin typically has a Bloom value ranging from as low as 0 to 270 g (20) compared to the high Bloom values for bovine or porcine gelatin, which have Bloom values of 200–240 g. Some gelatins of species of warm-water fish have been reported to exhibit relatively high Bloom values, close to that of

**Table 4.** Amino acid compositions of scales and gelatin.

| | Scales, mg/100 g | Sea bass scale gelatin, mg/100 g |
|---|---|---|
| Glycine | 5905 ± 12 [a] | 24,406.5 ± 187.5 [b] |
| Proline* | 4257 ± 10 [a] | 15,751 ± 2 [b] |
| Alanine | 3804 ± 4 [a] | 10,377 ± 11 [b] |
| Hydroxyproline * | 2965 ± 4.3 [a] | 10,223 ± 10 [b] |
| Serine | 965 ± 1.5 [a] | 5808.5 ± 9.5 [b] |
| Lysine | 1260.5 ± 12 [a] | 4264.5 ± 252.5 [b] |
| Threonine | 1114 ± 3 [a] | 4207.5 ± 56.5 [b] |
| Glutamic acid | 1284 ± 8 [a] | 3468 ± 240 [b] |
| Arginine | 1300 ± 12 [a] | 2643 ± 14 [b] |
| Aspartic acid | 299 ± 30 [a] | 2368.5 ± 127.5 [b] |
| Leucine | 1122 ± 3.5 [a] | 2576 ± 2 [b] |
| Valine | 1080.5 ± 2.5 [a] | 2151 ± 8 [b] |
| Phenylalanine | 1250.5 ± 3.2 [a] | 2670 ± 12 [b] |
| Methionine | 784 ± 10 [a] | 2004.5 ± 23.5 [b] |
| Isoleucine | 595 ± 9.2 [a] | 1130 ± 6 [b] |
| Histidine | 818 ± 3 [a] | 1289.5 ± 84.5 [b] |
| Tyrosine | 565 ± 4 [a] | 662 ± 4 [b] |

*Imino acids. Arithmetic means and standard deviations, n = 3. Different superscripts show significant differences between the samples ($P < 0.05$).

pork gelatin. Such high gel strength characterizes only the gelatins extracted from the skins of warm-water fish such as tilapia (21). For example, Bloom values ranging from 128 to 273 g have been reported for tilapia gelatin (21,22). In the current study, the Bloom value of gelatin was also found to be high (305.34 ± 8.10 g), signifying that high-quality gelatin was recovered in this study. The GMIA (10) reported some criteria to identify the type or edibility of gelatin. Gelatins with pH between 3.8 and 5.8 are defined as type A and gelatins with pH values between 5.0 and 7.5 are defined as type B. In the current study, the pH value of sea bass scale gelatin was determined to be 5.52 ± 0.05. There is no agreement that identification can be done with pH alone. The next criterion, turbidity value, might resolve this confusion. The isoelectric point of the gelatin can give some clues about the type of gelatin, either A or B. Poppe (23) declared that the maximum turbidity always gives the isoelectric point of gelatin (Table 2). In the current study, the maximum value was determined at pH 9 in gelatin recovered from sea bass. The gelatin was therefore placed in the pH range of 7–9 as reported by Aewsiri et al. (11) and the GMIA (10). This proved that the produced gelatin was type A. By analyzing the data obtained, the gelatin could be classified as edible, high-Bloom type A gelatin.

Another important textural criterion in gelatin quality could be the textural profile of the gelatin gels. TPA gave higher results than those of the previous study of Wangtueai and Noomhorm (24). Obtained data from sea bass scale gelatin and the aforementioned study of lizardfish scale gelatin were as follows: for sea bass scale gelatin, hardness of 3956.64 ± 798.21, chewiness of 3075.03 ± 667.19, springiness of 0.95 ± 0.02, and resilience of 0.66 ± 0.04, and for lizardfish scale gelatin, hardness of 644 ± 21.2, chewiness of 616 ± 64.07, and springiness of 1.01 ± 0.07. Current results were also higher than those of bovine gelatins reported in the study of Wangtueai and Noomhorm (24) and higher than results for grouper skin gelatin and bovine-porcine mixed gelatin in a study by Rahman and Al-Mahrouqi (25). These comparisons show that, in the current study, high-quality fish scale gelatin was produced with a better textural profile. TPA results can be seen in Table 3.

FT-IR spectroscopy was used to compare the structures of purchased calf gelatin leaf and produced fish scale gelatin leaf. Comparison between gelatins structure was based on the comparison of absolute peak intensities. The amide I band, between 1600 and 1700 $cm^{-1}$, was the most useful for infrared red spectroscopic analysis of the structure of proteins (26). The intensity of the amide III band has also been associated with the triple helical structure (14). This technique was used to compare the similarity between the produced gelatin and calf skin gelatin from a database. In the current study, three regions were assigned to the bonds: amide A and B; amide I, II, and III; and amide IV, V, and VI. Similar results were also observed by Hashim et al. (27), Muyonga et al. (14), and Ahmad and Benjakul (28). These were amide A, B, I, II, III, IV, V, and VI (14,29). In the literature, peak shape, wave number at peak, intensity, and whole range of intensity wave number data are used to characterize the differences in the structure of a material. Similarity was calculated to be 92% by spectroscopy software. This means that the quality of sea bass scale gelatin is nearly the same as that of calf skin gelatin.

Amino acid composition of gelatin might depend on the species and the used tissue. Many studies can be found on aquatic gelatin amino acid content in the literature, but most of them were recovered from the skin of the species (19,30). Glycine content of sea bass scale gelatin (24,406.5) was nearly the same as in big eye snapper skin gelatin as reported by Benjakul et al. (30), but lower than in sole, megrim, and cod gelatin (18). On the other hand, the content of imino acids (proline and hydroxyproline) in the current gelatin were 15751 mg/100 g (proline) and 10,223 mg/100 g (hydroxyproline), and these results were higher than the values reported for bigeye snapper, Nile tilapia, and Amur sturgeon skin gelatins (18,30). Gelatin with high amounts of imino acid offers better viscoelastic properties as reported by Gomez-Guillen et al. (19). These imino groups have a major role in the stabilization of the triple helical structure of collagen (18,19). A high amount of alanine was also observed in the current study. The wide range of Bloom values found for the various gelatins arises from differences in proline and hydroxyproline contents in collagens of different species and is also associated with the temperature of the habitat of the animals. Badii and Howell (29) showed that hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, Phe, and Met) could also contribute to the high Bloom value of tilapia fish gelatin. They found a lower number of hydrophobic amino acids in the commercial nongelling cod gelatin compared to tilapia and horse mackerel gelatin. Data on amino acid composition are given before extraction (scale data) and after extraction (gelatin data) for comparison in Table 4. Significant differences were determined between the values ($P < 0.05$).

Increasing demand for fish gelatin may pave the way for further research and exploration of fish gelatin as an alternative for mammalian gelatin, as it fulfills the majority of consumer needs and complements the increasing global demand for gelatin. These types of studies may also help in evaluating the wastes of fish scales and have the potential to provide raw material for the industrial sector. Evaluating fish and seafood processing wastes by development of new products and new biomolecules that have commercial importance is an important area for researchers. In the

current study, good results were obtained and high-Bloom type A gelatin was identified. However, the production costs should be decreased for the commercial sector. Removing the lipids and the demineralization process steps increase the costs (isobutyl alcohol and EDTA prices). Future studies should focus on how to decrease these expenses, using alternative chemicals for commercial production.

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