Design and characterization of novel ecofriendly European fish eel gelatin-based electrospun microfibers applied for fish oil encapsulation

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Abstract

The present study is focused on developing microfibers based on the European fish eel skin gelatin (ESG) using the electrospinning process and evaluating its ability to encapsulate European fish eel oil (EO). Based on the scanning electron microscopy images, electrospinning of 15% (w/v) gelatin solution in ethanol/water 40% (v/v) was efficient to produce eco-friendly microfibers. Regarding electrospinning parameters, the increase of the voltage from 10 to 17 kV and the flow rate of feed solution from 0.04 to 0.2 mL/h improved the electrospinnability of ESG solution (15%, w/v). Furthermore, ESG-based microfibers, loaded with EO, were prepared. The effects of the EO/ESG ratios (1/2 and 1:4, w/w) and two emulsification methods, homogenization (H) and homogenization followed by ultrasonication (HS) treatment, on the electrospun microfibers formation were investigated. The success of EO encapsulation was confirmed by FTIR spectroscopy analysis. Data also revealed that the highest microencapsulation efficiency encapsulation (89.79%) was noted with EO-loaded microfibers prepared at EO/ESG ratio of 1:4 (w/w) by combining the two emulsification processes (HS). These results suggested that EO-loaded microfibers may be promising to be used as active encapsulating materials in food and nutraceutical fields.

Keywords: Electrospinning process; Microencapsulation; Microfibers; FTIR spectroscopy.

1. Introduction

With the rapid development of new materials from renewable resources, diverse choices of raw materials have been provided for electrospinning such as biopolymers (proteins polysaccharides) used for a broad range of applications given their advantages such as better biocompatibility, hydrophilicity and non-toxicity [1]. The biopolymers microfibers are used in the microencapsulation of sensitive molecules such as omega-3 polyunsaturated fatty acids, especially eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C22:6n-3, DHA) acids present in fish oil. These two fatty acids are responsible for various beneficial effects on human health, such as the prevention of cardiovascular diseases, the improvement of anti-inflammatory activity and the development of brain and eye retina, in order to protect them from thermo- and oxygen-sensitive deterioration or other degradation reactions [2]. When compared with other widely used encapsulation techniques, like emulsification-evaporation, spray drying or coacervation [3], electrospinning is an emerging, powerful and non-thermal technique used to fabricate continuous polymer fibers with different morphologies and structures and specific fiber arrangements which is of great importance for preserving the structure and enhancing the stability and functionality during food processing and storage [4]. Due to their high surface area-to-volume ratio, electrospun microfibers have been widely studied for different applications, such as filtration [5, 6], wound healing [7] and/or drug delivery systems [8].

The electrospun fibers were characterized in terms of morphology and diameter, which depend on the physico-chemical parameters of the polymer solution (concentration, solvent dielectric constant, viscosity, electrical conductivity and surface tension) and the process parameters (needle / collector distance, electrical voltage, *etc*.) [9-11]. The electrospun fibers formation is easier by dissolving polymer in organic solvents. However, the associated toxicity of these solvents reduces their use for food or biomedical applications [12, 13]. Therefore,

efforts are made to explore formation of microfibers using ecofriendly and food-grade solvents, such as water or ethanol [14].

Several biopolymers such as collagen, gelatin, chitosan, zein and silk fibroin have been successfully used to fabricate ultrafine fibers and nanofibers by the electrospinning process [15-17]. Among them, gelatin exhibits interesting physicochemical, rheological and functional properties (particularly texturizing and emulsifier agents) which allow its extensive use in food, photography, cosmetic, medical applications, tissue engineering and drug delivery [18-21]. Commercial gelatin was mainly obtained from mammalian skin and bones, such as those from pig and beef [22]. However, considering health and religion-related issues, several alternatives for extracting gelatin from fish by-products have been investigated [22]. In fact, fish gelatins have been produced from many fish species waste e.g. black-barred halfbeak (*Hemiramphus far*) skin [23], Chinese giant salamander (*Andrias davidianus*) skin [24], golden grey mullet (*Liza aurata*) skin [25], Octopus (*Octopus vulgaris*) skin [26] or tilapia (*Oreochromis niloticus*) scale gelatin [27].

European fish eel (*A. anguilla*) is a catadromous fish found in rivers draining into the North Atlantic, Baltic and Mediterranean Seas and it is one of the economically important species of Northern Africa and Europe. This species has been extensively used in fillet production, which generated an important amount of by-products (skin, viscera, *etc*.). These by-products are scarcely used by processing industries [28]. European fish eel is classified as a fatty fish with a lipid level of up to 20% in muscle at the silver phase [29]. In addition, the fatty acid composition of *A. anguilla* oil is relatively rich in polyunsaturated fatty acids of the n-3 family, including DHA and EPA which have beneficial effects on human health [30].

Therefore, the aims of the present study were focused on the formulation of European fish eel gelatin microfiber with electrospinning processes and to evaluate its ability to microencapsulate European eel oil (EO) through the emulsification process. The

physicochemical property, morphology, and microencapsulation efficiency of the EO-loaded microfibers were evaluated by Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

EO, containing 28.71% of saturated fatty acids (SFAs), 16.86% of poly-unsaturated fatty acids (PUFAs), and 54.34% of monounsaturated fatty acids, was extracted as described in our previous work [2]. Glycine and ammonium sulfate were purchased from Sigma Chemical Co. (St. Louis MO, USA). Sodium dodecyl sulfate (SDS), acrylamide, ammonium persulfate, N,N,N′,N′-tetramethyl ethylene diamine (TEMED), Coomassie Brilliant Blue R-250 were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Organic solvents, such as methanol, chloroform, ethanol, and hexane as well as acetic and hydrochloric acids were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. European fish eel skin gelatin extraction

A. anguilla was purchased from the fish market of Sfax City, Tunisia. After separating viscera, the skin was separated from the muscle and then rinsed. European fish eel skin gelatin (ESG) was extracted following the method reported by Jridi et al. [31], with slight modification. Briefly, small cube-shaped pieces of European fish eel skin were immersed in NaOH solution (0.05 M), at a ratio of 1:10 (w/v), for 2 h at room temperature to eliminate lipids and soluble proteins. The NaOH solution was replaced each 15 min. The treated skins were then washed with tap water and soaked in 100 mM glycin-HCl buffer (100 mM; pH 2.0) with a solid/solvent ratio of 1:10 (w/v) and incubated 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. The mixture was then incubated at 50 °C for 24 h and centrifuged at 9500 g for 20 min and 25 °C. The supernatant,

containing gelatin, was collected and freeze-dried (Modulyo D Freeze dryer, Thermo Fisher, USA).

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the ESG was carried out following Laemmli [32] and using molecular weight markers ranged from 53 to 212 kDa.

2.3. ESG solutions : preparation and characterization

ESG solutions were prepared at different concentrations (2, 4, 6, 8, 10 and 15 % w/v) in water, ethanol solution (20 % and 40 % (v/v)) and acetic acid solution (20 % and 60 % (v/v)).

The conductivity of each solution was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain) at room temperature. Surface tension was determined using the Wilhemy plate method in an Easy Dyne K20 tensiometer (Krüss GmbH, Hamburg, Germany) at room temperature. The zeta potential (ZP) was measured using a dynamic light scattering instrument (Malvern Zetasizer Nano ZS, Worcestershire, UK) at 25 °C. All measurements were made at least in triplicate. The viscosity of the solutions was measured using a rheometer model Anton Paar (MCR301) at 25 °C with a cone-plate geometry (CP25) whose diameter was 0.049 mm.

2.4. EO:ESG emulsions: Preparation and characterization

2.4.1. EO:ESG emulsions preparation

Prior to EO:ESG emulsion preparation, ESG solution was firstly prepared at 15% (w/v) in an ethanolic solution (40%, v/v). Then, EO was added to the ESG solution at EO:ESG weight ratio of 1:4 and 1:2 (w/w) ratios. The solutions were stirred at 12500 rpm for 5 min using an Ultra-Turrax apparatus (T-18, IKA®, Germany). The obtained emulsions were named, EO:ESG1:4H and EO:ESG1:2H, respectively.

In addition, EO:ESG emulsions at 1:4 and 1:2 (w/w) ratios were prepared by Ultraturrax homogenization and by a subsequent sonication treatment at 20% amplitude for 2 min using

148 Branson Digital Sonifier® (Model S-450D, Branson Ultrasonics Corporation, Dan-bury, USA).
149 These samples were labelled as EO:ESG1:4HS and EO:ESG1:2HS.

150 ***2.4.2. EO:ESG emulsions characterization***

151 The stability of the EO:ESG emulsions was assessed according to their ZP, droplet size
152 as described in section 2.3. and their creaming index (CI) following the formula proposed by
153 Surh et al. [33].

154 When the emulsions separated into two different phases, the height of the top opaque ('cream')
155 layer was measured (H_C), and the creaming index (CI) was calculated using Eq. (1):

156 $$CI = 100\ (H_C / H_E) \qquad \text{Eq. (1):}$$

157 where H_E is the total height of each emulsion in the tube.

158 Optical microscopy images of the emulsions were taken with a digital microscopy system
159 (Nikon Eclipse 90i) fitted by a 12 V, 100 W halogen lamp and operational with a digital camera
160 head (Nikon DS-5Mc). Nis Elements software was used for image capturing. The rheological
161 behavior of the different emulsions was investigated at 20 ºC ± 0.1 ºC using a rheometer model
162 AR-G2 (TA Instruments, USA) with a parallel plate geometry as described by Gómez-
163 Mascaraque et al. [34]. Briefly, the shear stress (σ) was determined as a function of the shear
164 rate (γ) from 0 to 200 s^{-1} (diameter = 60 mm; gap = 0.5 mm). The power law model Eq. (2) was
165 applied to determine the consistency index (*k*) and flow behavior index (*n*). Apparent viscosities
166 were determined at 100 s^{-1}

167 $$\sigma = k.\gamma^n \quad \text{Eq. (2)}$$

168 **2.5. Electrospinning microfiber processing**

169 The prepared ESG solution and emulsions were processed as reported by Gómez-
170 Mascaraque and Lopez-Rubio [35] using a homemade electrospinning apparatus, equipped with
171 a variable high-voltage power supply. Samples were introduced in a 10 mL plastic syringe and
172 were pumped through a stainless-steel needle (14.43 mm of inner diameter). The needle was

connected through a PTFE wire to the syringe, which was placed on a digitally controlled syringe pump. Processed samples were collected on a stainless-steel plate connected to the cathode of the power supply and placed facing the syringe in a horizontal configuration, at a distance of 10 cm. To determine the best conditions for uniform microfiber production, different voltages (10, 12, 15 and 17 kV) and flow rates (0.04, 0.1, 0.15, 0.2 and 0.5 mL/h) were tested. The best condition investigated to formulate ESG microfibers will be further used to encapsulate fish eel oil through the emulsification process in EO-loaded microfibers. Oil-free ESG microfibers were used as a control for the characterization and the biological potential evaluation of EO-loaded microfibers.

2.6. Electrospinning microfibers characterization

2.6.1. Microfibers morphological characterization

The morphology of the ESG and EO loaded-microfibers was assessed by scanning electron microscopy (SEM) using Hitachi microscope (HitachiS-4800) at an accelerating voltage of 10 kV and a working distance of 8-9 mm, before the sputter-coating of the samples with a gold-palladium mixture.

Particle diameters were measured from the obtained micrographs at their original magnification using the Image J software. Size distributions were obtained from a minimum of 200 measurements.

2.6.2. Microencapsulation efficiency analysis

The determination of microencapsulation efficiency (MEE) of EO was based on FTIR assay as reported by Gómez-Mascaraque and Lopez-Rubio [35].

2.6.3. Fourier-transform infrared (FTIR) spectra analysis

FTIR analysis of EO-loaded microfibers was performed in transmission mode using a Bruker (Rheinstetten, Germany) FTIR Tensor 37 equipment. The spectra were obtained by averaging 10 scans at 1 cm^{-1} resolution.

The ESG15% and free EO FTIR profiles were also determined. These measurements were carried out at room temperature in the range of 4000-450 cm^{-1}. The free EO and the ESG15% microfibers were used as a control for EO-loaded microfibers.

2.6.4. Thermal analysis

The thermal stability of EO-loaded microfibers was determined by thermogravimetric analysis (TGA) using TGA50H thermobalance (Corporation Shimadzu, Kyoto, Japan). The analysis was conducted under the following operating conditions: aluminium pan; dynamic nitrogen atmosphere with flow of 100 mL/min; heating rate: 10 °C min^{-1}; and temperature range: 50-700 °C. The ESG15% microfibers were used as a control for EO-loaded microfibers.

2.7. Statistical analysis

All measurements were made at least in triplicate. 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 European eel skin and ESG

The proximate composition of European eel skin and ESG powder was determined in our previous study [36]. Briefly, the skin was composed of 30.72% protein, 2.08% ash, 6.7% fat, and 58% moisture. The obtained ESG contained 90.5% proteins and 1.12% fat, suggesting the efficient elimination of fat during the gelatin extraction process. In addition, the moisture content was about 6.1%, which was within the limit prescribed for edible gelatin [37].

The SDS-PAGE pattern of ESG revealed three major bands ranging between 116-212 kDa (**Fig. 1a**), corresponding to α1-, α2- and β-bands [38]. The molecular weight of gelatin may be affected by the extraction process conditions, that contribute to the splitting of the peptide bonds and also the intermolecular crosslinks between peptide chains [39]. **Fig. 1b** shows the isoelectric point of ESG dispersions determined by Z-potential measurements. The net

electronic charge of the solution was found at pH 9, indicating that ESG is a type A gelatin [40].

3.2. Investigation of different electrospinning conditions

Several factors could influence electrospun fibers morphology such as the process parameters and polymer properties [41]. Thus, the effects of gelatin concentration, solvent nature, and electrospinning process parameters (conductivity, surface tension, and viscosity) on the electrospinnability of ESG were investigated.

3.2.1. Effect of concentration on the ESG solution properties

The electrospinning process fundamentally involves the transfer of electrical charge from the electrode to the polymer solution to initiate and elongate fluid jets. The effect of the ESG concentrations (2, 4, 6, 8, 10 and 15%, w/v), dissolved in water, on its conductivity is reported in Table 1. The conductivity values of the ESG solution increased with gelatin concentration. Indeed, the electrical conductivity increased from 2.29 mS/cm for 2% (w/v) ESG solution to 10.99 mS/cm for 15% (w/v) ESG solution, and values obtained in water are higher than those in ethanolic solution (40%, v/v). This finding is in line with the previous study of Ratanavaraporn et al. [42], who found that the electrical conductivity increased from 2.91 to 4.74 mS/cm for ESG solutions of 7.5% and 20% (w/v), respectively. Additionally, Okutan et al. [43] reported that optimum conductivity is required for microfiber formation during the electrospinning process to improve the transfer of electric charge from the electrode to the spinning droplet. Fluids with low conductivity will be subjected to insufficient elongation, while those with too high conductivity will not stretch [44].

The surface charge of the polymer solution at the Taylor cone has to overcome surface tension [44], which depends on solvent chemical composition [45]. As seen in Table 1, the solubilization of gelatin in water led to a decrease of surface tension from 70 mN/m of water to 41-39 mN/m. The surface tension has a critical role in the electrospinning process [46]. Low

surface tension is required to facilitate the elongation of the jet. This finding is in line with the study of Sharif et al. [47] showing an effective decrease in the surface tension of water even at low concentration of gliadin. As a result, protein addition significantly reduces the surface tension of water needed to stabilize the jet solution during electrospinning/electrospraying process [34].

The viscosity values of the ESG solution prepared at different concentrations were also determined and shown in Table 1. The viscosity of ESG solution increased with its concentration increase, which enhanced the formation of fibers. In fact, the increase of concentration improved the viscosity of ESG solution, which contributed to the stabilization of the jet and to the formation of well dried collected materials. Thus, the enhancement of electrospun fibers formation could be ascribed to the strong intermolecular cohesion between gelatin chains at higher concentration, which prevented the jet fragmentation during the electrospinning process [48].

3.2.2. Effect of ESG concentration on microfibers morphology

The effect of the ESG concentration on the morphology of the electrospun microfibers, obtained when applying a flow rate of 0.1 mL/h and a voltage value of 17 kV, was evaluated. Analysis of the data showed that no ESG fibers were produced at gelatin concentrations below 6%, as mentioned in supplementary data **(S1)**. In fact, at low polymer concentration, microfibers were not properly formed due to the lack of gelatin polymers entanglements, which contributes to the destabilization of the jet (**Fig. 2A** (a)), the decrease of viscosity and the formation of fine droplets in the collector [49]. Additionally, the results, illustrated in **Fig. 2A (b-d),** reveal the enhancement of fiber microstructure towards a more fibrillar morphology by the increase in ESG concentration. This finding was in accordance with the study of Li et al. [50] who reported that the increase of polymer content improves protein-protein cross-linking, leading to the development of elongated fluid, and hence generates microfibers structure. Thus,

the high concentration of polymer provides great stability and elongation of the jet from the Taylor cone [51].

Moreover, the morphology of the electrospun material can switch from pseudo-spherical particles geometry to a more elongated shape until they stretch completely to form fibers as a function of polymer concentration [52].

Additionally, the average diameters of fibers were examined and it appeared that the increase of ESG concentration in water from 6 to 15 % (w/v) increased the diameter size of the fiber from 1.21 to 1.9 µm (supplementary data **S2)**. Similar outcomes are reported in previous studies [53,54], which found that fiber diameter increased with increasing gelatin concentration.

Therefore, it could be concluded that 15% ESG (w/v of water) provided continuous fibers, although beaded fibers were obtained and a fused mass of material could be seen in the background, probably because of the inadequate drying of the structures. The morphology of the ESG fibers is strongly affected by ESG concentration and solvent.

3.2.3. Effect of solvents on microfibers formation

Based on the fiber microstructure, the ESG concentration of 15% (w/v) was selected for further investigation of the effect of different solvents, including water, aqueous acid acetic (20% and 60% (v/v)) and ethanol solutions (20% and 40% (v/v)). Results revealed that the morphology of the ESG fibers is strongly affected by solvent nature and its concentration. However, no gelation of the ESG solutions (water, acetic acid solution) during the electrospinning process was detected. Electrospinnability of gelatin in acetic acid has been widely described in the literature [47, 55, 56]. In the present study, the SEM image shows the absence of microfibers development, when ESG is dissolved in the aqueous acetic acid solvent (Data not shown). Moreover, the use of aqueous solutions to prepare gelatin microfibers is recommended as an alternative to substitute electrospun materials prepared with organic solvents. Kwak et al. [57] reported the development of microfibers using water as a solvent in

the different tested flow rate values. Nevertheless, as previously reported in **Fig 2.A** (a-d), the limitation of water usage consists in its low evaporation rate, which contributes to the formation of materials covered by dripping [58]. Hence, to increase solvent evaporation and improve structure formation of fibers, ethanol (20 and 40%, v/v) was also tested as solvent to prepare 15% ESG solutions. The SEM image, shown in **Fig. 2A** (e), reveals that dissolving gelatin in 20% ethanol solution was not enough to avoid solution dripping. Interestingly, using 40% ethanolic solution, properly dried ESG microfibers were obtained during the electrospinning process, as illustrated in **Fig. 2A (f)**.

According to **Table 1**, the conductivity of ESG solution (15%, w/v) dissolved in 40% ethanol solution was lower than that prepared in water (5.25 mS/cm *vs* 10.99 mS/cm). Furthermore, at a concentration of 15%, the surface tension of ESG prepared in 40% ethanol (33 mN/m) was lower than that of ESG dissolved in aqueous solution (41.37 mN/m). This finding goes in line with the previous study of Gomes et al. [59]. Indeed, ethanol is considered as a safe solvent that enhanced evaporation and, therefore, helped the drying of the collected material and increased the viscosity of ESG solutions (**Table 1).**

The pH values of ESG solutions are presented in **Table 1**. pH is a crucial parameter as gelatin degrades at pH∼1.6 or lower, regardless of any direct effect on electrospinning [60]. The effect of pH on the gelatin solubility can be attributed to the increase of electrostatic repulsion of the protonated or deprotonated gelatin molecules leading to the increase of solubility. Results demonstrate that the pH of ESG dissolved in water was 5.9 at the different concentrations tested (**Table 1**), which was similar to the pH of gelatin dissolved in ethanolic solution 40% (w/v). Thus, the pHs of all tested solutions were far from the isoelectric point of gelatin (which was pH 9.0 as described above), indicating the high solubility of gelatin.

3.2.4. Effect of the feeding rate and voltage on the electrospinning process

Several parameters can influence the morphology and the properties of the ESG microfibers, including electrospinning process variables (voltage, flow rate, distance and ambient relative humidity) [46]. The effect of feed flow rate on electrospinnability of ESG15% solution, prepared at a concentration of 15% (w/v) in 40% ethanol solution, is presented in supplementary data **(S3)**. The increasing feed rate of ESG15% solution from 0.04 to 0.1 mL/h led to the collection of more material during the electrospinning process. In contrast, at flow rates greater than 0.1 mL/h, ESG solution started to drip during the electrospinning process. Thus, the application of a high flow rate caused the formation of beaded and branched microfibers due to the insufficiency of time for solvent evaporation [61]. Hence, a feeding rate of 0.1 mL/h was required to form regular ESG microfibers.

In addition, the voltage is an important parameter in the electrospinning process, providing surface charge to the electrospinning jet to obtain fibers. The effect of the voltage on the electrospinning process is presented in supplementary data **(S3)**. The result reveals that increasing voltage values from 10 to 17 kV at a flow rate of 0.1 mL/h improved the spinnability of ESG (15% (w/v)) solution prepared in 40% ethanol solution, by accelerating solvent evaporation. Hence, at a voltage of 17 kV, the electric field was high enough to overcome the surface tension of the fluid. Indeed, the electrical forces as well as surface tension create a protrusion where the charges were accumulated. Therefore, a very thin filament, that is strongly stretched, is formed reaching the diameter 1.66 µm, which is similarly described by Reneker & Chun [62]. Previous studies also reported the decrease of the morphology and the diameter size of fibers at high voltage [47, 63].

Thus, an applied feed flow rate of 0.1 mL/h and a voltage of 17 kV might support the development of microfibers from 15% of gelatin prepared in 40% (v/v) ethanol at 15 % (w/v) concentration, were selected to encapsulate fish oil.

3.3. EO:ESG emulsions characterization

The ability of ESG solution (15%, w/v) to encapsulate EO by the electrospinning method was studied. For this purpose, the effects of EO:ESG weight ratios (1:2 and 1:4, w/w) and the emulsification processes (homogenization by ultraturrax (H) or homogenization followed by sonication (HS)), on the emulsion properties were studied. EO:ESG emulsions were characterized in terms of surface tension, conductivity and viscosity to evaluate their electrospinning ability (**Table 2**). The creaming index (CI) and zeta potential (ZP) were also investigated.

The emulsion stability, determined by the creaming index, is a crucial parameter to evaluate the emulsions stability. All emulsions (EO:ESG, 1:4 and 1:2 (w/v) were stable (CI=100%) after 24 h of storage at room temperature **(**supplementary data **S4).** In fact, gelatin is an important natural amphiphilic macromolecule and can act as emulsifiers in oil-in-water emulsions via the hydrophobic interaction of gelatin chain and oil [64]. Based on its good surface-activity capacity, gelatins can migrate to the oil/water interface and stabilize the emulsion [65].

In the other hand, the data, presented in Table 2, reveal also that the incorporation of oil to ESG solution decreased its conductivity ($p<0.05$). Overall, the conductivity values of the EO:ESG emulsions were slightly lower (3.99-4.3 mS/cm) than that of ESG solution (15%) prepared in ethanolic solution 40% (v/v) (5.25 mS/cm). In addition, the emulsification technique slightly affected the conductivity value of the different emulsions ($p<0.05$).

Furthermore, the surface tension of emulsions was determined and the results, illustrated in Table 2 show that the ST decreased from 33 mN/m for ESG solution to 31.37-31.9 mN/m for EO:ESG emulsions. Thus, the reduction of surface tension causes the formation of thinner fibers and reduces, therefore, the bead formation [66]. It is worth noting that the EO:ESG ratio and the emulsification methods did not affect the surface tension of the emulsions.

The ZP of EO:ESG emulsions was determined to estimate the stability of droplet in emulsion [67]. Regarding emulsions only prepared by homogenization, ZP slightly increased

with increasing EO content from 6.75 mV to 8.89 mV for EO:ESG emulsions prepared using EO:ESG ratios of 1:4 and 1:2, respectively (**Table 2**). It is interesting to note that the increase of ZP of emulsion provided high-energy barrier between emulsion droplets, which delivers a good electrostatic repulsion and increase their stability [68]. Furthermore, the application of HS emulsification steps reduced the ZP of the EO:ESG emulsions ($p<0.05$), compared to those prepared without the sonication step (H).

The average of size droplet was also investigated showing smaller and homogeneous droplets distribution in all EO:ESG emulsions (**Fig. 3A)**. This finding was in accordance with the optical microscopic observations presenting a small and homogeneous droplet size of the different EO:ESG emulsions (**Fig. 3B**). Besides, result revealed that the size of droplet in the EO:ESG emulsion decreased with increasing the ESG concentration, reaching 5.38 and 3.10 µm for EO:ESG1:2 and, EO:ESG1:4, respectively [2]. In contrast, the emulsification process did not significantly affect size droplet for EO:ESG (1:4 and 1:4) **(Table 2)**.

The rheological property, such as viscosity, is one of the important factors affecting the emulsion stability and the microstructural properties of microfibers [43]. Thus, solutions with a high viscosity cannot be ejected from the spinneret, whereas solutions with very low viscosity do not produce continuous fibers [46]. The rheological properties of ESG solution (15% (w/v)) and the different emulsions were studied and results are presented in the supplementary data **S5** and **S6**. All samples displayed shear-thinning behavior, which can be attributed to the shear-induced breakdown of the polymer network and macromolecule interconnections [69].

The viscoelastic force within the polymer and emulsion charged jet is the key force acting against the Coulombic repulsion, which is the main force leading to elongation of the jet after from the Taylor cone apex. Ratanavaraporn et al. [42] reported that the rheological behavior of the feed solution is one of the most crucial parameters during the electrospinning process.

The rheological analysis of ESG solution (15% (w/v)) and the different emulsions were suitable to the Ostwald de Waale model (Eq. 1). This finding reflects the pseudoplastic behavior determined by means of shear rate with regard to shear stress of samples and shear rate in terms of viscosity (supplementary data **S5** and **S6**). The values of the correlation coefficient (r^2) for different samples ranged between 0.974 and 0.997. The result of rheological parameters (**Table 3**) revealed that consistency index (*k*) and behavior index (*n*) of ESG 15% (w/v) increased with EO incorporation, which is mainly ascribed to the viscosity of the European fish eel oil introduced. In fact, the behavior index (*n)* increased from 0.878 for ESG 15% (w/v) to 0.97 and 0.99 for EO:ESG1:2 and EO:ESG1:4 respectively. Furthermore, apparent viscosities determined at 100 s^{-1} slightly increased with the increase of the EO ratio from 0.11 Pa.s for EO:ESG1:4 to 0.12 Pa.s for EO:ESG1:2. Moreover, the sonication step had no effect neither on the rheological parameters (*k* and *n*) nor on the apparent viscosity for the emulsions. This finding is in line with the characterization of emulsion microfibers indicating that the methods of emulsification did not significantly affect the average droplet size in the emulsion, in the diameter and in the microstructures of emulsion microfibers.

3.4. EO-loaded microfibers characterization

ESG-based electrospun microfibers loaded with EO were developed using the electrospinning process. The different emulsions were produced under the processing conditions established previously (feed flow rate of 0.1 mL/h and a voltage of 17 kV).

3.4.1. SEM Analysis

All formulations were able to produce microfibers with the presence of some beads, as shown in the Fig. (4), that can be correlated with the emulsion quality, as the beads in the microfibers can be a result of the clusters in the forming emulsions and the almost purely fibrous structure derived from the cluster-less emulsion. This is in line with the previous study of Gianneti et al. [70]. In addition, results show that the average diameter of microfibers reaching

1.125 µm was not significantly affected by the EO/ESG weight ratio and the emulsification methods ($p<0.05$) (supplementary data **S6**). Similarly, the previous study of García-Moreno [71] indicated that the increase of the loaded-oil content to whey protein isolate solution did not affect the fiber diameter. Gómez-Mascaraque et al. [34] reported that the change of EO-loaded microfibers diameters mainly depended on wall polymer concentration.

3.4.2. FTIR spectra analysis

The FTIR analysis was adopted to confirm the success and the effective loading of EP into electrospun ESG microfibers (**Fig. 4A**). The infrared spectra of ESG powder presented the specific absorbance bands of ESG at 3285-3334 cm^{-1} (Amide A, N-H or O-H), 2909-2912 cm^{-1} related to the CH_2 asymmetric and symmetric stretching vibration, mainly from the glycine and proline lateral-chains [72], 1625-1662 cm^{-1} (Amide I, C=O and C=N), 1541-1554 cm^{-1} (Amide II, C-N and N-H), 1340 cm^{-1} (C-H bending), 1237-1241 cm^{-1} (Amide III). Regarding EO profile, the presence of distinctive bands of fish oil at 1750 cm^{-1} attributed to C=C stretching and the characteristic band of omega3-PUFA at 3012 cm^{-1} related to C-H stretching of cis-alkene (HC=CH-) groups was also identified [73]. The EO-loaded microfibers spectra presented both the characteristic peaks of ESG and oil, confirming the ability of gelatin to entrap the fish eel oil in their matrix. This finding is in accordance with the microencapsulation efficiency analysis.

3.4.3. Microencapsulation efficiency

High encapsulation efficiency is of great importance to protect EO since it can minimize the exposure of free bioactive compounds to oxygen [56]. The FTIR analysis was used to assess the MEE of EO into ESG microfibers. Based on the measurements of absorbance intensities from the isolated spectral bands from the protein matrix and the bioactive EO at 1640 cm^{-1} and 3010 cm^{-1}, respectively, a calibration curve (R^2= 0.952) was constructed using physical

445 mixtures of gelatin and EO of known relative concentrations. As seen in **Table 2**, regardless of
446 the emulsification technique, the MEE increased with increasing wall material concentration,
447 i.e. a greater protein content was required for efficient oil encapsulation. The microfibers
448 prepared at EO:ESG weight ratio of 1:4 showed higher MEE (76.11-89.8%) than those prepared
449 at EO:ESG weight ratio of 1:2 (26 - 27%). Several studies have reported the use of gelatin as
450 wall material for oil encapsulation [34, 35, 74]. Regarding its emulsifying capacity, film-
451 formation, water-solubility, gelatin is used as an emulsifier to reduce the surface tension of
452 solution by adsorbing oil-water interface, making small droplets [43, 75, 76]. Therefore, the
453 ratio of European oil (core material) to ESG was the key variable affecting encapsulation
454 efficiencies of European oil microfibers which is in line with the study of who reported that
455 the encapsulation matrix composition was effective in higher loading efficiency encapsulated
456 fish oil [77]. Thus, high encapsulation efficacy of fish eel oil (76.11-89.8%) is of great
457 importance for preserving the structure and enhancing the stability and functionality during
458 food processing and storage. These structural and functional advantages make electrospinning
459 a preferred technique for developing active food packaging [78].

460 ***3.4.4. Thermogravimetric analysis***

461 In order to evaluate the thermal stability of the EO-loaded microfibers, the
462 thermogravimetric analysis was carried out (**Fig. 4B** and **Fig. 4C**). The maximal degradation
463 temperatures (T_{max}), which correspond to the highest amount of weight loss, are depicted
464 through DTG thermograms. The TGA curve of ESG microfibers reveals the presence of two
465 steps of degradation (**Fig. 4B**). The first step, detected around 100 °C, is attributed to the
466 moisture loss from microfibers. The second step of degradation took place in the range of 275-
467 400 °C (T_{max} at 306 °C), assigned to gelatin decomposition. All ESG microfibers presented
468 higher thermal stability, which is important to avoid thermal damage into the material for
469 industrial food or biomedical application.

Based on TGA profiles of EO-loaded microfibers, EO and ESG degradation stages occurred at 361 °C-368 °C and 306 °C, respectively. The entrapped oil temperature degradation slightly decreased to 361-368.5 °C, compared to the non-protected fish eel oil previously determined (397.63–417.50 °C) [2], which may be attributed to a slight EO structural change after emulsification and during the electrospinning process.

It is interesting to note that the temperature degradation of EO-loaded microfibers increased with increasing gelatin content. The T_{max} of electrospun microfibers prepared at EO: ESG weight ratios of 1:2 and 1:4 were, respectively, 361-362°C and 364-368 °C (**Fig. 4C**). It means that increasing gelatin ratio improved the thermal stability of the EO-loaded microfibers. This finding is in line with our previous work which reported the use of anguilla protein isolate as wall material to encapsulated EO using spray-drying technique [2]. As a result, EO: ESG ratio of 1:4 led to the formation of microfibers with improved microencapsulation efficiency and with stable encapsulated-EO. Indeed, the protective effect of EO-loaded microfibers could be considered as a better alternative to avoid the degradation of polyunsatured fatty acid and the formation of toxic aldehydes after fish oil oxidation [79].

4. Conclusion

The gelatin extracted from European fish eel skin was successfully used as a biopolymer for microfibers production and then for microencapsulation of the European fish eel oil. The optimization of electrohydrodynamic processing revealed that increasing ESG concentration to 15% improved the microstructure of material obtained through electrospinning. In addition, the use of ethanolic solution 40% (v/v) improved the evaporation of feeding solution using a voltage of 17 kV and a flow rate of 10 mL/h. The EO:ESG emulsions showed higher creaming index reflecting their higher stability, which was confirmed by SEM observation. The increase of EO content induced the decrease of conductivity and the increase of the viscosity in the emulsion but it did not significantly affect the average diameter of microfibers. In addition, the

rheological analysis showed that European fish eel polymer and its emulsion displayed a shear thinning behavior. The characterization of EO-loaded microfibers revealed that ESG1:4 had the highest MEE (89.79% for ESG1:4HS). This work demonstrates that ESG microfibers could be used as a novel ecofriendly alternative to encapsulate sensitive molecules using only food grade ingredients and to replace the use of synthetic polymer and toxic solvent, thus, have potential applications in the food, nutraceutical and medical fields.

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Highlights

1. The European fish eel skin gelatin (ESG) microfibers were successfully produced using electrospinning technique.

2. The ability of ESG microfibers to microencapsulate fish eel oil was investigated.

3. The ESG emulsions showed their higher stability by creaming index and zeta potential.

4. The FTIR analysis and encapsulation efficiency confirmed the proper fish eel oil encapsulation.

Graphical abstract

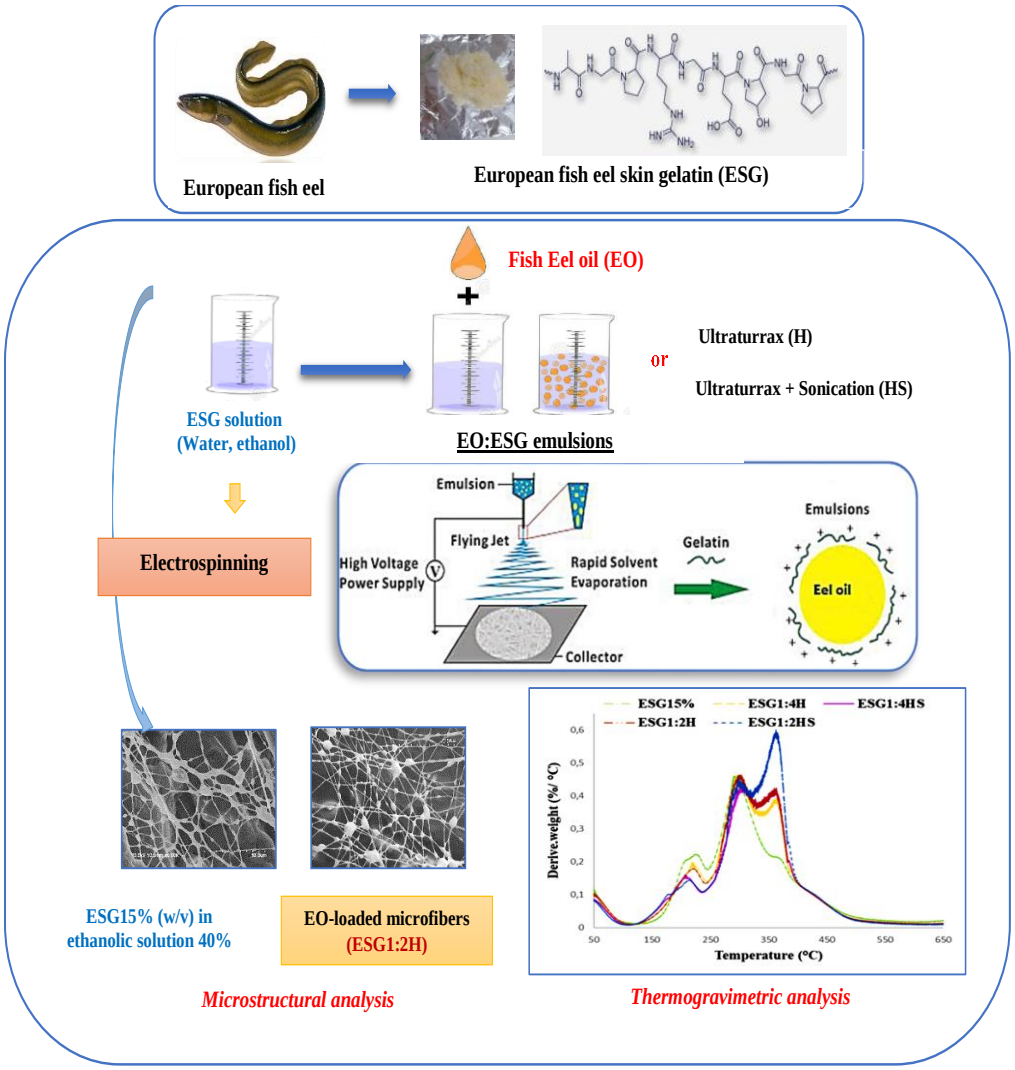


Figure captions

Fig. 1. Characterization of European fish eel skin gelatin (ESG). (a) Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) profile of ESG. Line 1 (L1) correspond to the ESG and line 2 (L2) correspond to the molecular weight marker. (b) Zeta potential of ESG solution at different pHs.

Fig. 2. Microstructural analysis of European fish eel skin (ESG) and European eel oil (EO)-loaded microfibers with Scanning electron (SEM) using the flow rate 0.10 mL/h, distance 10 cm and voltage 17 Kv

A. (a)~(d) refer to microstructural analysis of European fish eel skin (ESG) 6%, 8%, 10% and 15% (w/v) in aqueous solution, respectively. (e) and (f) refer to 15% ESG (w/v) in 20% and 40% ethanolic solution (v/v), respectively.

B. Microstructural analysis of European fish eel oil (EO)-loaded microfibers with Scanning electron (SEM) by means of two processes, homogenization with ultraturrax (H) and homogenization+ Sonication (HS) using the flow rate 0.10 mL/h, distance 10 cm and voltage 17 kV (based on the European fish eel skin (ESG) solution condition ESG15% in ethanolic solution (40%, v/v)) (a) the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:4 (w/w) and prepared with homogenization with ultraturrax (H) (ESG1:4H); (b) the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:4 (w/w) and prepared with homogenization+ Sonication (HS) (ESG1:4HS); (c) the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:2 (w/w) and prepared with homogenization with ultraturrax (ESG1:2H) (d) the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:2 (w/w) and prepared with homogenization+ Sonication (HS) (ESG1:2HS).

Fig. 3. Characterization of European fish eel oil: European eel skin gelatin (EO:ESG) emulsions

(A) Particles size distribution of European eel oil: European eel skin gelatin (EO:ESG) emulsions prepared with the ratios of 1:4 and 1:2 (w/w) using the two emulsification processes, homogenization with ultraturrax (H) and homogenization+ Sonication (HS) and named EO:ESG1:4H (a), EO:ESG1:4HS (b), EO:ESG1:2H (c), EO:ESG1:2HS (d), respectively.

(B) Optical microscopy images of the European eel oil: European eel skin gelatin (EO:ESG) emulsions after 24 h, prepared with the ratios of 1:4 and 1:2 (w/w) using two emulsification

processes, homogenization with ultraturax (H) and homogenization+ Sonication (HS) and named EO:ESG1:4H, EO:ESG1:4HS, EO:ESG1:2H, EO:ESG1:2HS respectively.

Fig. 4. Structural and thermal characterization of European fish eel oil (EO)-loaded microfibers **A.** Fourier-transform infrared spectroscopy (FTIR) spectra analysis of EO,
European fish eel skin gelatin (ESG15%) and EO-loaded microfibers. **B.** Thermogravimetric analysis (TGA curve) of ESG15% and EO-loaded microfibers. **C.** The derivative thermogravimetry (DTG) thermograms analysis of ESG15% and EO-loaded microfibers.

The European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:4 (w/w) and prepared with homogenization with ultraturrax (H) (ESG1:4H); the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:4 (w/w) and prepared with homogenization+ Sonication (HS) (ESG1:4HS); the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:2 (w/w) and prepared with homogenization with ultraturrax (ESG1:2H); the European fish eel oil (EO)-loaded microfibers obtained with EO:ESG ratio of 1:2 (w/w) and prepared with homogenization+ Sonication (HS) (ESG1:2HS).

Fig. 1

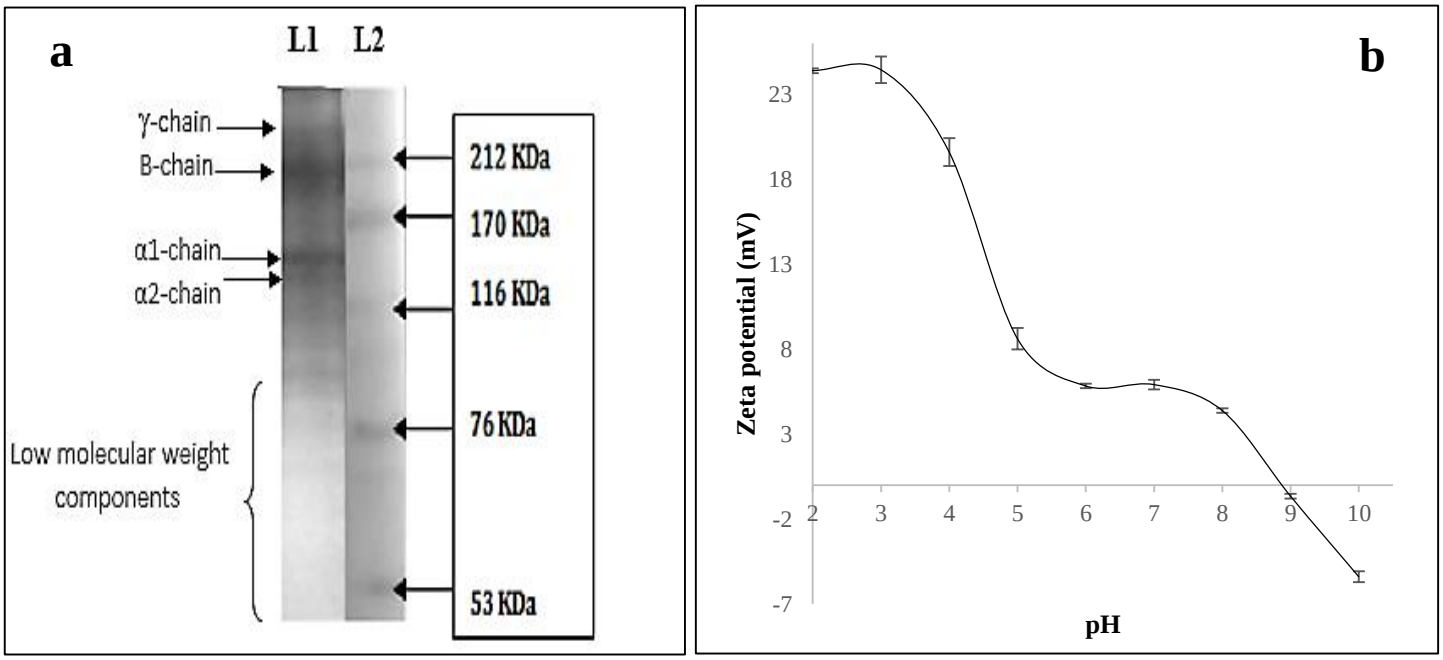

a
L1
L2
γ-chain
B-chain
α1-chain
α2-chain
Low molecular weight components
212 KDa
170 KDa
116 KDa
76 KDa
53 KDa
b
Zeta potential (mV)
23
18
13
8
3
-2
-7
2
3
4
5
6
7
8
9
10
pH

Fig. 2.

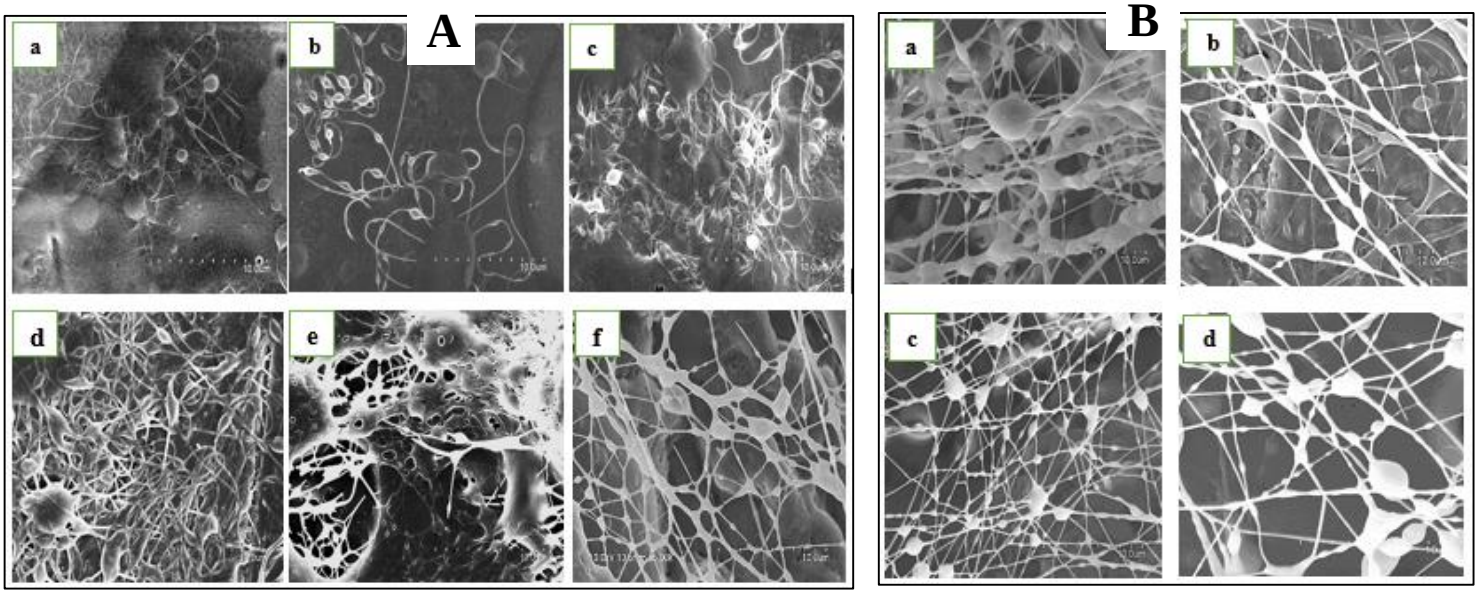
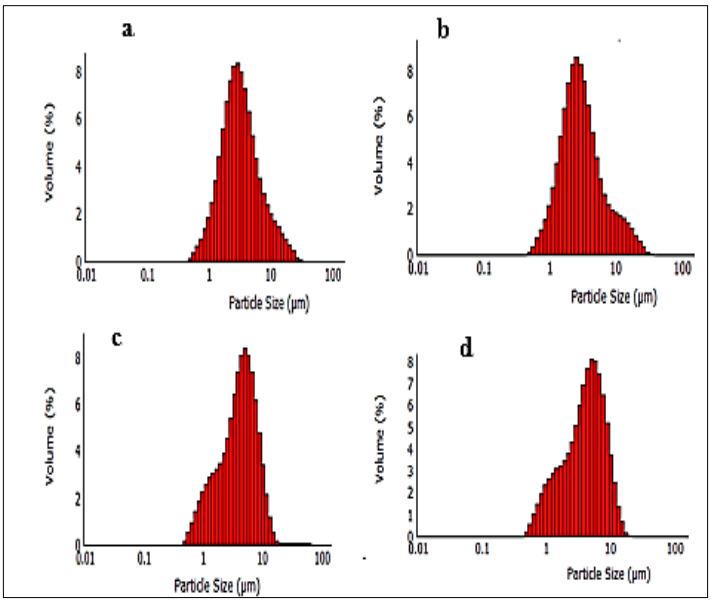

A
a
b
c
d
e
f
B
a
b
c
d

Fig. 3

A


a
b
c
d
Volume (%)
Particle Size (µm)
0.01
0.1
1
10
100

B

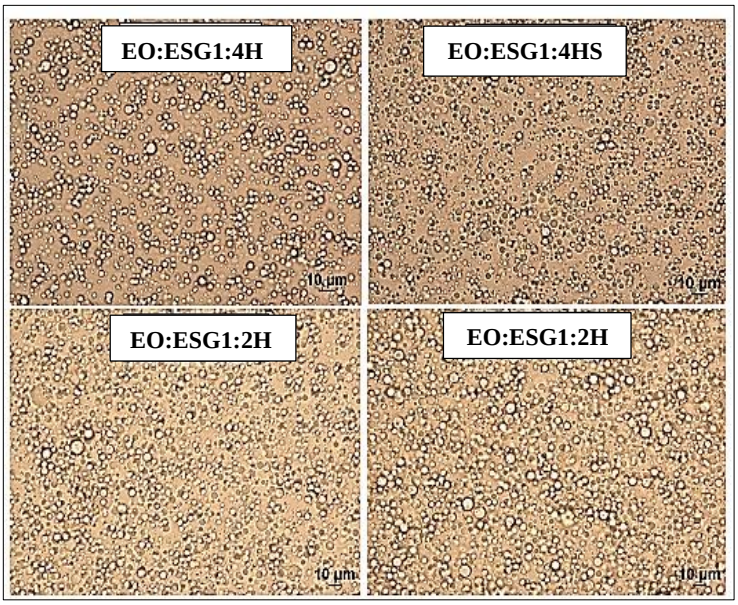
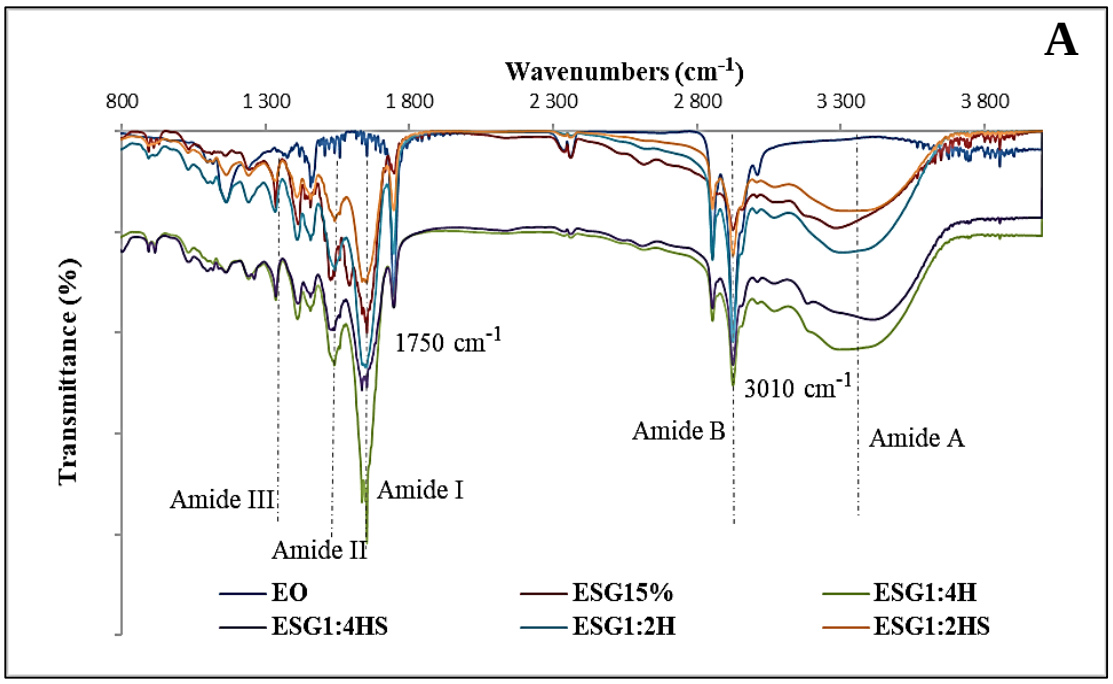
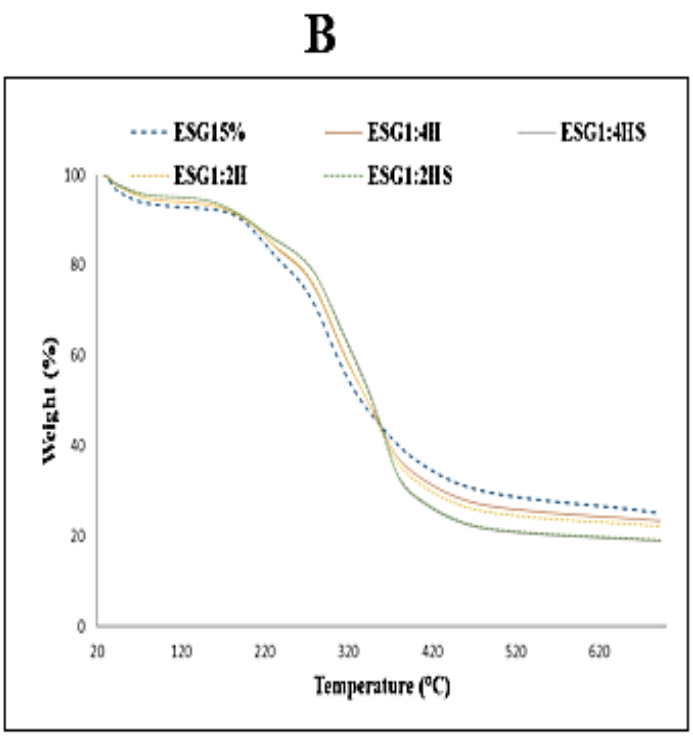

EO:ESG1:4H
EO:ESG1:4HS
EO:ESG1:2H
EO:ESG1:2H
10 µm

Fig. 4


A
Wavenumbers (cm-1)
800
1 300
1 800
2 300
2 800
3 300
3 800
Transmittance (%)
1750 cm-1
3010 cm-1
Amide III
Amide II
Amide I
Amide B
Amide A
EO
ESG15%
ESG1:4H
ESG1:4HS
ESG1:2H
ESG1:2HS


B
ESG15%
ESG1:4H
ESG1:4HS
ESG1:2H
ESG1:2HS
Weight (%)
Temperature (°C)

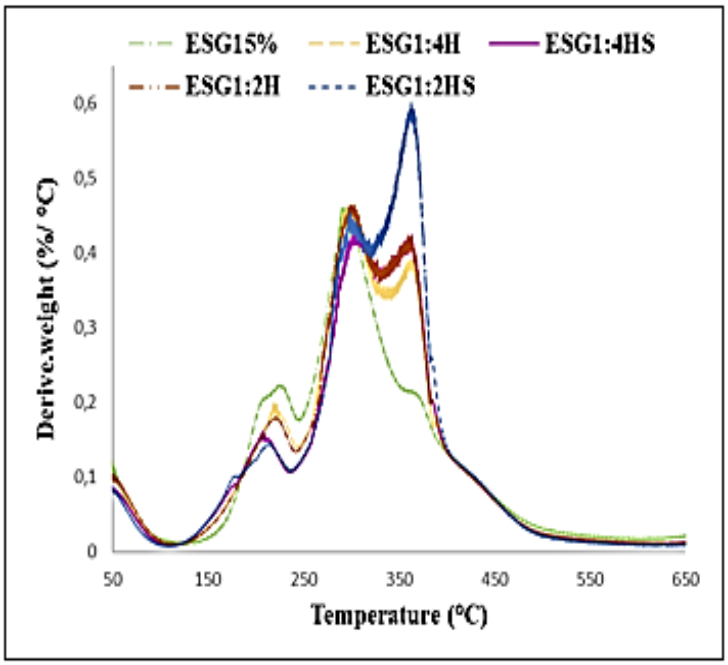

C
ESG15%
ESG1:4H
ESG1:4HS
ESG1:2H
ESG1:2HS
Derive.weight (%/ °C)
Temperature (°C)

Table 1: Effects of solvent and European fish eel skin gelatin (ESG) concentration (w/v) on the conductivity (mS/cm), surface tension (ST, mN/m) and viscosity (mPa.s) at 25 °C**.**

Solvent	Concentration ESG (w/v)	Conductivity (mS/cm)	Surface Tension (ST) (mN/m)	pH	Viscosity (mPa.s)
Water	2	2.29 ± 0.01^{a}	39.00 ± 1.69^{a}	5.96 ± 0.03^{a}	0.009
	4	3.3 ± 0.02^{b}	39.73 ± 0.64^{a}	5.96 ± 0.02^{a}	0.013
	6	4.67 ± 0.03^{c}	39.87 ± 0.06^{a}	5.97 ± 0.01^{a}	0.021
	8	6.41 ± 0.01^{d}	39.87 ± 0.15^{a}	5.95 ± 0.04^{a}	0.026
	10	8.05 ± 0.01^{e}	39.97 ± 0.06^{a}	5.98 ± 0.04^{a}	0.030
	15	10.99 ± 0.04^{f}	41.37 ± 0.21^{b}	5.98 ± 0.04^{a}	0.076
Ethanolic solution 40% (v/v)	2	0.90 ± 0.00^{a}	32.83 ± 0.23^{ab}	5.89 ± 0.02^{a}	0.007
	4	1.58 ± 0.06^{b}	32.93 ± 0.12^{ab}	5.91 ± 0.04^{a}	0.011
	6	1.93 ± 0.01^{c}	32.70 ± 0.17^{b}	5.91 ± 0.01^{a}	0.033
	8	2.64 ± 0.05^{d}	32.97 ± 0.06^{ab}	5.91 ± 0.01^{a}	0.039
	10	3.06 ± 0.05^{e}	32.97 ± 0.10^{ab}	5.91 ± 0.01^{a}	0.069
	15	5.25 ± 0.01^{f}	33.00 ± 0.15^{a}	5.91 ± 0.01^{a}	0.092

Values are given as mean ± standard deviation (n=3). $^{a\text{-}f}$ different letters in the same column indicate significant differences ($p<0.05$).

Table 2: Surface tension (ST), conductivity, Zeta potential (ZP), average droplet size and microencapsulation efficiency (MEE) of emulsions at different European fish eel oil: European fish eel skin gelatin (EO:ESG) emulsions using the EO:ESG ratio 1:2 and 1:4 (w/w)

Samples	Emulsification methods	n	k (Pa.s)^{n}	r^2	η_{ap} (Pa.s) ($\gamma=100^{-1}$)
ESG15%		0.878	0.863	0.974	0.107
EO:ESG1:2	**H**	0.968	0.921	0.997	0.122
	HS	0.9715	0.925	0.996	0.122
EO:ESG1:4	**H**	0.994	0.936	0.994	0.110
	HS	0.999	0.924	0.996	0.111

Values are given as mean ± standard deviation (n=3). a-e different letters in the same column indicate significant differences ($p<0.05$). European fish eel skin gelatin solution 15% (w/v) dissolved in ethanolic solution (40%, v/v)); The European fish eel oil: European fish eel skin gelatin emulsions (EO:ESG emulsion), prepared with the ratios of 1:2 and 1:4 (w/w) were named EO:ESG1:4 and EO:ESG1:2 respectively using two emulsification processes, homogenization with ultraturrax (H) and homogenization+ Sonication (HS).

Table 3:

Rheological parameters of the electrospun solutions: Ostwald de Waale model parameters (*n*, *k* and r^2) and apparent viscosity (η_{ap}) at 100 s^{-1}, at 25 °C.

Samples	Emulsification methods (E.M.)	Conductivity (mS/cm)	ST (mN/m)	ZP (mV)	Average droplet size (µm)	MEE (%)
ESG15%		5.25 ± 0.01^{a}	33.03 ± 0.15^{a}	-	-	-
EO:ESG 1:2	**H**	4.09 ± 0.03^{d}	31.37 ± 0.71^{b}	8.89 ± 0.40^{a}	5.38 ± 0.53^{a}	27.01 ± 1.43^{b}
	HS	3.99 ± 0.01^{e}	31.47 ± 0.06^{b}	7.43 ± 0.18^{b}	4.69 ± 0.45^{a}	26.06 ± 1.59^{b}
EO:ESG 1:4	**H**	4.30 ± 0.02^{b}	31.53 ± 0.21^{b}	6.75 ± 0.29^{c}	3.10 ± 0.30^{b}	76.11 ± 7.98^{a}
	HS	4.22 ± 0.02^{c}	31.90 ± 0.50^{b}	5.74 ± 0.13^{d}	2.70 ± 0.26^{b}	89.79 ± 0.77^{a}

European fish eel skin gelatin solution (ESG) 15% (w/v) dissolved in ethanolic solution (40%, v/v)); The European fish eel oil: European fish eel skin gelatin emulsions (EO:ESG emulsion), prepared with the ratios of 1:2 and 1:4 (w/w) were named EO:ESG1:4 and EO:ESG1:2 respectively using two emulsification processes, homogenization with ultraturrax (H) and homogenization+ Sonication (HS); Behavior index *(n)*; Consistency index (*k)*; Correlation coefficient (r^2)

Supplementary Data

S1. Effect of European fish eel skin gelatin (ESG) concentration on electrospinning process and microstructure.

S2. Average diameter of European fish eel skin gelatin (ESG) microfibers (ESG6% and ESG15%) and European fish eel oil (EO)-loaded microfibers.

S3. Investigation of different electrospinning (ES) conditions of European fish eel skin gelatin (ESG): Effect of rate and voltage on ES and microstructure.

S4. Stability of European fish eel oil: European fish eel skin gelatin (EO:ESG) emulsion at t=0 hand t=24 h.

S5. Rheological properties of European fish eel skin gelatin (ESG) and European fish eel oil: European fish eel skin gelatin (EO:ESG) emulsions shear stress in terms of shear rate. EO:ESG emulsions were prepared with the ratios of 1:4 and 1:2 (w/w) using two emulsification processes, homogenization with ultraturrax (H) and homogenization+ Sonication (HS) and named EO:ESG1:4H, EO:ESG1:4HS, EO:ESG1:2H, EO:ESG1:2HS respectively. a) ESG15%; b) EO:ESG1:4H, c) EO:ESG1:4HS, d) EO:ESG1:2H, e) EO:ESG1:2HS.

S6. Rheological properties of European fish eel skin gelatin (ESG) and European fish eel oil: European fish eel skin gelatin (EO:ESG) emulsions viscosity in term of shear rate. EO:ESG emulsions were prepared with the ratios of 1:4 and 1:2 (w/w) using two emulsification processes, homogenization with ultraturrax (H) and homogenization+ Sonication (HS) and named EO:ESG1:4H, EO:ESG1:4HS, EO:ESG1:2H, EO:ESG1:2HS respectively. a) ESG15%; b) EO:ESG1:4H, c) EO:ESG1:4HS, d) EO:ESG1:2H, e) EO:ESG1:2HS.

S1.

ESG concentration (%)	Water/Ethanolic solution 40%
4	ES (-) SEM (-)
6	ES (+/-) SEM (-)
8	ES (+) SEM (+)
10	ES (+) SEM (+)
15	ES (+) SEM (+)

European fish eel skin gelatin (ESG); SCANNING ELECTRON Microscopy observation (SEM); Electrospinning process (ES); Absence of drops during the electrospinning process (ES +); Presence of drops in the collected materials during the electrospinning process (ES -)

S2.

Samples	Average diameter of microfibers (µm)
ESG6%	1.21 ± 0.148
ESG15%	1.9 ± 0.636
EO-loaded microfibers	1.125 ± 1.063

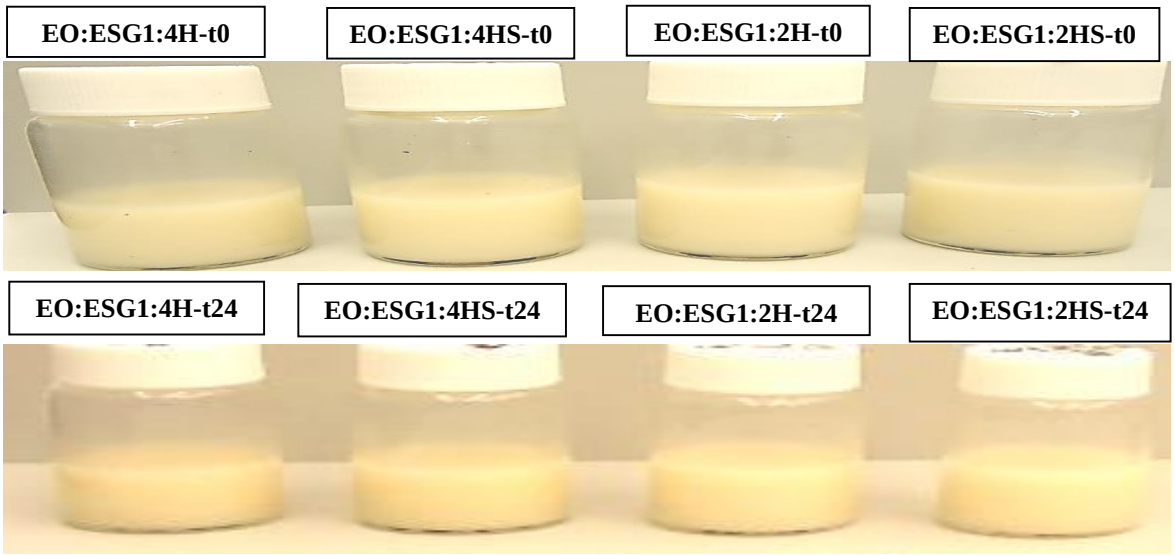
European fish eel skin gelatin (ESG); European fish eel oil (EO)-loaded microfibers

S3.

European fish **eel skin gelatin (ESG) 15% in water**				
Flow rate (mL/h)	**Voltage (kV)**	**ES**	**SEM**	
0.04	**10**	**-**	**-**	**This rate is below the optimum feeding rates of ESG solutions.**
	12	**-**	**-**	
	15	**-**	**-**	
	17	**-**	**-**	
0.1	**10**	**-**	**-**	**The best feeding rate allowing the evaporation of solvent and the obtaining of solid microfibers by increasing the voltage to 15 and 17.**
	12	**-**	**-**	
	15	**+**	**+**	
	17	**+**	**+**	
0.15	**10**	**-**	**-**	**The feeding rate is below the optimum (0.1 mL/h). ESG start dripping after a period of time which affect the quality of microfibers.**
	12	**-**	**-**	
	15	**+/-**	**+/-**	
	17	**+/-**	**+/-**	
0.5	**10**	**-**	**-**	**The flow rate is too high so solution was dripping during electrospinning process, even by increasing voltage the microstructure was covered by the solution drops.**
	12	**-**	**-**	
	15	**+/-**	**+/-**	
	17	**+/-**	**+/-**	

European fish eel skin gelatin (ESG); SCANNING ELECTRON Microscopy observation (SEM); Electrospinning process (ES); Absence of drops during the electrospinning process (ES +); Presence of drops in the collected materials during the electrospinning process (ES -)

S4.

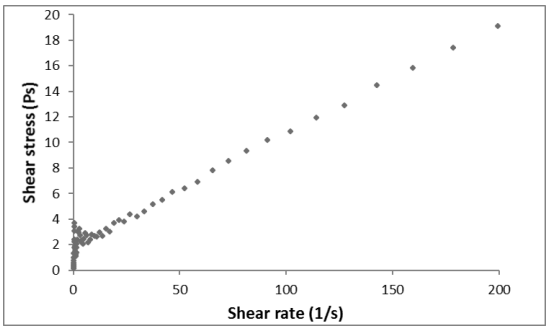


European fish eel skin gelatin (ESG); European fish eel oil (EO); European fish eel oil: European fish eel skin gelatin (EO:ESG) emulsions after 24 h, prepared with the ratios of 1:4 and 1:2 (w/w) using two emulsification

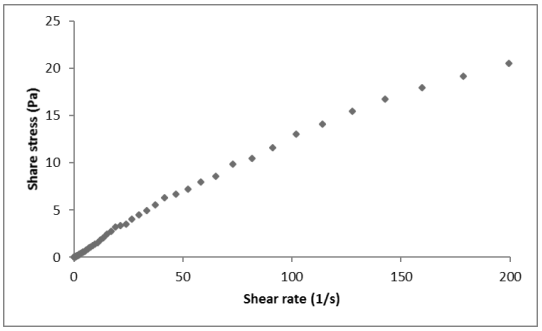
processes, homogenization with ultraturax (H) and homogenization+ Sonication (HS) and named EO:ESG1:4H, EO:ESG1:4HS, EO:ESG1:2H, EO:ESG1:2HS respectively.

S5.

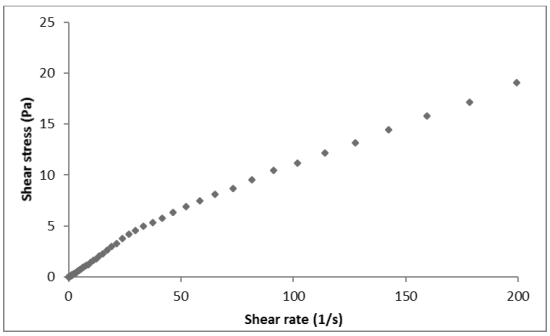
a)



b)



c)



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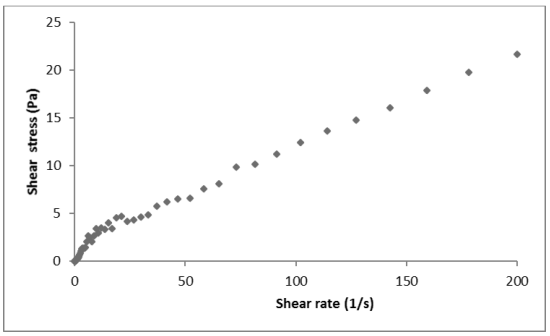

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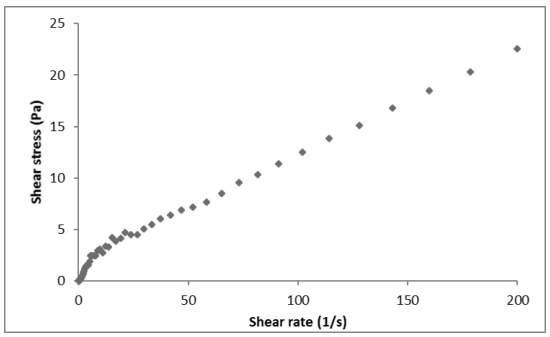
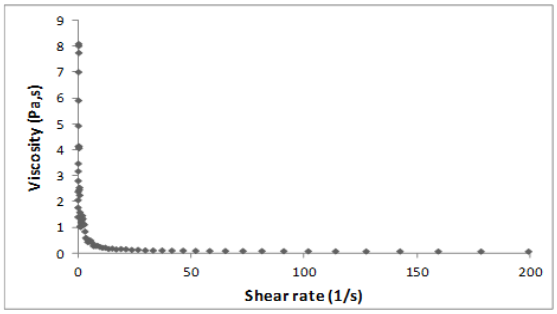
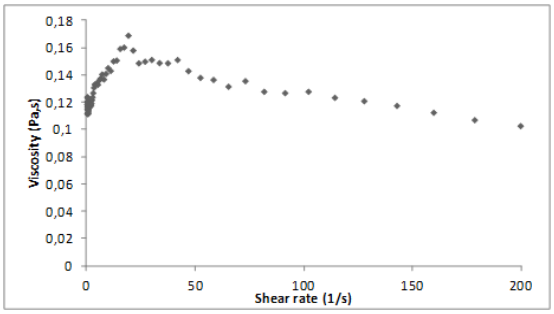


S6.

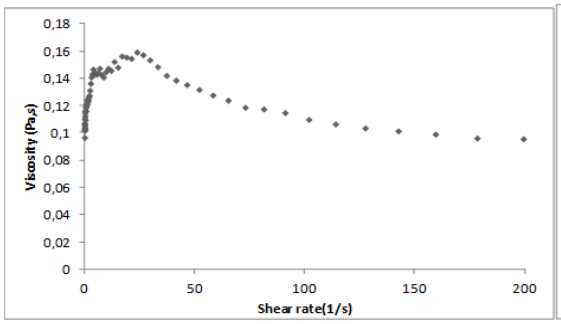
a)


Viscosity (Pa,s)
Shear rate (1/s)

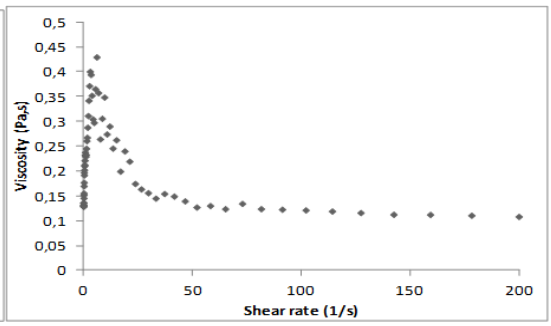
b)


Viscosity (Pa,s)
Shear rate (1/s)

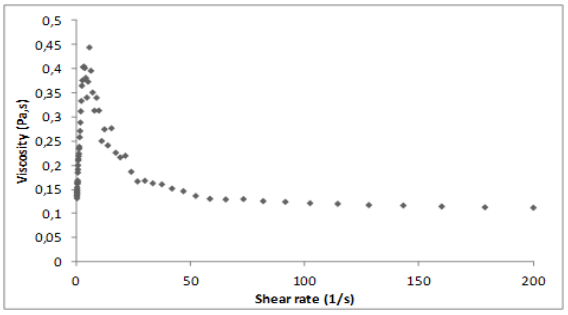
c)


Viscosity (Pa,s)
Shear rate(1/s)

d)


Viscosity (Pa,s)
Shear rate (1/s)

e)


Viscosity (Pa,s)
Shear rate (1/s)