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Development of emulsion gelatin gels for food application: Physicochemical, rheological, structural and thermal characterization

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A B S T R A C T

The current work aimed to prepare emulsion gels based on European eel skin gelatin (ESG). The results revealed that the ESG exhibited interesting antioxidant and functional properties in a dose-dependent manner. The ESG has a gel strength of 354.86 g and high gelling and melting temperatures of about 33 and 43 °C, respectively. Hence, based on its interesting gelling ability, the ESG-based gel was employed to stabilize European eel oil (EO) emulsions. In this context, two emulsions were prepared by homogenization or homogenization followed by sonication at EO:ESG weight ratios of 1:2 and 1:4. The physicochemical, textural, structural and thermal properties of emulsion gelatin-based gels (EGGs) were evaluated. The EGGs had a rigid and a cohesive gel network, according to the textural and microstructural analysis. Structural and thermogravimetric analyses showed the effective entrapment of EO in the ESG gel network.

Keywords:

Emulsion gelatin-based gel
Rheological property
Thermogravimetric analysis

1. Introduction

Nowadays, the food industry is constantly developing innovative technologies to obtain competitive functional foods with high interest, particularly protein gels enriched with highly bioactive compounds such as fatty acid [1]. In fact, polyunsaturated fatty acids (PUFAs) in fish oil are gaining increasing interests in the food, agriculture, and pharmaceutical industries, owing to their natural and safe status, various bio-functional properties and health benefits to human [2].

However, due to their unsaturation, PUFAs are chemically unstable and susceptible to oxidation producing free radicals which are deemed to negatively affect the shelf-life, sensory properties and overall acceptability of the food products [3]. Several studies have described the efficiency of some competitive biopolymers including krill protein isolate [4], European eel protein isolate [5], whey protein, carbohydrates [6], and gelatin [2] as wall of protection matrices of sensitive molecules. Owing to its abundance, low cost and great ability to carry wide compounds, gelatin is extensively used in food, photography, cosmetic, coating, pharmaceutical, and medical applications [7–11]. Recently fish gelatins have emerged as a promising alternative to bovine and porcine gelatins, due to religious dietary issues and bovine spongiform encephalopathy contamination risk,

and could be motivating for the fish-processing industry in order to solve the problem of waste disposal [12].

Emulsified gels are defined as an edible semi-solid material composed by a gel network structure protecting oil droplets [13]. The interesting gelation behavior, textural and emulsification properties of fish gelatin contribute to the wide diversity of food products and other potential applications, according to different dispersion systems such as gels, emulsions or emulsified gels [14]. Indeed, emulsified gelatin or emulsion filled based gels are a promising alternative, which provides different formulation with interesting structural and mechanical properties, consistently with foods [15]. Emulsions gels have fascinated considerable attention in recent decades. Several studies focus on protein emulsion-based gels instead of the conventional emulsions to avoid protein aggregation issues, coalescence, and thermal denaturation spawning a weak stabilities of protein emulsion [16]. Further, emulsion gels possess a higher stability due to their specific gel-like complex structure and solid-like mechanical features [17]. Indeed, the first step for the formation of gel product fortified with fish oil is the producing of an emulsion. The hydrophobic droplets of fish oil can be dispersed, in aqueous systems containing several hydrocolloids, (gelatin, alginate, pectin) by emulsification. This process led to force fish oil droplets dispersion into a continuous aqueous phase, using different methods, including low pressure by membrane emulsification, high pressure by high-pressure homogenizer, ultrasonication used to form nano-sized particles, and microfluidization techniques which enhance the loading

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capacity and the protection fish oil against oxidation [18]. The second step is the preparation of the emulsion gels by thermal, enzymatic or chemical procedures. Emulsion gels formed by a protein-stabilized oil-in-water is an emulsion with a gel-like network structure and solid-like mechanical properties [19], which has several applications such as improving food structure [20], developing healthy products [21] and adjusting heat-sensitive ingredients [22].

In our previous study, gelatin was extracted from the catadromous European eel and was successfully used as a promising biopolymer in food packaging films, incorporated with European eel oil, to improve the shelf life of bio-packaged foods [23]. The present work focuses on the physicochemical, textural, rheological, techno-functional and biological properties of the European eel skin gelatin (ESG) and on its use for the preparation of emulsion gels.

2. Materials and methods

2.1. Materials

European eel oil (EO), contained 28.71% of saturated fatty acids, 54.34% of monounsaturated fatty acids and 16.86% of PUFAs, extracted as described previously [5]. Butylated hydroxyanisole (BHA), Ferrozine, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), linoleic acid and other chemicals, such as potassium ferricyanide, trichloroacetic acid (TCA), FeCl₃, FeCl₂, EDTA, sodium hydroxide, and other solvents such as ethanol, hexane, methanol, and chloroform were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. European eel gelatin extraction

Gelatin was extracted from European eel skin as described previously [23]. Briefly, European eel skins were cut into small pieces and then soaked in NaOH solution (0.05 M) with a sample/alkaline solution ratio of 1:10 (w/v). The mixture was stirred at room temperature for 2 h. The NaOH solution was replaced each 15 min. After washing to a near-neutral pH, the mixture was then soaked in 100 mM glycine-HCl buffer (100 mM; pH 2.0) with a solid/solvent ratio of 1:10 (w/v) for 18 h at room temperature to extract the collagen. After incubation, the pH of the mixture was raised to 7.0 using NaOH solution. For gelatin extraction, the treated skins were incubated at 50 °C for 18 h. The mixture was centrifuged at 8000 rpm for 20 min and 4 °C, using a refrigerated centrifuge to remove insoluble material. The supernatant containing European eel skin gelatin (ESG) was collected and freeze-dried (Moduloyd Freeze dryer, Thermo Fisher, USA).

2.3. European eel skin gelatin characterization

2.3.1. Size exclusion chromatography

The molecular weight (MW) distribution of ESG was carried out by size-exclusion HPLC on Biobasic SEC-120 column (ThermoFisher, LC1260, USA) with UV detection at 215 nm eluted at 0.35 ml/min with 0.5 M phosphate buffer (pH 7.0), which was previously calibrated with thyroglobulin (304 kDa), bovine serum albumin (66.4 kDa) and egg albumin (45 kDa).

2.3.2. Determination of gel strength

ESG gel strength was determined according to the method described by Bloom [24]. The gelatin solution, prepared at a concentration of 6.67% (w/v) in distilled water, was poured into a 25-ml beaker and cooled down at 4 °C for 18 h. The gel strength of gelatin gel was determined using a Model TA-TX2 texture analyzer with a 5 kN load cell equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger.

2.3.3. Rheological 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 plate geometry (cone angle 4°, gap 2 mm, diameter 25 mm) [25]. For the flow behavior test, ESG solution was subjected to a controlled shear ramp ranged from 10¹ to 10² s⁻¹ at 5 and 20 °C. The flow behavior was determined by the variation of shear viscosity (Pa·s) as a function of shear rate (s⁻¹). The linear and dynamic viscoelasticity tests were investigated in the linear regime under a sinusoidal shear of frequency $f = 1$ Hz, and where the variation of the conservation modulus (G') and the loss modulus (G'') as a function of the strain ($\gamma = 0.1$ –1000%) was measured at 5 and 20 °C.

For the thermo-viscoelasticity properties, the temperature sweep was carried out by heating from 5 to 60 °C and then cooling from 60 °C to 5 °C under constant shear ($f = 1$ and $\gamma = 1\%$).

2.3.4. Functional properties of ESG

The emulsification activity index (EAI) and emulsification stability index (ESI) of ESG at 0.5%, 1% and 2% concentration were carried out according to the method of Pearce & Kinsella [26]. Foam expansion (FE) and foam stability (FS) of ESG were conducted referring to the method of Shahidi et al. [27]. Water binding capacity (WBC) was measured referring to Mac-Connel et al. [28] and oil binding capacity (OBC) was carried out according to Lin et al. [29].

2.3.5. Antioxidant activity

The DPPH free radical-scavenging activity of ESG was determined as described by Bersuder et al. [30]. A volume of 500 μ l of each sample at different concentrations (1–5 mg/ml) was added to 375 μ l of 99.5% ethanol and 125 μ l of 0.02% DPPH in 99.5% ethanol. The mixtures were then kept at room temperature in the dark for 60 min, and the reduction of DPPH radical was measured at 517 nm using a UV-Visible spectrophotometer (T70, UV/VIS spectrometer, PG Instruments Ltd., China). The control was conducted in the same manner, except that distilled water was used instead of sample. The DPPH radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100$$

where A_c is the absorbance of the control reaction and A_s is the absorbance of the sample. DPPH has an absorption at 517 nm, which disappear upon reduction by an antiradical compound. The lower absorbance of the reaction mixture indicated higher DPPH radical-scavenging activity. BHA was used as positive control. The test was carried out in triplicate.

The ability of ESG to reduce iron (III) was determined as described by Yildirim et al. [31]. An aliquot of 1 ml of gelatin at different concentrations (1–6 mg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide solution. The reaction mixtures were incubated for 30 min at 50 °C. After incubation, 2.5 ml of 10% (w/v) TCA was added and the reaction mixtures were then centrifuged at 10,000 $\times g$ for 10 min. Finally, 2.5 ml of the supernatant solution from each sample mixture was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride. After a 10 min reaction time, the absorbance of the resulting solutions was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power. The control was conducted in the same manner, except that distilled water was used instead of sample. Values presented are the mean of triplicate analyses.

Total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH [32]. An aliquot of 0.1 ml of sample solution at different concentrations was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated at 90 °C for 90 min. After that, each sample was allowed to cool to room temperature and the absorbance was measured at

695 nm against a control. The control solution consists of 1 ml of reagent solution and 0.1 ml distilled water. The activity of gelatin samples was expressed as α -tocopherol equivalents using the following linear equation:

$$A = 0.011x + 0.0049; R^2 = 0.987$$

where A is the absorbance at 695 nm and C the concentration as α -tocopherol equivalents ($\mu\text{mol/ml}$). The values of antioxidant analysis are presented as the means of triplicate analyses.

The ABTS radical (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging capacity of ESG was evaluated as described by Re et al. [33]. This test is based on the ability of antioxidant molecules to quench the long-lived ABTS⁺ species. The ABTS⁺ radical was generated by mixing 7 mM ABTS⁺ solution with 2.45 mM potassium persulphate. This solution was then diluted with ethanol to adjust the absorbance to approximately 0.7 at 734 nm. 100 μl of distilled water containing gelatin samples were added to 900 μl of the diluted ABTS⁺ solution. A solution without samples was recorded as a control. The mixtures were incubated at 25 °C for 10 min. The absorbance was then determined at 734 nm and ABTS⁺ radical-scavenging capacity was computed using the following equation:

$$\text{ABTS}^+ \text{ radical scavenging activity (\%)} = \frac{AC + AB - AR}{AC} \times 100$$

where AC is the absorbance of the control ABTS⁺ solution; AR is the absorbance of gelatin samples with ABTS⁺ solution and AB is the absorbance of blank tubes containing sample without addition of ABTS⁺ solution. The values represent mean of triplicate analyses.

2.4. Emulsion gelatin-based gel (EGGs) preparation

ESG was initially dissolved in aqueous solution at a concentration of 15% (m/v) and then stirred for 30 min at 40 °C. Then, ESG solution was heated at 90 °C during 10 min. The emulsion gelatin-based gels (EGGs) were carried out by the incorporation of EO to the ESG solution at ratios of 1:4 and 1:2 (EO:ESG, w/w) at 60 °C. Two emulsification processes were applied: the homogenization and the homogenization followed by sonication. The homogenization process (H) was carried out using only Ultra-turrax (T-18, IKA®, Germany) operated at 12,500 rpm for 5 min. Then, the emulsions were cooled in a refrigerator at 4 °C (maturation temperature) for 18 h. The homogenization followed by sonication treatment (HS) using Branson Digital Sonifier® (Model S—450D, Branson Ultrasonics Corporation, Dan-bury, USA) was carried out at 20% amplitude for 2 min at room temperature. EGG 1:2 H and EGG 1:4 H refer to EGGs prepared at EO/ESG weight ratios of 1:2 and 1:4 (w/w), respectively, using only the homogenization (H) process, while EGG 1:2 HS and EGG 1:4 HS refer to EGGs prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using the homogenization and sonication (HS) treatments. Gelatin based gel (GG) without EO, was prepared with the same way of EGGs and was considered as control gel.

2.5. Characterization of the prepared EGGs

2.5.1. Emulsion release content

Emulsion release content (ERC) of EGGs was measured following the method of Rawdkuen & Benjakul [34]. Briefly, 100 mg of gel samples were cut into small square pieces and placed into filter papers for 2 min at 4 °C. Then, the EGGs were removed from the papers and weighed again. The expressible emulsion release content was calculated using the subsequent formula, where X and Y are the initial and final weights of the gel samples, respectively.

$$\text{ERC(\%)} = [(X - Y) / X] \times 100$$

2.5.2. Total pigment content

The total pigment content (TPC) in the gel samples was determined as described by Lee et al. [35]. Briefly, 1 g of each gelatin gel was mixed with 9 ml of the solvent composed by a mixture of acetone: deionized water: HCl (90:8:2 v/v/v). The obtained mixture was stirred and putted undisrupted for 1 h at 25 \pm 2 °C. The TPC was calculated using the following formula:

$$\text{TPC (mg/100g gel)} = A_{640} \times 680$$

where A₆₄₀ is the absorbance of the samples at 640 nm (pic of EO absorbance due to the presence of tocopherol) and 680 is the conversion factor of hematin.

2.5.3. Textural properties of emulsified gelatin-based gel

The texture profile analysis (TPA) of EGGs was performed using the TA-TX2 texture analyzer (Lloyd Instruments, Ltd., West Sussex, UK). Pieces of EGGs (15 \times 25 mm, height \times diameter) were placed into a cylindrical cell and a probe (diameter = 12 mm) then, compressed in a double cycle to 50% of their original height (rate = 30 mm/min). From the resulting force-deformation curves, hardness (N), cohesiveness adhesion (N) and elasticity (mm) were determined as reported by Bourne et al. [36].

2.5.4. Microstructural properties

The microstructure of gel samples was conducted using scanning electron microscope (SEM, Hitachi S-4800) at an accelerating voltage of 10 kV and a working distance of 8–9 mm. Samples were sputter-coated with a gold-palladium mixture under vacuum prior to examination. Size distributions were obtained from a minimum of 200 measurements.

2.5.5. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis of samples were performed using a Perkin - Elmer spectrometer (Spectrum 65, France) equipped with an attenuated total reflectance accessory with a Zn Se crystal. A total of 32 scans were collected with 4 cm^{-1} resolution over the wavenumber range from 650 to 4500 cm^{-1} . Calibration was done using background spectrum recorded from the clean and empty cell at 25 °C.

2.6. Thermogravimetric analysis of EGGs

The thermal stability of EGG and GG was monitored via the thermogravimetric analysis TGA Q500 High Resolution, TA Instruments (Corporation Shimadzu, Kyoto, Japan) under the following operating conditions: alumina pan; dynamic nitrogen atmosphere through the range of temperature 50–600 °C, the flow 100 ml/min and the heating rate: 10 °C min^{-1} .

2.7. Statistical analysis

Statistical analyses were performed with Statgraphics ver. 5.1, professional edition (Manugistics Corp., USA) using ANOVA analysis. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Characterization of ESG

3.1.1. Molecular weight distribution

The molecular weight (MW) distribution of ESG, presented in Fig. 1 showed mainly two peaks. The major MW fraction was located between 66.4 and 304 kDa, which correspond to the MW of the beta chain (190 kDa), while the second peak could be assigned to the elution of α 1 and α 2-chains. The presence of polypeptide chains with high molecular

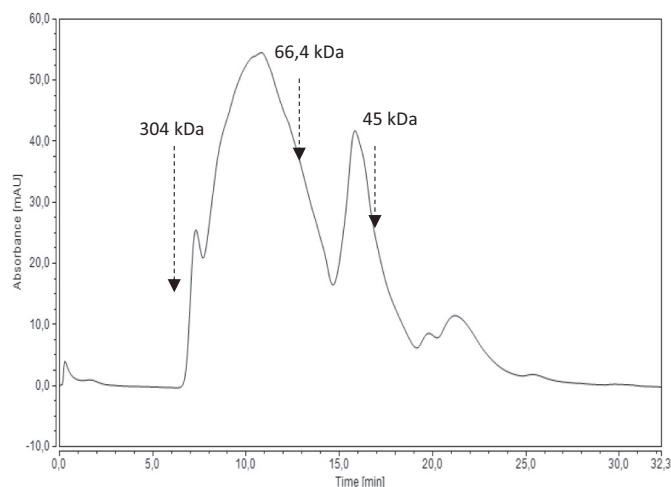


Fig. 1. Molecular weight distribution of European skin gelatin (ESG) profile by size-exclusion HPLC. Sample volume: 20 μ l; Buffer: 20 mM phosphate buffer, 280 mM NaCl, pH 7.4; Flow rate: 1 ml/min; Detection: 215 nm.

weight (α -chain and β -chain) influences positively the gelatin properties such as gel strength, viscosity, melting point [37] and gelatin structures [38]. Whereas gelatin with low molecular weight polypeptide chains displays various biological activities such as antioxidant, antihypertensive, and enzyme-inhibitory activities [39].

3.1.2. Gel strength

Bloom strength is the most important physical property of gelatin gel, reflecting its hardness, firmness, and compressibility. The results indicate that the ESG presented a high gel strength of 354.86 g (data not shown). Similar Bloom values have been reported for gelatins extracted from sole (350 g) and megrim (340 g) skins [25]. Interestingly, gelatin of European eel skin was more strength than those obtained from tilapia (125.6 g) skins [40], thornback ray (140 g) [41], cuttlefish (192 g) [42], and carp (267 g) [43]. Generally, the properties of gelatins are affected by several parameters, including the chemical composition, the MW distribution, and the amino acid composition [44–46]. Indeed, gelatin of high molecular weight gives the stronger gel. Furthermore, Mizuno et al. [45] stated that a high imino acid content, especially hydroxyproline, gives a high bloom strength of gelatin gel. Amino acids are associated with the stability of the triple-helix of collagen and gel structure through hydrogen bonding [46,47].

3.1.3. Rheological properties

The viscoelastic properties of ESG solution (6.67%, w/v) were evaluated by recording the storage (G') and loss (G'') moduli at 5 and 20 $^{\circ}$ C (Fig. 2). The flow behavior analysis, presented in Fig. 2a, shows the shear rate (s^{-1}) in terms of shear viscosity (Pa.s). As expected, the viscosity value decreased with the increase of shear rate, suggesting that the ESG solution is a non-Newtonian fluid and follows the shear-thinning performance. Additionally, the viscosity values of the ESG solution at 5 and 20 $^{\circ}$ C were, respectively, 1419 Pa.s and 809 Pa.s at a shear rate of 0.1 s^{-1} . Viscosity depends on the molecular weight and polydispersity of gelatin solution [48].

The strain sweep measurement of ESG solution by recording the storage (G') and loss (G'') moduli changes is illustrated in Fig. 2b. For gel incubated at 5 $^{\circ}$ C, strain range below 615% corresponds to the linear viscoelastic region of ESG, in which modulus values remain constant, regardless of the strain value. Under these conditions, the ESG gel exhibited viscoelastic gel-like behavior ($G' > G''$). At a critical strain of 800%, the G' value becomes lower than that of G'' , indicating the transition from a solid to a liquid viscoelastic behavior (gel-sol).

Fig. 2c presents the thermoviscoelastic behavior of ESG solution. Changes of G' and G'' moduli during heating (5–60 $^{\circ}$ C) and cooling

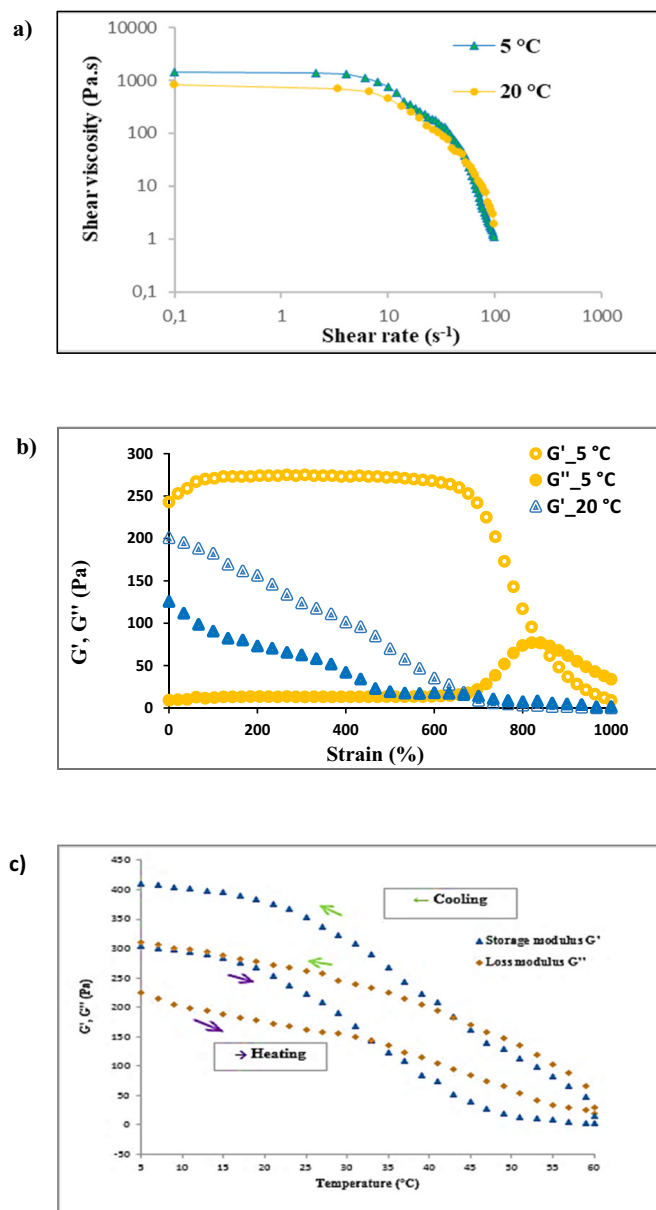


Fig. 2. a) Share Rate in terms of share viscosity b) Elastic modulus G' (open symbols) and loss modulus G'' (solid symbols) in terms of Strain c) Elastic modulus G' and loss modulus G'' in terms of temperature.

(60–5 $^{\circ}$ C) are used to determine melting and gelling temperatures of samples, respectively. During the heating stage, G' was higher than G'' , suggesting a solid viscoelastic gel behavior. The melting point was obtained at 33 $^{\circ}$ C when the crossover points of G' and G'' curves occurred. Above melting point, the gel crosslinking starts to break, and the triple helices content decreased. So, G'' became greater than G' , demonstrating a liquid viscoelastic behavior, where gelatin molecules are in single-strand arrangement.

Conversely, during the cooling, G' and G'' increased rapidly. At 43 $^{\circ}$ C, a crossover of the curves of G' and G'' arises, indicating the gelation point of the ESG sample (sol-gel transition). The formation of gelatin gels is based on the single - strand to triple - helix transition of gelatin chains via hydrogen bonding, ionic interaction, and hydrophobic interactions [49]. Indeed, the rapid junction zone formation in the protein network was established by the increases in G' values during cooling [50]. Interestingly, ESG exhibited higher gelation and melting points than seabass skin gelatin (26.3–27 $^{\circ}$ C) [51] and catfish skin gelatin (20.8 $^{\circ}$ C) [52]. The gelling and melting points of ESG are positively

Table 1

Emulsifying activity (EAI) and stability (ESI), foaming capacity (FC) and foaming stability (FS), water holding capacity (WHC) and oil binding capacity (OBC) of ESG.

| Concentration | EAI (m ² /g) | ESI (min) | FC (%) | FS (%) | WHC (g/g ESG) | OBC (g/g ESG) |
|---------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| 0.5% | 96.37 ± 3.34 ^a | 11.18 ± 0.18 ^c | 39.98 ± 0.46 ^c | 20.15 ± 0.49 ^c | 1.88 ± 0.23 ^a | 13.4 ± 0.31 ^a |
| 1% | 82.08 ± 0.50 ^b | 15.94 ± 0.30 ^b | 55.95 ± 0.64 ^b | 36.55 ± 0.62 ^b | 1.65 ± 0.04 ^a | 9.99 ± 0.16 ^b |
| 2% | 75.64 ± 2.92 ^c | 35.82 ± 0.87 ^a | 64.15 ± 0.49 ^a | 44.0 ± 1.41 ^a | 1.10 ± 0.02 ^b | 8.38 ± 0.34 ^c |

Values are given as mean ± standard deviation ($n = 3$). a–c different letters in the same column indicate significant differences between samples ($p < 0.05$).

influenced by its higher gel strength, which allows its use in many applications [53,54].

3.1.4. Functional properties

The EAI of ESG was presented in Table 1. The EAI decreased significantly ($p < 0.05$) with the increase of ESG concentration increase from 96.37 m²/g at a concentration of 0.5% to 75.64 m²/g at a concentration of 2%, which is in line with the study of Ktari et al. [54]. Several parameters affect the adsorption of protein to the oil–water interface during homogenization, including protein concentration. Dickinson & Lorient [55] stated that proteins adsorb to the emulsion surface during the homogenization process and forms a protective interfacial layer around the droplets. At higher concentrations, the aggregation of proteins in the aqueous phase occurs, leading to a decrease in the oil droplet concentration [56]. Nevertheless, data show that the emulsion stability increased with the increase of ESG concentration increase ($p < 0.05$). The emulsion stability depends on the amino acid composition, mainly the content of proline and hydroxyproline residues, and the concentration and the MW of α and β chains [57]. These findings were approved by optical microscopic observation of gelatin emulsion. As seen in Fig. 3, homogeneous emulsion distribution was obtained and in which the droplet size decreased with the increasing of ESG concentration. Indeed, the small droplet size had higher stability than large ones [5].

The WHC and OBC of gelatin are important functional properties that affect the food system texture. WHC and FBC of ESG were determined and, the results are reported in Table 1. The highest WHC (1.88 g water/g) and OBC (13.4 g oil/g) were obtained at 0.5% of ESG concentration. In a food system, the functional properties of proteins depend, in part, on their WHC, which refers to the aptitude of the protein to retain water inside their matrix. Many factors could influence WHC, e.g., pH solution, amino acids composition, number of polar groups inside the particle, accessibility of hydrophilic spots, ionic strength, temperature and protein concentration [58]. Lawal [56] stated that OBC depends on the presence of non-covalent bonds, such as hydrophobic, electrostatic and hydrogen bonding forces involved in lipid-protein interactions.

Foaming capacity and stability of ESG at different concentrations are presented in Table 1. It was noticeable that FC and FS increased with increasing gelatin concentration ($p < 0.05$), reaching 64.15% and 44%, respectively, at 2% concentration, which is in line with the previous study of Ktari et al. [54]. High protein concentration gives a stable air-water

interface due to the thickness increase of the interfacial films which reflect the absorption kinetic of macromolecules at the interface and strength of the adsorbed layer, and to improve the stability of macromolecules foam [59]. The hydrophobicity of the unfolded proteins had a positive correlation with foaming characteristics. Hydrophobic residues can form a large hydrophobic sphere and ameliorate the FC [60].

3.1.5. Antioxidant activity

The antioxidant activity of ESG was evaluated based on DPPH and ABTS scavenging activities, total antioxidant activity and reducing power (Fig. 4). ESG exhibited antioxidant activities in a dose-dependent manner. ESG displayed a high ability to scavenge ABTS radical reaching 99.94% at 5 mg/ml (Fig. 4a). However, a moderate scavenging activity of DPPH radical (43.43%) was obtained at 5 mg/ml (Fig. 4b). In addition, the total antioxidant activity of ESG (5 mg/ml) reached 27.19 expressed as α -tocopherol equivalents (Fig. 4c). However, a low reducing power was obtained by ESG (0.23 at 5 mg/ml) (Fig. 4d). This result agrees with previous work of Lassoued et al. [41].

3.2. Characterization of emulsion gelatin-based gel (EGG)

3.2.1. Emulsion release content

ERC, presented in Table 2, indicated the EGGs ability to retain water in the gel network and to compare the emulsion release from GG and EGGs. ERC values of EGGs were ranged from 8.36 to 18.61 g/100 g gel. The EGG 1:2 showed higher ERC level compared to the EGG 1:4. The increase of ERC values of EGGs could be attributed to low ability of emulsion gel to entrap water molecule into their gel network [61]. However, the lower ERC in the GG is associated to their higher ability to retain water and to the breaking force enhancement [62]. The water holding capacity in the EGGs depends on fish oil and gelatin contents and on the interesting physicochemical properties of gelatin due its hydrophilic and hydrophobic side chains influence the adsorption capacity of the oil and water interface interaction in the emulsion gel [63,64].

3.2.2. Total pigment content

TPC of GG and EGGs was determined (Table 2). Data reveals that GG presented the lowest pigment content. The incorporation of EO increased the TPC values of EGG ($p < 0.05$) from 14.79 mg/100 g (EGG 1:4 H) to 48.59 mg/100 g gel (EGG 1:2 H). This phenomenon may be

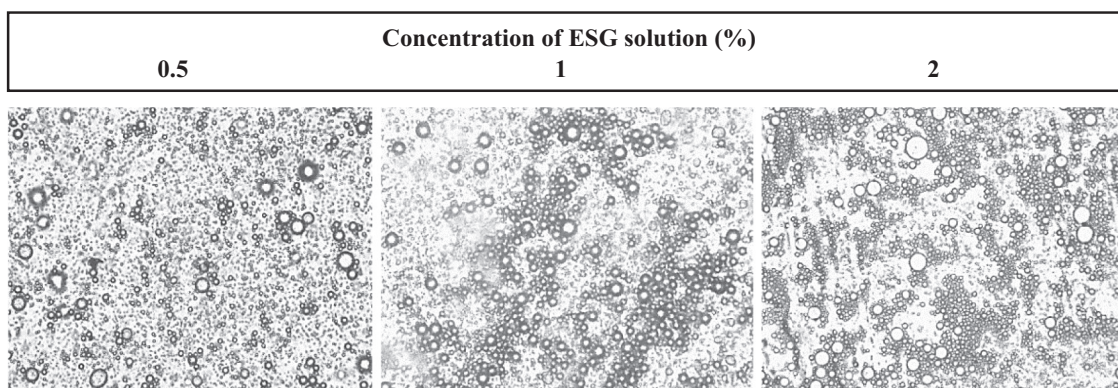


Fig. 3. Optical microscopic observation ($\times 400$) of emulsions based on 0.5%, 1% and 2% ESG.

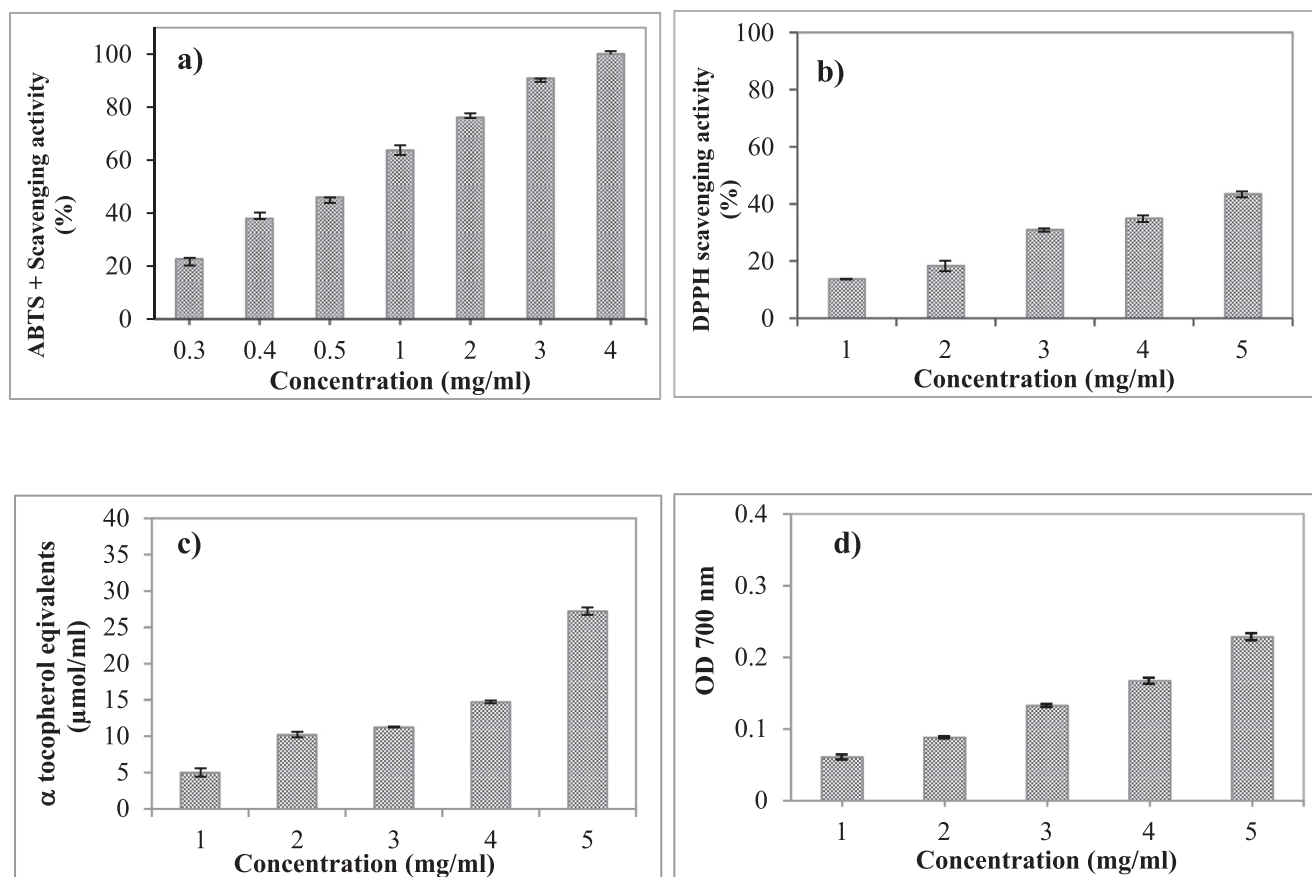


Fig. 4. Antioxidant activities of European skin gelatin (ESG) a) 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity; b) 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging method; c) total antioxidant activity and d) reducing power.

Table 2
Emulsion release content (ERC) and total pigment content (TPC) of EGGs.

| Samples | ERC (g/100 g gel) | TPC (mg/100 g gel) |
|------------|---------------------------|---------------------------|
| GG | 9.26 ± 0.41 ^{bc} | 10.22 ± 0.06 ^c |
| EGG 1:4 H | 8.36 ± 0.35 ^c | 14.79 ± 3.61 ^c |
| EGG 1:4 HS | 11.58 ± 0.10 ^b | 25.40 ± 3.44 ^b |
| EGG 1:2 H | 18.61 ± 0.50 ^a | 48.58 ± 2.44 ^a |
| EGG 1:2 HS | 18.30 ± 1.89 ^a | 46.96 ± 3.69 ^a |

Values are given as mean ± standard deviation (n = 3). a–c different letters in the same column indicate significant differences between samples (p < 0.05); GG: gelatin gel (control); EGG: emulsion gelatin-based gel. EGG1:2 H and EGG1:4 H present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4 (w/w), respectively, using only the homogenization (H) process. EGG1:2 HS and EGG1:4 HS present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using the homogenization and sonication (HS) treatments.

Table 3
Textural properties of GG and EGGs.

| Samples | Hardness (N) | Elasticity (mm) | Masticability (Nmm) | Breaking Strength (N) | Rigidity (N/mm) |
|------------|--------------------------|--------------------------|----------------------------|---------------------------|---------------------------|
| GG | 7.87 ± 2.39 ^a | 9.16 ± 0.13 ^a | 35.94 ± 10.18 ^a | 4.72 ± 0.03 ^a | 1.29 ± 0.14 ^a |
| EGG 1:4 H | 3.25 ± 0.58 ^b | 9.18 ± 0.05 ^a | 20.04 ± 2.87 ^b | 2.70 ± 1.28 ^{ab} | 0.70 ± 0.34 ^{ab} |
| EGG 1:4 HS | 3.74 ± 1.26 ^b | 7.67 ± 2.20 ^a | 14.94 ± 6.06 ^b | 1.01 ± 1.24 ^b | 0.72 ± 0.07 ^{ab} |
| EGG 1:2 H | 3.03 ± 1.27 ^b | 9.00 ± 0.36 ^a | 15.49 ± 5.89 ^b | 2.71 ± 1.19 ^{ab} | 0.76 ± 0.40 ^{ab} |
| EGG 1:2 HS | 1.52 ± 0.17 ^b | 8.48 ± 0.29 ^a | 6.64 ± 2.17 ^b | 0.76 ± 0.91 ^b | 0.24 ± 0.02 ^b |

Values are given as mean ± standard deviation (n = 3). a–b different letters in the same column indicate significant differences between samples (p < 0.05); GG: gelatin gel (control); EGG: emulsion gelatin-based gel. EGG1:2 H and EGG1:4 H present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using only the homogenization (H) process. EGG1:2 HS and EGG1:4 HS present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using the homogenization and sonication (HS) treatments.

explained by the interesting content of tocopherols in EO incorporated into the gel matrix.

3.2.3. Textural properties

The textural properties (hardness, cohesion, elasticity, masticability, breaking strength and rigidity) of GG and EGGs were determined. Results, illustrated in Table 3, revealed no significant difference in the elasticity of the different gels, even after the oil incorporation which is associated to the strong eel oil droplet emulsion interaction into EGG matrix [65]. This parameter is a perception of gel texture in the mouth and is a measure of how much the gel structure is broken down by the initial compression [66]. Furthermore, the highest hardness value was obtained with the GG (7.87 N) which decreased after the EO incorporation. Hardness is related to the strength of the gel structure under compression. The values of masticability, breaking strength and rigidity of GG were higher than those of EGGs. This finding is in accordance with the study of Dun et al. [67] who found that the addition of oil reduced

the firmness and the breaking strength of the starch gel. Gómez-Guillén et al. [25] stated that the emulsion gelatin-based gel was more flexible compared to the gelatin gel.

3.2.4. Microstructural properties

The visual appearance of GG and EGGs showed the higher whiteness and the lower transparency of EGGs compared to GG (Fig. 5). Furthermore, the microstructural images using the SEM revealed the rougher and homogeneous surface of EGGs compared to GG, which could be attributed to the higher gelatin emulsion stability and incorporation. Moreover, GG and EGGs have compact structure, indicating the ability of European eel gelatin to form a strong gel, which is in line with the textural analysis. Additionally, regarding oil content, the EGG had a porous structure, in which the pore diameters increased with the increase of EO content, as seen in SEM images. In fact, EGG1:4 showed compact gel with a smaller porosity, compared to the EGG1:2. This result could be

explained by the higher interfacial gelatin concentration, which facilitated the droplet distribution and better greater homogeneity and improved the ESG molecules affinity to interact with EO [68], owing to reducing the porosity. Furthermore, the gel porosity increased with the ultrasonication treatment.

3.2.5. Fourier transform infrared analysis

The FTIR analysis of the different samples was determined to understand the molecular interaction between ESG and EO. As seen in Fig. 6, the FTIR spectra of the GG showed the characteristic bands of the gelatin ascribed to the amide A (3270 cm^{-1} , N—H or O—H stretching), amide B (2920 cm^{-1} , C—H bending), amide I (1630 cm^{-1} , C=O and C=N), amide II (1540 cm^{-1} , C—N and N—H), C—H bending at 1380 cm^{-1} , and amide III (1240 cm^{-1}) bands [69]. The FTIR spectra of EO showed the most representative bands of fish oil at 1750 cm^{-1} , 1711 cm^{-1} and 1640 cm^{-1} , associated to the C=C, C=O and C=C-C=C bending,

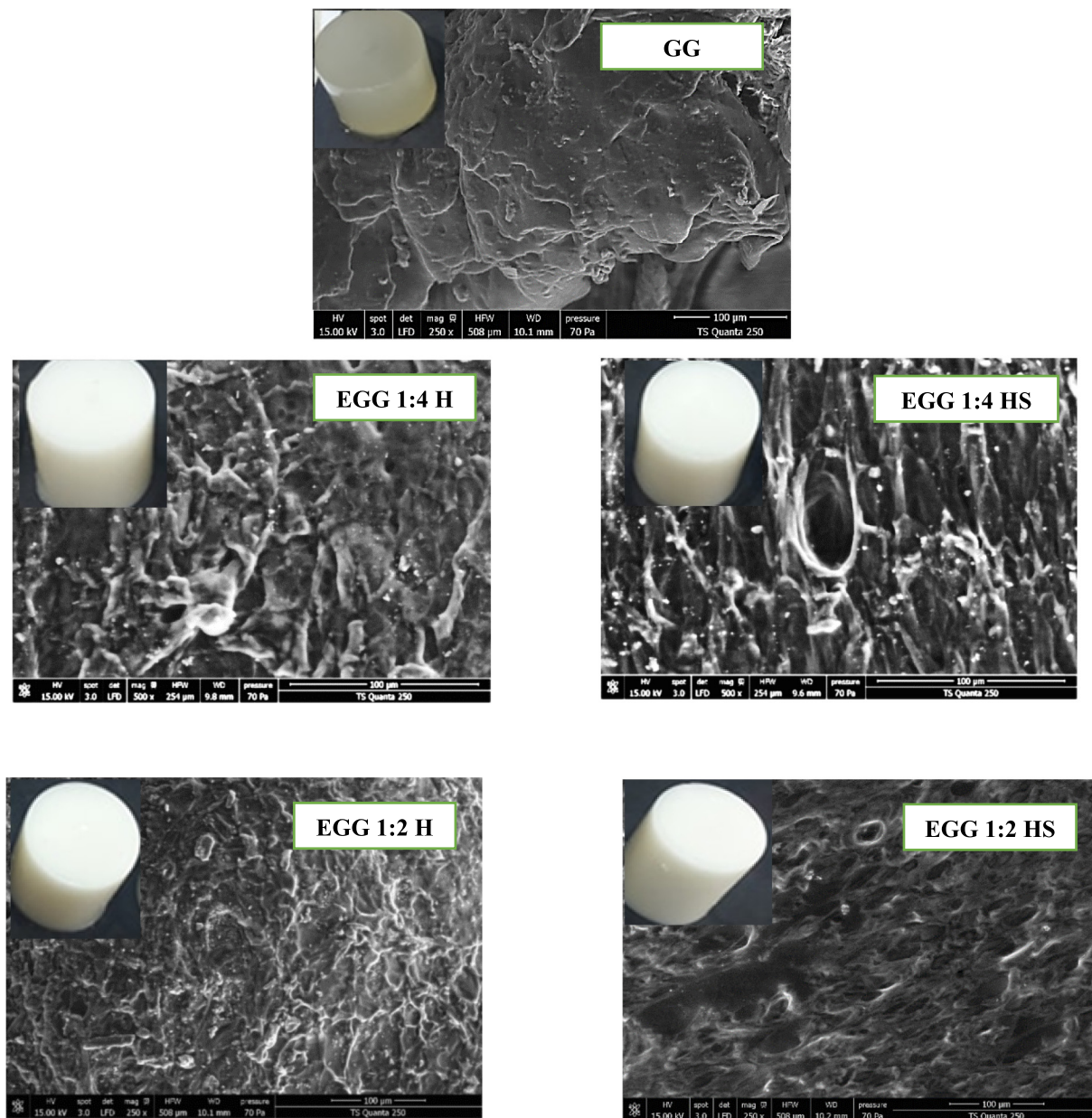


Fig. 5. Microstructures of European eel gelatin gel (GG) and emulsion gelatin-based gels (EGGs). EGG 1:2 H and EGG 1:4 H present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4 (w/w), respectively, using only the homogenization (H) process. EGG 1:2 HS and EGG 1:4 HS present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using the homogenization and sonication (HS) treatments.

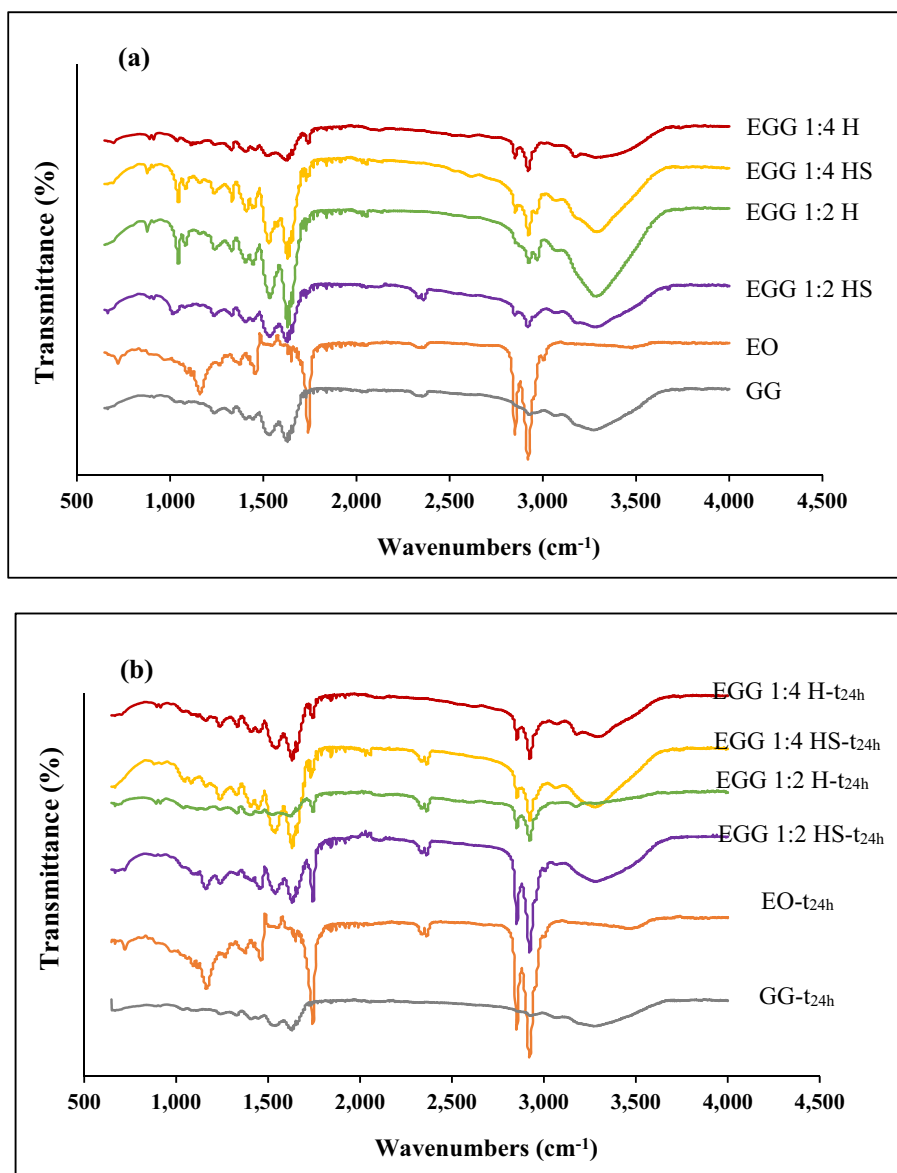


Fig. 6. Fourier transform infrared (FTIR) spectrum of European eel oil (EO), European eel skin gelatin gel (GG) and emulsion gelatin-based gels (EGGs) before ($t = 0$) (a) and after thermal treatment at $90\text{ }^{\circ}\text{C}$ for 24 h ($t = 24\text{ h}$) (b). EGG 1:2 H and EGG 1:4 H present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4 (w/w), respectively, using only the homogenization (H) process. EGG 1:2 HS and EGG 1:4 HS present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using the homogenization and sonication (HS) treatments.

respectively [70,71]. In addition, the specific absorption band of omega-3 PUFA at 3012 cm^{-1} , which corresponds to the C—H stretching of cis-alkene ($-\text{HC}=\text{CH}-$) groups, was also detected [72].

For FTIR spectra of the emulsion gelatin-based gels, the characteristic bands of both gelatin and fish oil were distinguished, indicating the incorporation of oil into the gelatin matrix (Fig. 6a). Regarding the emulsification methods, a shift in the wavenumbers of the peak associated to the C=C band from 1750 cm^{-1} for EGG1:4-H to 1730 cm^{-1} for EGG1:4-H was noted, which could be related to the effect of the ultrasonication treatment. Additionally, the wavenumber of the amide A in the EGG prepared by the ultrasonication treatment (HS) decreased to a low wavenumber (from 3300 to 3180 cm^{-1}). This result shows that the changes in the hydrogen bonding structure of the proteins after ultrasonication step could increase the hydrophobic interactions between the oil droplets and expose more hydrophobic side chains in the EGG [70,73].

The FTIR analysis of EO and EGG after heat treatment at $90\text{ }^{\circ}\text{C}$ for 24 h was presented in Fig. 6b. The spectra of treated EGG at $90\text{ }^{\circ}\text{C}$ for 24 h

revealed a decrease in the wavenumber of oil characteristic band from 1750 to 1680 cm^{-1} , which could be related to the C=C band oxidation during the heat treatment [74]. Regarding the emulsification process, no changes in the FTIR spectra were observed. However, regarding EO content, the spectra of EGG1:2H showed an increase in the wavenumbers of CH and of C=C-C=C bindings from 2890 to 2960 cm^{-1} and from 1630 to 1650 cm^{-1} , respectively, after 24 h of storage at $90\text{ }^{\circ}\text{C}$. It implies that higher amount of bioactive eel oil could be partially degraded in the gel EGG1:2 after heat treatment [70].

3.3. Thermogravimetric analysis

The TGA and DTG curves of GG, EO and EGG were conducted to evaluate their thermal stability (Fig. 7). The GG spectra displayed weight loss in three stages. The first weight loss stage occurred within the range of $80\text{--}150\text{ }^{\circ}\text{C}$, suggesting the loss of humidity. Water evaporation above $100\text{ }^{\circ}\text{C}$ has been assigned for the formation of hydrogen bonds in the gel samples [75]. The second and third stages occurred from 155 and

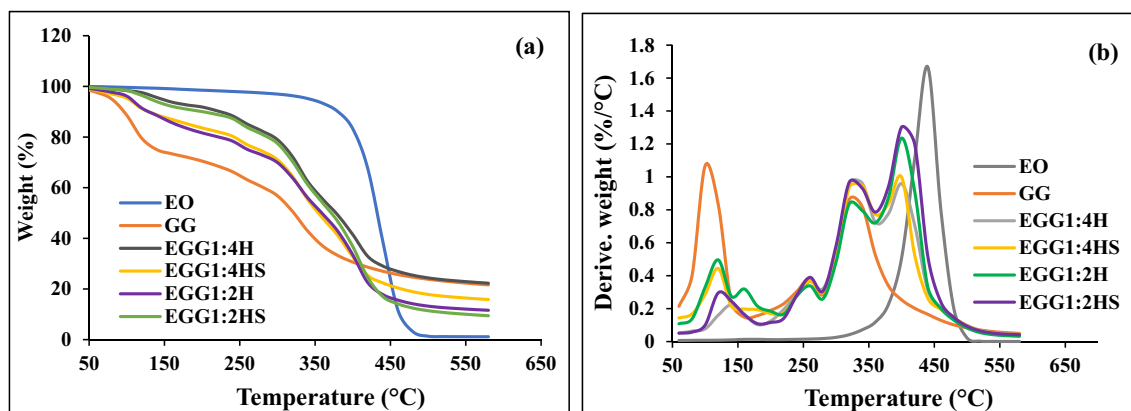


Fig. 7. Thermogravimetric analysis (TGA curves) and its derivative (DTG curves) of European eel oil (EO), European eel skin gelatin gel (GG) and emulsion gelatin-based gels (EGGs). EGG 1:2 H and EGG 1:4 H present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4 (w/w), respectively, using only the homogenization (H) process. EGG 1:2 HS and EGG 1:4 HS present emulsion gelatin-based gels prepared at EO/ESG weight ratios of 1:2 and 1:4, respectively, using the homogenization and sonication (HS) treatments.

250 °C and from 250 and 380 °C, respectively, indicating the decomposition of gelatin macromolecule in the GG. For the EO profile, only one weight loss, between 360 et 500 °C, is observed and related to its degradation rate which is similar to the mass losses of corn oil in the range of 400–440 °C [76]. The DTG curves of the EGG network displayed the presence of both characteristic profiles of thermal degradation associated to the gelatin and EO. Moreover, the main degradation stages for EO were present in the EGG, obtained with the ratio of EO:ESG, 1:4 and 1:2, and had its temperature of maximum degradation rate (T_{max}) at 400 °C. These results indicate the interaction of EO and ESG into the gel emulsion network which is in line with the FTIR spectra analysis [70]. Therefore, EGG matrices could be considered as a novel biomaterial with composed of food grade compounds (gelatin and fish oil) with interesting thermal properties potential indicating a good prospect in thermal application [77].

4. Conclusion

A novel bioactive EGG was successfully produced from ESG and EO. The characterization of ESG revealed high gel strength and viscosity, interesting functional properties and good antioxidant activities. The emulsion release content and total pigment content of emulsified gelatin-based gel increased with EO content increasing ($p < 0.05$). It was also demonstrated, using the data from the FTIR and TGA analyses, the ability of the EGG1:2 and EGG1:4 to load EO into gel matrices. The SEM images showed firm, and compact gel structure, reflecting the ability of European eel gelatin to form a strong gel which goes in accordance with the visual observation and the textural properties of gelatin gel. These results will promote in the progress of emulsion gelatin-based gel in the field of fortified food products.

CRediT authorship contribution statement

Wafa Taktak: Conceptualization, Data curation, Writing Original draft preparation; **Marwa Hamdi:** Investigation and Methodology; **Imen chentir:** Reviewing and Editing; **Youssra Ben Azaza:** Investigation and Methodology; **Soumaya Boughriba:** Investigation and Methodology; **Suming Li:** Conceptualization, Funding acquisition; **Moncef Nasri:** Visualization; **Maha Karra-Chaâbouni:** Supervision, Editing and Validation; **Rim Nasri:** Funding acquisition, Supervision, Editing and Validation.

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