**Gelatin prepared from European eel (*Anguilla anguilla*) skin:
physicochemical, textural, viscoelastic and surface properties**

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Graphical abstract

Research Highlights

- Gelatin was extracted from the skin of European eel.
- European eel skin gelatin (EESG) had excellent functional properties.
- Textural and viscoelastic properties of EESG were investigated.
- EESG could be used as a replacer for bovine or porcine gelatin.

Abstract

Gelatin (EESG) was extracted from the skin of European eel (*Anguilla anguilla*) with a yield of $8.69 \pm 0.42\%$ (dry weight basis). It was mainly composed by protein ($89.27 \pm 0.31\%$), while moisture ($6.42 \pm 0.83\%$), fat ($0.56 \pm 0.05\%$) and ash ($1.88 \pm 0.09\%$) contents were low. The amino acid profile of the gelatin revealed a high proportion of glycine and imino acid residues. The EESG contained α - and β -chains as predominant components. The absorption bands of gelatin in FTIR spectra were mainly situated in the amide band region (amide A, amide B, amide I, amide II and amide III). Textural properties of European eel skin gelatin were also investigated. The gelling and the melting temperature of the extracted gelatin were $14\text{ }^{\circ}\text{C}$ and $21\text{ }^{\circ}\text{C}$, respectively. The EESG showed excellent surface properties, which were governed by the protein concentration. The results showed that European eel skin can be a good source for gelatin.

Keywords: European eel; skin gelatin; textural parameters; surface properties; viscoelastic properties.

1. Introduction

Seafood plays an important role in human nutrition as a source of nutrients like polysaccharides, protein, vitamins and healthy fats (omega-3 and omega-6 fatty acids) [1]. Seafood processing operations generate more than 60% of the raw material as by-products comprising of viscera, head, bones and skin. Those by-products are usually discarded and constitute a serious environmental problem [2]. Alternatively, they are converted into low economical value products, as animal feed, fish meal or fertilizer. Hence, development of novel means of processing is required to convert seafood by-products into forms that are noble, marketable, safe and acceptable to the consumer.

Seafood by-products contain several valuable components such as protein, enzymes, glycosaminoglycans, chitin, minerals, carotenoids and collagen [3]. Gelatin is obtained by partial thermal hydrolysis of collagen. It is an important hydrocolloid and a high molecular-weight biodegradable macromolecule [4]. A large number of applications within a multitude of different product areas (food, cosmetic, pharmacy, material and photography industries) make gelatin one of the most versatile biopolymers.

Gelatins are traditionally produced from bovine and porcine skins and bones, and often do not meet the requirements for halal and kosher foods due to religious dietary restrictions.

Additionally, gelatin from aquatic sources has been recognized to be free of viral infection or the risk of contamination with bovine spongiform encephalopathy. Therefore, fish skin gelatins provide an ideal alternative to gelatin from mammals [5]. The fish gelatin does not have such restrictions and additionally, and from the economic point of view, the use of fish by-products to obtain gelatin could be quite interesting for the fish-processing industry [6]. Some studies have reported the extraction and characterization of skin gelatins from several fish and cephalopod species such as *Lates calcarifer* [7], *Barbus callensis* [8] and *Loligo vulgaris* [9], among others.

European eel (*Anguilla Anguilla*) is a catadromous fish found in rivers draining into the North Atlantic, Baltic and Mediterranean Seas. Young eels live in freshwater, where they stay for a period of 6 - 12 years for males and 9 - 18 years for females. When the eels become sexually mature, they migrate to the sea [10]. *A. anguilla* is one of the economically important species of Northern Africa and Europe. It has been widely used for fillet production, in which a large amount of by-products (scales, bone, skin...) is generated. These by-products are scarcely used by processing industries. So, the objective of the present work was to extract and characterize the gelatin from European eel (*A. anguilla*) skin in order to know if it could be used to obtain high-value products. Additionally, viscoelastic, functional and textural properties of extracted gelatin were investigated.

2. Materials and Methods

2.1. Reagents

Sodium dodecyl sulphate (SDS), acrylamide, ammonium persulphate, N,N,N',N'-tetramethyl ethylene diamine (TEMED), Coomassie Brilliant Blue R-250 were from Bio-Rad Laboratories (Hercules, CA, USA). Porcine pepsin, glycine, bovine hemoglobin, ammonium sulphate and trichloroacetic acid were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade.

2.2. Raw material and preparation of skin

Skins of European eel were obtained from the local fish market of Sfax, Tunisia. The samples were placed in ice and transported to the laboratory. Upon arrival, skins were washed with tap water and residual meat was removed manually. The skin was then cut into small pieces.

2.3. Extraction of European eel skin gelatin

Gelatin was extracted according to the method of Nalinanon et al. [11]. European eel skins were soaked in 0.05 mol/l NaOH in order to remove non-collagenous proteins. The mixture was stirred for 2 h at room temperature (25 ± 1 °C). The alkaline solution was changed every 30 min. The alkaline-treated skins were then washed with distilled water until neutral pH was obtained. The alkaline-treated skins were soaked in 0.2 mol/L acetic acid (ratio 1:10) and subjected to limited hydrolysis with commercial porcine pepsin (pH 2.0, 15 units/g of alkaline-treated skin), as described by Abdelmalek et al. [9]. The mixtures were stirred for 48 h. The pH of the mixtures was then raised to 7.5 using 10 mol/l NaOH. The mixtures were stirred for 1 h at 4 °C, and then incubated at 50 °C for 18 h with continuous stirring to extract the gelatin from the skin. The mixtures were centrifuged for 30 min at 10,000 x g using a refrigerated centrifuge (Rotina 380 R centrifuge, Hettich, Germany) to remove insoluble material. The supernatant was collected and freeze-dried (CHRIST, ALPHA 1-2 LD plus, Germany) to obtain the European eel skin gelatin (EESG).

2.4. Calculation of gelatin yield

The gelatin yield was calculated as follows:

$$\text{Yield (\%)} = (\text{weight of dried gelatin (g)} / \text{weight of dry matter of European eel skin (g)}) \times 100.$$

2.5. Physicochemical characterization

2.5.1. Chemical composition

Moisture and ash contents were determined according to the AOAC standard methods 930.15 and 942.05, respectively [12]. Crude fat was determined gravimetrically after Soxhlet extraction of dried samples with hexane. Total nitrogen content was determined using the Kjeldahl method. Conversion factors of 5.55 (gelatin) and 6.25 (skin) were used for the conversion of nitrogen content to protein content.

2.5.2. Color and water activity evaluation

Color of the EESG was determined with a tristimulus colorimeter (CHROMA METER CR-400/410, KONICA MINOLTA, Japan) using the CIE Lab scale ($C/2^\circ$), where L^* , a^* and b^* refer to the parameters measuring lightness, redness, and yellowness, respectively. A standard white plate was used as a reference. Water activity (A_w) was measured at room temperature ($25 \pm 01^\circ\text{C}$) by a NOVASINA aw Sprint TH-500 apparatus (NOVASINA, Pfäffikon, Switzerland).

2.5.3. Amino acid composition

Gelatin was dissolved (1 mg/ml) in ultrapure water and further hydrolyzed in vacuum-sealed glass at 110°C for 24 h in presence of continuously boiling 6 N HCl containing 0.1% phenol and norleucine as internal standard (Pharmacia, Barcelona, Spain). After hydrolysis, the sample was again vacuum-dried, dissolved in application buffer, and injected onto a Biochrom 20 amino acid analyser (PHARMACIA, Barcelona, Spain). A mixture of amino acids was used as standard (Sigma Aldrich, Inc., St Louis, MO, USA). The amount of hydroxyproline and

hydroxylysine in the sample was determined by using standard internals of both residues, while the amount of Tryptophane was not calculated.

2.6. Electrophoretic study (SDS-PAGE)

The molecular weight distribution of the European eel skin gelatin was determined by SDS-polyacrylamide gel electrophoresis. Gelatin solution (2 mg/ml) was dissolved in loading buffer (2% SDS, 5% mercaptoethanol, and 0.002% bromophenol blue) at room temperature (25 ± 01 °C). It was heat-denatured at 90 °C for 5 min and further analysed in a Mini Protean II unit (Bio-Rad Laboratories, USA) using 4% stacking gels and 7.5% resolving gels [13]. The loading volume was 15 μ L in all lanes. Protein bands were stained with Coomassie brilliant blue R-250. Commercial fish gelatin (Rousselot S.A.S, France) was used as a marker for α_1 -chain, α_2 -chain and β -chain mobilities. Furthermore, a molecular weight standard kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) composed of glutamic dehydrogenase (53 kDa), transferrin (76 kDa), β -galactosidase (116 kDa), α_2 -macroglobulin (170 kDa) and myosin (212 kDa) was also used to determine the approximate molecular weight of the gelatin fractions.

2.7. FTIR Spectroscopic Analysis

The FTIR spectrum of the prepared gelatin was recorded between 600 and 4000 cm^{-1} in a NICOET spectrometer (Thermo Fisher Scientific, Waltham, MA USA). The transmission spectrum of the sample was recorded using the KBr pellet containing 0.1% of the sample.

2.8. Ultraviolet absorption spectrum

The gelatin solution (0.5%) (w/v) was heated at 60 °C for 30 min and subsequently cooled at room temperature (25 ± 1 °C). The ultraviolet (UV) absorption spectrum of gelatin solution was recorded in the wavelength range of 200 - 350 nm using a UV-visible spectrophotometer (UV-mini 1240, UV/VIS spectrophotometer, SHIMADZU, China).

2.9. Textural properties

The textural parameter analysis (TPA) was performed on gelatin gel at 6.67% (w/v) concentration. Gelatin gel was prepared by mixing the dry European eel skin gelatin in distilled water at 60 °C for 30 min. Then, the gelatin solution was cooled at 7 °C (maturation temperature) in a refrigerator for 18 h. Textural properties of gelatin gel were determined using a texture analyzer (Texture Analyser *TA plus*, LLOYD, INSTRUMENTS™, AMETEK®, England), equipped with a 1000 (N) load cell, 0.05 (N) detection range. The Texture Analyzer was interfaced with a computer, which controls the instruments and analyses the data, using the software supplied by Texture Technologies Corp. Textural parameters (hardness, cohesiveness, elasticity, elasticity index, chewiness and adhesiveness) were obtained from the TPA curve.

2.10. Viscoelastic properties

Dynamic oscillatory study of gelatin solution at 6.67% (w/v) concentration was carried out on a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle 4°, gap 0.15 mm). Cooling from 35 to 02 °C and back to 35 °C took place at a scan rate of 01 °C/min, frequency of 0.5 Hz, and target strain of 0.5%. Before starting the heating ramp, the sample was left to stand at 02 °C for 6 min. The elastic modulus (G' ; Pa) and viscous modulus (G'' ; Pa) were plotted as a function of temperature.

Frequency sweep was done after 6 minutes of cold maturation at 05 °C by applying oscillation amplitude within the linear region ($\gamma = 0.005$) over the frequency range 0.1-10 Hz. The elastic modulus (G' ; Pa) and viscous modulus (G'' ; Pa) were plotted as a function of frequency. To characterize the frequency dependence of G' over the limited frequency range, the power law model was used:

$$G' = G_0' \omega^n$$

Where G_0' is the energy stored and recovered per cycle of sinusoidal shear deformation at an angular frequency of 1Hz, ω is the angular frequency and n is the power law exponent; which for gels exhibiting an ideal elastic behavior should be near-zero.

2.11. Surface properties

2.11.1. Foaming properties

The foam expansion and foam stability of EESG solutions (0.5 to 3%) were tested using the method described by Shahidi et al. [14].

2.11.2. Emulsifying capacity: microscopic observation of emulsion droplet

Emulsions were prepared as described by Abdelmalek et al. [9]. Different concentrations of EESG from 0 to 3% were tested. A drop of the emulsions prepared for evaluating the emulsifying properties of the gelatin was placed on a microscope slide and covered by a cover slip. The emulsion structure was observed using an optical microscope (Olympus CX41, Japan) with a 40 $\times$ objective; images were captured with the attached camera (QImaging MicroPublisher 3.3 RTV, Canada).

2.12. Statistical analysis

All experiments were carried out in triplicate. All data were subjected to Analysis of Variance (ANOVA) and differences between means were evaluated by Duncan's Multiple Range Test. The SPSS software package (SPSS, Chicago, IL) was used for data analysis.

3. Results and discussion

3.1. Yield, proximate composition, water activity and color of the extracted gelatin

The yield of gelatin obtained from European eel skin was $8.69 \pm 0.42\%$ based on dry weight basis (Table 1). This yield was similar to those obtained by Gomez-Guillen et al. [15] and Sila et al. [8] with sole and barbell skin gelatin, respectively. On the other hand, the yield

obtained in the present study is higher than that reported by Abdelmalek et al. [9] with squid skin gelatin (6.82%), but lower than those obtained by Bougatef et al. [16] with smooth hound skin gelatins (8.94 - 11.78%). The yield of gelatin is affected by many factors such as season, age, species, type of process employed.

Table 1 shows also the proximate composition of skin and dried skin gelatin (EESG) from European eel. The skin was composed by $21.06 \pm 1.73\%$ protein, $1.75 \pm 0.13\%$ ash, $6.68 \pm 0.2\%$ fat and $70.12 \pm 0.96\%$ moisture. Regarding EESG, it was characterised by high protein content ($89.27 \pm 0.31\%$). The fat ($0.56 \pm 0.05\%$) content was low, suggesting efficient removal of fat from the skin material. The moisture content was in the vicinity of 6% which was within the limit prescribed for edible gelatin [17]. The low ash content ($1.88 \pm 0.09\%$) indicates that the extracted gelatin was of good quality [18]. The EESG showed low water activity (0.567 ± 0.01), which is known to prevent mould, yeast and bacteria development. The instrumental color measurement (expressed in terms of L^* , a^* and b^*) of gelatin extracted from European eel skin is also presented in Table 1. The lightness (L^*) value of EESG was 89.28 ± 0.21 and indicated a light white color. The L^* value of gelatin obtained from the skin of different fishes has been reported to be in the range of 90-93 [19]. The a^* (redness) and b^* (yellowness) values of EESG were -0.12 ± 0.02 and 11.70 ± 0.08 , respectively. Color of gelatin is an important aesthetic property, depending on the application for which the gelatin is intended. Meanwhile, color did not affect functional properties of gelatin [20].

3.2. Amino acid analysis

Amino acid composition (expressed as number of residues per 1000) of the extracted gelatin is also given in Table 1. The properties of gelatin are largely influenced by the amino acid composition and their molecular weight distribution [21]. Glycine was the dominant amino acid (360 residues per 1000 residues) followed by alanine (134 residues per 1000 residues) and

proline (109 residues per 1000 residues). Similar values were found in gelatin from Nile tilapia skin [22]. Glycine, alanine and proline are the main amino acids in gelatin [23]. In fact, the triple-chain helical structure of collagen contains glycine in every third position in the sequence and a high content of proline and hydroxyproline. In general, collagen contains approximately 33% glycine, 12% proline and 11% alanine [24]. Imino acids (Proline and Hydroxyproline) impart considerable rigidity to the collagen structure and a relatively limited imino acid content should result in a less sterically hindered helix and may affect the dynamic properties of the gelatin [25]. The imino acid content of gelatin from European eel skin (176 residues per 1000 residues) was higher than that reported in salmon skin (166 residues per 1000 residues) [26], but lower than that reported by Sinthusamran et al., [7] in seabass skin gelatin (198 - 202 residues per 1000 residues). Gelatin with high levels of imino acids tends to have high gel strength, viscoelastic properties and melting point [27]. They were essentially low in histidine and tyrosine, while the cysteine content was very low (1 per 1000 residues). Similar values were reported by Sila et al. [8] in barbel skin gelatin. The presence of cysteine is considered to reflect the presence of certain stroma proteins such as elastin [28]. The total number of essential amino acids (Ile, Leu, Lys, Met, Phe, Thr, Val and His) was 123 residues per 1000 residues. Furthermore, the content of hydrophobic amino acids, such as Pro, Ala, Val, Met, Gly, Ile, Leu and Phe, in the EESG was calculated, and it was found to be 674 residues per 1000 residues.

3.4. Protein pattern: molecular weight distribution

In order to characterise differences in molecular weight, the extracted gelatin and commercial fish gelatin (Rousselot S.A.S, France) were analysed by polyacrylamide gel electrophoresis in the presence of SDS (Fig. 1). SDS-PAGE profiles of gelatins were characterised by a typical molecular weight distribution. Both gelatins showed the presence of β , α_1 and α_2 chains. Similar protein patterns were observed by Khiari et al. [29] for gelatin prepared

from fish by-products. Multiple peptide bands were observed up to 76 kDa in the European eel skin gelatin, indicating the formation of low molecular weight peptides. This could be due to extensive hydrolysis of collagen during the extraction with commercial pepsin. Abdelmalek et al. [9] reported that gelatin from squid skin extracted using commercial pepsin contained a lot of peptides with molecular weight lower than that of the α -chain. During gelatin extraction, the conversion of collagen to gelatin with varying molecular mass took place, due to the cleavage of inter-chain cross-links [30].

3.5. Infra-red spectroscopic analysis (FT-IR)

Infra-red spectrum of the European eel skin gelatin was analysed (Fig. 2.A). FT-IR spectroscopy has been used to study the changes in functional groups and secondary structure of gelatin. Gelatin sample had the major peak in amide region. Generally, all the gelatins showed similar spectra [7]. The amide I band of the EESG appeared at 1625 cm^{-1} . The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the C-N stretch, C-C-N deformation and in-plane N-H bending modes. The absorption peak at amide I was characteristic for the coil structure of gelatin [31]. The absorption in the amide I region is probably the most useful for infrared spectroscopic analysis of the secondary structure of proteins [32]. Amide II band of gelatin was found at wavenumber ranging from 1541 cm^{-1} . Amide II arises from bending vibration of N-H groups and stretching vibrations of C-N groups [31]. The amide III represents the combination peaks between C-N stretching vibrations and N-H deformation from amide linkages as well as absorptions arising from wagging vibrations from CH₂ groups from the glycine backbone and proline side-chains [31]. The amide III band for European eel skin gelatin was found at 1234 cm^{-1} . Amide A band, arising from the stretching vibrations of the N-H group, appeared at 3276 cm^{-1} . The amide A band associated with the N-H stretching vibration showed the existence of hydrogen bonds. Normally, a free N-H stretching

vibration occurs in the range of 3400 - 3440 cm^{-1} . When the N-H group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequency. The result suggested that hydrogen bonds stabilising collagen structure were broken by enzymatic hydrolysis during gelatin extraction [33]. The amide B was observed at wavenumbers of 3069 cm^{-1} , corresponding to asymmetric stretch vibration of $=\text{C}-\text{H}$ as well as $-\text{NH}^{3+}$ [31].

3.6. Ultraviolet absorption spectrum

The UV absorption spectra of EESG solution is given in in Fig.2. B. Higher absorption was recorded in the wavelength region of 220 to 230 nm indicating the presence of peptide bonds in the polypeptide chains of gelatin. The shape of the UV absorption spectrum is typical of that previously published for gelatin prepared from the swim bladders of freshwater fish [34] and for gelatin extracted from Yak skin [35]. A small hump in the region of 270 to 280 nm in the gelatin samples may be due to a few aromatic residues.

3.7. Textural properties

Textural parameters analysis (hardness, cohesiveness, elasticity, elasticity index, chewiness and adhesiveness) of European eel skin gelatin gel at 6.67% are shown in Table 2. In addition, Fig. 3. shows the corresponding TPA curve of EESG gel. The hardness value of EESG was about 16.01 ± 2.2 N. Muyonga et al. [36] reported hardness values of 20.16 ± 1.96 and 22.40 ± 2.40 N in gelatin from young and adult Nile perch skins, respectively. Hardness is related to the strength of the gel structure under compression. The values of elasticity, chewiness and adhesiveness of extracted gelatin were 14.16 mm, 62.49 N mm and 4.41 N, respectively. Elasticity is a perception of gel in the mouth, and is a measure of how much the gel structure is broken down by the initial compression [37]. Furthermore, chewiness is related to the work needed to masticate a solid food to a state ready for swallowing. The gelatin from European eel skin had a cohesiveness of 0.276 ± 0.03 . Cohesiveness is a measure of the degree of difficulty in breaking down the gel's internal

structure [37]. The cohesiveness value of EESG was lower than that reported by Muyonga et al. [36] for Nile perch skins gelatin (~ 0.9). High TPA values are generally considered to be indicative of the good quality of gelatin.

3.8. Viscoelastic properties

Viscosity is the second most important commercial physical property of gelatin [25]. Dry extracted gelatin was dissolved in distilled water and viscoelastic properties were further analysed. Fig 4. (A and B) represents the modulus of elasticity (G') and modulus of viscosity (G'') during both cooling (from 35 to 02 °C) and subsequent heating (from 02 to 35 °C) ramps of the EESG. The temperature at which the crossover of the curves of G' and G'' occurs can be considered as the gelling point and is close to the sol-gel transition temperature. From the graph the gelling point of gelatin was found to be 14 °C. Varying gelling temperatures were reported for gelatin from swim bladders of freshwater fish (13.7 °C) [34], amur sturgeon skin (14 °C) [38], and seabass skin (19.5 - 20 °C) [7]. The melting temperature of the European eel skin gelatin was found to be 21 °C. The melting point of EESG was lower than that reported for the gelatin from amur sturgeon skin (22 °C) and seabass skin (26.3 - 27 °C) [7,38] and higher than that reported by [39] for African catfish skin gelatin (20.8 °C). The gelatin from fish sources possesses a lower gelling and melting temperature compared to a mammalian source due to a lower imino acid content. Fish species, temperature of the environment and imino acids content affects the gelling and melting temperatures of the resultant gelatin [21].

Elastic (G') and viscous (G'') modules as a function of the angular frequency of EESG are shown in Fig. 4.C. From the higher value of G' as compared to G'' we can deduce that the prepared gelatin was able to form a gel at low temperature. For gels exhibiting an ideal elastic behavior, exponent value (n) should be near-zero [40]. This is the case of European eel skin gelatin gels ($n = 0.175$).

3.9. Surface properties

The properties of the gelatin foam are important and very useful in food such as marshmallows [41]. Foam expansion and foam stability of European eel skin gelatin at different concentrations are shown in Table 3. The formation of foam is generally controlled by the reorganization, transport and penetration of protein molecules in the air/water interface and influenced by the amount of hydrophobic amino acids, intrinsic properties of the protein, the protein source and the protein conformation in solution [42]. Foam expansion of EESG increased with the increment of gelatin concentration. This is in accordance with the works of Sila et al. [8] and Abdelmalek et al. [9]. Foams with higher concentration of proteins were denser and more stable because of an increase in the thickness of interfacial films. Foam expansion was determined at 10, 30, and 60 min after whipping to evaluate the foam stability of EESG. After 10 min of whipping, the amount of foam decreased in a range between 9% and 35%, depending on the concentration used initially. The foam stability of protein solutions is usually positively correlated with the molecular weight of peptides [42]. Furthermore, the foam stability depends on the nature of the film and indicates the degree of protein–protein interactions within the matrix [41].

The emulsifying capacity of the European eel skin gelatin was evaluated by microscopic observations of the resulting emulsions (Fig. 5). The emulsions showed significant structural diversities between the different concentrations of EESG. The diameter of droplet size gradually decreased with the increment of protein concentration. In fact, emulsion droplets evolved gradually from large and coarse image at 0.5% to a small and fine appearance at 3%. Similar results were observed by Zarai et al. [43] for gelatin prepared from *Helix aspersa*. Protein at higher concentration might form a stronger layer surrounding the oil droplets, thereby preventing emulsion collapse [44]. At higher particle concentration, we envisage that, in addition to

adsorption at the interface, the particles in the aqueous phase are probably contiguous with those adsorbed, so the gelatin particles from different oil droplets are bound together in a three-dimensional network, which, in turn, traps the oil droplets in the gel matrix [45]. Therefore, the increment in particle concentration leads to more stable and stronger gel-like emulsion. Besides protein concentration, molecular weight is the key factor influencing the ability of gelatin to form and stabilize oil/water emulsions. The emulsifying properties of gelatin from fish species is reported to be lower than those of mammalian gelatin [15]. This possibly resulted from the differences in the intrinsic properties, composition and conformation of gelatins from both sources [46].

4. Conclusion

Gelatin with interesting viscoelastic and textural properties can be obtained from European eel (*A. anguilla*) skin. The extracted gelatin was also noted to contain α - and β -components. Additionally, this gelatin possesses good functional properties, including fat-binding and water-holding capacities, foam capacity and emulsion stability, which suggests that *A. anguilla* skin gelatin could have applications in food. Therefore, gelatin from European eel skin could potentially be used as a replacer for bovine or porcine gelatin.

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Figure caption

Figure 1. SDS polyacrylamide gel (7.5%) electrophoretic profile of European eel skin gelatin (line 1) and commercial fish gelatin (line 2) at 2 mg protein/mL. (MWM: molecular weight marker).

Figure 2. European eel skin gelatin: **(A)** Fourier transform infrared (FTIR) spectrum in the ranges of 600 - 4000 cm^{-1} (A_A : Amide A, A_B : Amide B, A_I : Amide I, A_{II} : Amide II and A_{III} : Amide III European eel skin gelatine). **(B)** Ultraviolet absorption spectrum in the wavelength region of 220 to 320 nm.

Figure 3. Texture profile analysis curve of EESG gel.

Figure 4. Viscoelastic properties of European eel skin gelatin: Changes in the modulus of elasticity G' and the modulus of viscosity G'' upon **(A)** cooling and **(B)** heating. **(C)** Elastic modulus (G' , Pa) and viscous modulus (G'' , Pa) as a function of the angular frequency of EESG at 5 °C ($y = 1893.8 x^{0.175}$ and $r^2 = 0.9665$).

Figure 5. Optical micrographs (40 x) of soybean oil emulsions prepared with different aqueous concentrations of European eel skin gelatine at 0 **(A)**, 0.5 **(B)**, 1 **(C)**, 2 **(D)** and 3% **(E)**.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Table 1. Physicochemical composition, water activity, color, yield and amino acid composition of European eel skin gelatin.

Physicochemical composition	European eel skin	European eel skin gelatin (EESG)
Moisture [¥]	70.12 ± 0.96	6.42 ± 0.83
Protein ^{¥¥}	21.06 ± 1.73	89.27 ± 0.31
Ash ^{¥¥}	1.75 ± 0.13	1.88 ± 0.09
Fat ^{¥¥}	6.68 ± 0.20	0.56 ± 0.05
aw	-	0.567 ± 0.01
Color		
<i>L</i> *	-	89.28 ± 0.21
<i>a</i> *	-	-0.12 ± 0.02
<i>b</i> *	-	11.70 ± 0.08
Yield ^{¥¥¥}	-	8.69 ± 0.42
Amino acids ^{¥¥¥¥}		
Aspartic acid	-	43
Threonine	-	19
Serine	-	42
Glutamic acid	-	67
Glycine	-	360
Alanine	-	134
Cysteine	-	01
Valine	-	13
Methionine	-	18
Isoleucine	-	06
Leucine	-	19
Tyrosine	-	02
Phenylalanine	-	15
Histidine	-	07
Lysine	-	26
Hydroxylysine	-	08
Arginine	-	43
Proline	-	109
Hydroxyproline	-	67

[¥] % ; ^{¥¥} g/100 g of fresh matter ; ^{¥¥¥} g of gelatin / 100 g of European eel skin ; ^{¥¥¥¥} Number of residues/1000 ; Values are given as mean ± SD from triplicate determinations.

Table 2. Textural properties of European eel skin gelatin (EESG).

Textural properties	Hardness (N)	Cohesiveness	Elasticity (mm)	Elasticity Index	Chewiness (Nmm)	Adhesiveness (N)
EESG	16.01 ± 2.2	0.276 ± 0. 03	14.16 ± 2.1	1.06 ± 0.2	62.49 ± 5.3	4.41 ± 0.7

Values are given as mean ± SD from triplicate determinations.

Table 3. Foam expansions and foam stability of European eel skin gelatin (EESG).

		EESG concentration (%)			
		0.5	1	2	3
Foam Expansions (%)		100.97 ± 2.07 ^{aA}	110.81 ± 2.14 ^{aA}	121.76 ± 3.50 ^{bA}	129.5 ± 4.14 ^{bA}
Foam Stability (%)	10 min	65.60 ± 1.07 ^{aB}	87.33 ± 4.36 ^{bB}	103.14 ± 1.59 ^{cB}	118.82 ± 2.07 ^{dA}
	30 min	47.54 ± 1.80 ^{aC}	75.19 ± 0.84 ^{bB}	83.25 ± 2.42 ^{bC}	100.13 ± 1.56 ^{cB}
	60 min	20.1 ± 0.55 ^{aD}	39.53 ± 1.71 ^{bC}	63.92 ± 1.30 ^{cD}	71.04 ± 1.15 ^{cC}

Values are given as mean ± SD from triplicate determinations ; ^{a-d} Different letters in the same row within the same parameter indicate significant differences ($p < 0.05$) ; ^{A-D} Different capital letters in the same column within the same concentration indicate significant differences ($p < 0.05$).