DOI: 10.51745/najfnr.9.SI.S40-S49

ORIGINAL ARTICLE

Special Issue - Potential of Food By-Products

Potential of Food By-Products

Acid-Induced Gelation of European Eel Protein Isolate Fortified with Fish Oil: Enhancement of Physicochemical, Microstructural and Thermal Properties

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ABSTRACT

Aims: This study aimed to investigate the gelation capacity of European Eel Protein Isolate (EPI) through the development of novel emulsion protein isolate-based gels (EPIGs). Furthermore, the research evaluated the extent to which fortification with bioactive European eel oil (EO) modulates the structural and functional attributes of these acid-induced gel matrices.

Methods: EPIGs were synthesized via thermal treatment (90°C for 60 min) of EPI solubilized in 20% (v/v) acetic acid, followed by emulsification with EO at two distinct weight ratios: 1:2 and 1:4 (EO:EPI, w/w). The resulting gel structures were characterized using Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), texture profile analysis (TPA), and scanning electron microscopy (SEM).

Results: A 4% (w/v) EPI solution in acetic acid achieved gelation at 54°C, demonstrating superior thermal stability and significant gelling potential. FTIR analysis confirmed the successful incorporation of EO into the gel matrix, while TGA results indicated a slight increase in thermal degradation onset temperatures associated with higher EO concentrations, increasing from 375°C (EPIG1:4) to 382°C (EPIG1:2). Texture analysis revealed that incorporation of EO modestly augmented gel elasticity (from 12.93 mm to 13.19 mm), a phenomenon attributed to the stabilizing interactions between the lipid droplets and the protein network. Microstructural appraisal via SEM indicated that a higher EO load (EPIG1:2) induced the formation of larger pores, whereas the EPIG1:4 formulation exhibited a more cohesive and compact architecture with reduced porosity.

Keywords: European Eel; Emulsion Protein Isolate-based Gel; FTIR; Thermogravimetric; SEM.

Article Information

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Received: December 02, 2025
Revised: December 14, 2025
Accepted: January 18, 2026
Published: February 27, 2026

Article edited by:
Prof. Meghit Boumediene Khaled
Article reviewed by:
Dr. Sawssen Hajji
Dr. Imen Ben Atitallah

Cite this article as: Taktak, W., Affes, S., Hamdi, M., Li, S, Nasri, M, Nasri, R, Karra-Chaabouni, M. (2025). Acid-induced gelation: A powerful strategy to enhance the physicochemical, microstructural and thermal properties of European eel protein isolate fortified with fish oil. *The North African Journal of Food and Nutrition Research, 9*(SI): S40–S49. https://doi.org/10.51745/najfnr.9.SI.S40-S49

1 INTRODUCTION

Recently, significant scientific and industrial focus has been directed toward the development of functional foods fortified with bioactive and nutraceutical compounds. These products are engineered to provide physiological benefits and alleviate chronic disease risk beyond basic nutritional requirements (Jamshidi *et al.,* 2020). Research has extensively characterized the efficacy of various competitive biopolymers which are used as protective encapsulation matrices and renewable alternatives to produce robust delivery-systems (García-Moreno *et al.,* 2017). Among these biopolymers, protein and carbohydrates are primarily applied to enhance the bioavailability of bioactive molecules within food matrices (Nasri *et al.,* 2020). Several studies absorbed on the exciting techno-functional properties of protein isolate to improve textural properties and to protect sensitive biomolecules of interest into gel networks (Datta *et al.,* 2020; Yang *et al.,* 2014). The gelation behaviors and emulsifying properties of protein depend on their amino acid sequence and secondary

structure (Souissi *et al.,* 2017). These characteristics allow for proteins to contribute to the structural and the textural properties of a diverse dispersion systems such as hydrogels, emulsions or emulsion gels (Hattrem *et al.,* 2015). Furthermore, protein have been successfully employed to entrap numerous lipophilic molecules, such as fish oil, via emulsification techniques (Li *et al.,* 2018). Emulsions involve the dispersion of two immiscible phases, where hydrophobic droplets are stabilized within an aqueous system using surface-active carriers. Various methodologies facilitate this process, including low-pressure membrane emulsification, high pressure homogenization, ultrasonication (for de-agglomeration to form nano-sized particles), and microencapsulation by micro-fluidization (for microencapsulation) (Jamshidi *et al.,* 2020).

Fish oil emulsions may be utilized directly in food systems—such as beverages or processed meats—or converted into microencapsulated powders through solvent removal. Consequently, these systems have seen widespread application across the pharmaceutical, cosmetic, and biotechnological sectors. Emulsion gels are defined as edible, semi-solid materials characterized by a cross-linked network structure enriched with dispersed oil droplets. These systems offer superior rheological and sensorial properties, making them ideal candidates for the development of fat-reduced products (Dickinson, 2015). Structurally, emulsion gels consist of a dispersed phase immobilized within a three-dimensional (3D) polymer matrix (Farjami & Madadlou, 2019), as observed in common food matrices such as yogurt, cheese, and restructured meats.

The fabrication of emulsion gels generally involves a two-stage process: emulsification followed by gelation. Various method could be employed to enhance efficiency encapsulation of bioactive molecules with the emulsion gel systems. Indeed, the gelation can be originated from proteins via thermal, ionic (salt-induced), acidic, or enzymatic approaches (Lu *et al.,* 2020), resulting in the formation of stable, semi solid materials (Ye & Taylor, 2009). Protein-based emulsion gels represent a safe alternative in the industry (McClements *et al.,* 2017) and recently utilized to protect active compounds and to improve the nutraceutical product accessibility (Geremias-Andrade *et al.,* 2017; Li *et al.,* 2018). In medical and food field the emulsion application is constrained by challenges related to emulsion instability. A substantial improvement in the emulsion stability could greatly achieved by embedding oil droplets within a cross-linked three-dimensional solid such as hydrogel (Farjami & Madadlou, 2019).

The present study seeks to evaluate the gelation capacity of European eel protein isolate (EPI) within an acetic acid solution. The objective was to formulate a novel emulsion EPI-based gel system fortified with eel oil (EO)—previously characterized as a model of bioactive molecules (Taktak *et al.,* 2019)—and to evaluate its physicochemical, structural, textural, microstructural and thermal properties.

2 Materials and Methods

2.1 Materials

European eel oil, characterized by a fatty acid profile comprising 54.34% monounsaturated fatty acids, 28.71% saturated fatty acids (SFAs), and 16.86% polyunsaturated fatty acids (PUFAs), was extracted according to the protocol detailed in our previous study (Taktak *et al.,* 2019). Analytical grade organic solvents, including methanol, chloroform, ethanol, and hexane, as well as acetic and hydrochloric acids, were procured from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Fish Sample Preparation

Specimens of *Anguilla anguilla* were purchased from the local fish market in Sfax, Tunisia. Species authentication was performed based on standard morphological markers including body morphology, gill raker counts, and fin architecture. Samples were sealed in polyethylene bags, maintained on ice, and transported to the laboratory within 30 minutes. The muscle tissue was excised, separated from the viscera, and rinsed with distilled water to eliminate salts and contaminants. The tissue was then stored at −20 °C pending further analysis.

2.3 Extraction of European Eel Protein Isolate

The extraction of European Eel protein isolate (EPI) from muscle tissue was performed employing the alkaline solubilization-isoelectric precipitation method described by Taktak *et al.* (2018). Briefly, fish mince was homogenized with chilled distilled water (4°C) at a ratio of 1:9 (w/v) ratio. The pH of the homogenate was adjusted to 11.5 utilizing 2 N NaOH and stirred for 60 min. The mixture was subsequently centrifuged at 9,500 × *g* for 20 min at 4°C. The protein-rich supernatant was acidified to the nominal isoelectric point (pH 5.5) with 2 N HCl to induce precipitation. The resulting precipitate was recovered via centrifugation (9,500 × *g*, 20 min), resuspended in distilled water, and neutralized to pH 7.0 with 2 N NaOH. Finally, the EPI was dehydrated employing a freeze-dryer (Moduloyd-230, Thermo Fisher Scientific, USA) at −50°C and 121 mbar.

2.4 Preparation of EO: EPI Emulsions

EO:EPI emulsions were prepared by solubilizing the protein isolate (4 wt%) in a 20% (v/v) acetic acid solution. The solution was subjected to thermal treatment at 90°C for 60 min to induce protein unfolding and partial aggregation. After cooling to ambient temperature, EO—extracted via the Folch method (Folch *et al.,* 1957)—was gradually

incorporated into the EPI solution. Primary emulsification was achieved using a high-shear homogenizer (Ultra-Turrax IKA T18 basic, Wilmington, USA) at 6,000 rpm for 5 min. Finally, the cold-gelation was introduced by modifying the solvent conditions to endorse protein aggregation (Kharlamova *et al.,* 2018).

Two weight ratios of EO:EPI (1:2 and 1:4 w/w) were established. Samples prepared solely via homogenization were designated as E1:2-H and E1:4-H. To investigate the impact of intensified processing, a subset of emulsions underwent supplementary ultrasonication (Model S-450D, Branson Ultrasonics, USA) at 160 W and 20 kHz for 2 min. (30 s on/off cycles). These samples were designated as E1:4-HS and E1:2-HS.

2.5 Characterization of EO: EPI Emulsions

The zeta-potential of the emulsions was determined using a Malvern Zetasizer (NanoZS, Worcestershire, UK) at 25°C in triplicate. The physical stability of the emulsions was evaluated through the creaming index method as described by Surh *et al.* (2006).

2.6 Determination of EPI Gelling Point

Dynamic oscillatory study was determined employing Physica MCR 301 rheometer (Anton Paar, GmbH, France.) to identify the sol-gel transition point of the EPI. The elastic modulus (G'; Pa) and viscous modulus (G"; Pa) were recorded during the above dynamic rheological measurements. The diameter of the cup was 28.5 mm and the diameter of the bob was 25 mm, giving a gap between the cup and the bob of 1.0 mm. Heating from 10 to 90 °C and back to 10 °C took place at a scan rate of 01 °C/min, frequency of 1 Hz, and target strain of 1%. The elastic modulus (G'; Pa) and viscous modulus (G"; Pa) were plotted as a function of temperature to characterize the large deformation rheological properties.

2.7 Preparation of Emulsion EPI-Based Gels

Emulsion-based gels (EPIGs) were formulated by solubilizing 4% (w/v) EPI in 20% (v/v) acetic acid. Following thermal treatment at 90°C for 60 min, the solution was cooled to 60 °C and EO was incorporated at ratios of 1:4 and 1:2 (w:w), resulting in EPIG1:4 and EPIGG1:2, respectively. Emulsification was performed using either high-shear homogenization (H) at 12,500 rpm for 5 min or a combined homogenization-ultrasonication (HS) approach (20% amplitude for 2 min). The resulting mixtures were stored at 4°C for 24 h to facilitate gel maturation (Chaijan *et al.,* 2010).

2.8 Characterization of EPIGs

2.8.1 FTIR Analysis

FTIR spectra of EPIGs were acquired using a Perkin-Elmer Spectrum 65 (France) equipped with a ZnSe attenuated total reflectance (ATR) accessory. A total of 32 scans were recorded over a spectral range of 650 – 4000 cm^{-1} with a resolution 4 cm^{-1}. Background spectra were obtained calibration is obtained through the unsoiled and empty cell at 25 °C. Data processing was performed using Spectrum Suite ES software.

2.8.2 Thermal Properties

Thermogravimetric analysis (TGA) was conducted employing a TGA50H thermobalance (Shimadzu Corp, Kyoto, Japan). Samples were heated in an alumina pan from 50 – 550 °C at a constant rate of 10 °C min^{-1} under a dynamic nitrogen atmosphere (flow rate: 100 mL min^{-1}).

2.8.3 Textural Properties

Texture Profile Analysis (TPA) was performed using a TA-TX2 texture analyzer (Lloyd Instruments, Ltd., West Sussex, UK). Cylindrical gel samples (15 × 25 mm, height × diameter) underwent a two-cycle compression to 50% of their original height at a rate of 30 mm/min using a 12-mm diameter probe. Hardness (N), cohesiveness (N), and elasticity (mm), were calculated from the resulting force-deformation curves.

2.8.4 Microstructural Analysis

The microstructure of the EPIGs was visualized via Scanning electron microscopy (SEM) employing a Hitachi S-4800 microscope at an accelerating voltage of 10 kV and a working distance of 8 – 9 mm.

2.9 Statistical Analysis

All experiments were conducted in triplicate, and data are presented as mean ± standard deviation. Statistical significance was determined via one-way Analysis of Variance (ANOVA) using Statgraphics version 5.1 (Manugistics Corp., USA). Differences between means were considered statistically significant at $p < 0.05$.

3 Results and Discussions

3.1 Physicochemical Characterization of European Eel Muscle and EPI

The characterization of European eel muscle and the subsequent extraction of EPI were established in our previous investigations (Taktak *et al.,* 2018). Given its rich amino acid profile, EPI is considered an essential nutritional constituent for human consumption. Furthermore, the techno-functional properties of EPI revealed substantial emulsifying and foaming capacities, which peaked under both highly acidic and alkaline conditions. This behavior is primarily attributed to the enhanced solubility of EPI at extreme pH values. The molecular configuration of EPI facilitates the development of a viscoelastic network, which is instrumental in the formation

of cohesive gels at alkaline pH (Taktak *et al.,* 2018). Moreover, EPI has demonstrated efficacy as a functional, and interactive biopolymer microencapsulation, effectively protecting fish oil against oxidative degradation (Taktak *et al.,* 2019).

Data presented in Table 1 delineate the isoelectric point (pI) of EPI dispersions, determined across a range of pH through the ζ-potential analysis. The pI is defined as the pH at which the zeta potential curve crosses zero (ζ-potential = 0 mV), corresponding to a net neutral surface charge and minimum electrostatic stabilization.

Table 1. Zeta Potential of the European Protein Isolate (EPI) at different pHs

pH	Zeta potential (MV)	Mobility (µmcm/Vs)	Conductivity (mS/cm)
3	34.83 ± 2.60 ^{a}	2.73 ± 0.20 ^{a}	0.90 ± 0.020 ^{b}
3.5	30.9 ± 1.750 ^{b}	2.42 ± 0.130 ^{b}	0.32 ± 0.002 ^{cd}
4	22.76 ± 0.45 ^{c}	1.78 ± 0.030 ^{c}	0.09 ± 0.001 ^{de}
5	-6.37 ± 0.26 ^{d}	-0.49 ± 0.020 ^{d}	0.14 ± 0.001 ^{de}
5.5	-25.1 ± 0.36 ^{g}	-1.97 ± 0.020 ^{g}	0.04 ± 0.001 ^{e}
6	-22.23 ± 1.02 ^{f}	-1.74 ± 0.080 ^{f}	0.10 ± 0.002 ^{de}
7	-25.16 ± 0.20 ^{g}	-1.97 ± 0.014 ^{g}	0.07 ± 0.0004 ^{e}
8	-25.63 ± 1.30 ^{g}	-2.01 ± 0.100 ^{g}	0.10 ± 0.001 ^{de}
9	-17.8 ± 0.70 ^{e}	-1.39 ± 0.050 ^{e}	0.525 ± 0.006 ^{c}
10	-18.5 ± 2.72 ^{e}	-1.45 ± 0.210 ^{e}	3.54 ± 0.390 ^{a}

Data are given in mean ± SD. a-g different letters in the same column indicate significant differences between sample ($p < 0.05$).

The surface charge of protein isolate remained positive up to pH 4, with values decreasing from 34.83 mV at pH 3.0 to 22.00 mV at pH 4.0, before transitioning to a negative charge at higher pH levels. Therefore, the pI of EPI was identified within the pH 4.0 – 5.0 range. At this threshold, electrostatic repulsion is minimized, promoting aggregation and flocculation mediated by van der Waals attractions and hydrophobic interactions.

3.2 Characterization of EO:EPI Emulsion

The EO:EPI emulsions (formulated at weight ratios 1:2 and 1:4) were evaluated based on ζ-potential, surface tension, and creaming index (CI) to determine their emulsion stability.

As illustrated in Table 2, all EO:EPI formulations exhibited exceptional stability, maintaining a CI of 100% following 24 hours of incubation at room temperature (Supplementary Data). The high degree of stability is related to the inherent emulsifying properties of EPI determined in a previous study (Taktak *et al.,* 2019) and to the emulsification processes. This finding was confirmed by the high ζ-potential

Table 2. Surface Tension (ST), zeta potential (ZP) and Creaming Index (CI) of 4% EPI (w/v) and (EO: EPI) emulsion (E) at different ratio 1:4 and 1:2 (w/w) obtained by homogenization

Samples	ST (mN/m)	ZP (mV)	CI (%)
EPI4 %	35.27 ± 1.10 ^{c}	34.83 ± 2.6 ^{d}	-
E1:4H	39.5 ± 0.53 ^{a}	40.63 ± 0.55 ^{a}	100
E1:4HS	38.8 ± 0.26 ^{a}	37.76 ± 0.68 ^{bc}	100
E1:2H	37.07 ± 0.45^{b}	36.7 ± 0.51 ^{c}	100
E1:2HS	37.63 ± 0.31^{b}	40.1 ± 0.40 ^{ab}	100

Data are given in mean ± SD. a-d different letters in the same column indicate significant differences between sample ($p < 0.05$).

values (ranging from 36 to 40 mV) for the different EO:EPI emulsions, which was usually used for predicting the emulsion stability of emulsion systems (Cho, Lee, & Frey, 2012).

Surface tension decreased with increasing the EO:EPI ratio, reaching 39.5 to 37.07 for E1:4H and E1:2H respectively, and not with Ultrasonication step as presented in Table 2. This is attributed to the ability of ultrasonication to provide mechanical energy to create sub-micron droplets and do not alter the chemical affinity of the emulsifier for the interface. Therefore, the ultrasonication step creates a shift in ζ-potential within the same ratio (e.g., from 40.63 to 37.76 for E1:4H and E1:4HS, respectively) could be attributed to the intense ability of ultrasonication to provide the shear necessary for modifying the interfacial adsorption and the resulting surface charge of droplets (O'Sullivan *et al.,* 2016).

Moreover, the high ζ-potential values supplied the high energy barrier among emulsion droplets, providing an appropriate electrostatic repulsion (Li *et al.,* 2019). The surface tension of the EO: EPI emulsions for the different ratios was in the range of 37.5 and 39.05 mN/m revealing a slight increase of surface tension in emulsion systems compared to EPI solution (35.27 mN/m). The interplay of these physicochemical parameters serves as a reliable predictor for the structural stability of EPI-based emulsion gels.

3.3 Determination of the EPI Gelation Point

In this study, the gelation point of EPI was determined by identifying the crossover point of the elastic modulus G' and the viscous modulus G'' (Harsch *et al.,* 2008). The EPI 4% (acetic acid 20%) gelling point is presented in Figure 1.

In the initial phase (20 – 54°C), G' remained significantly elevated than G'' indicating a predominantly elastic, solid-like behavior. The increase in G' during cooling suggests the rapid formation of junction zones within the protein network (Saha & Bhattacharya, 2010). The crossover point occurred at 54°C, which is formally defined as the gelation temperature for this specific formulation. Within the 54 – 90°C range, G'

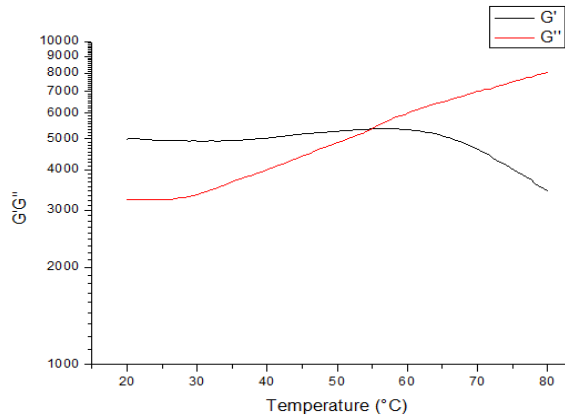

Figure 1. Determination of the Gelling Point of European Protein Isolate (EPI)

decreased while G'' increased, signifying a transition toward a predominantly viscous, liquid-like viscoelastic state. The relatively high gelation temperature observed for EPI is indicative of superior protein quality and suggests broad industrial applicability (Tabarestani *et al.,* 2010).

The robust gelling capacity of 4% EPI is induced by thermal treatment, which facilitates a thermos-irreversible sol-gel transition. During this heat-induced process, myofibrillar protein molecules associate through a complex interplay of ionic bonds, hydrogen bonding, hydrophobic interactions, and covalent cross-linking (Yan *et al.,* 2020).

The identification of gelation points at 54°C offers distinct strategic advantages:

- **Food science application:** This temperature is optimal for the development of heat-set gels under mild pasteurization conditions. This allows for the production of protein-based snacks or dairy analogues without compromising the integrity of heat-sensitive micronutrients (McClements, 2015).
- **Biomedical applications**: the gelation points of 54°C presents a strategic advantage for externally activated in situ gelation systems. The formulation remains injectable at room temperature but can undergo a sol-gel transition at body temperature via a mild thermal stimulus (e.g., a warm compress). Furthermore, certain cellular structures can tolerate transient exposure to these temperatures if the stimulus is precisely targeted, making this system a promising candidate for controlled drug delivery depots minimally invasive tissue fillers (Taylor *et al.,* 2017).

3.4 Characterization of EPIGs

3.4.1 FTIR Spectra Analysis

FTIR spectroscopy was employed to elucidate the molecular interaction between the lipid phase (EO) and the proteic matrix (EPI) within the emulsion the gel (Figure 2). The spectra exhibited characteristic absorption bands indicative of the proteinaceous structure, notably **Amide A** (3281 cm^{-1}, associated with N-H or O-H stretching), **Amide B** (2920 cm^{-1}, representing C-H bending), **Amide I** (1623 cm^{-1}, primarily C=O and C=N), **Amide II** (1543 cm-1, involving C-N stretching and N-H bending), C-H bending at 1380 cm^{-1}, and **Amide III** (1241cm^{-1}) (Chen *et al.,* 2006).

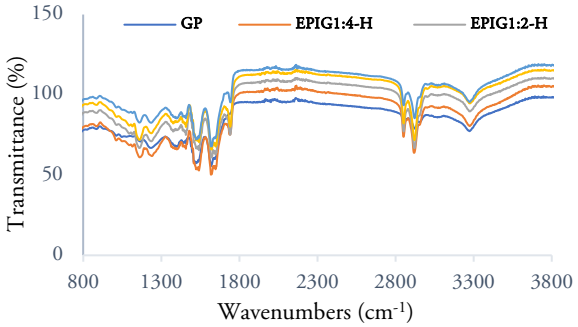

Figure 2. FTIR Spectra Analysis of Unloaded Protein Isolate Gel (GP) and Emulsion Protein Isolate-Based Gel EPIG1:4H, EPIG1:4HS, EPIG1:2H, and EPIG1:2HS

Furthermore, the incorporation of European eel oil as a bioactive constituent introduced distinctive peaks at 1750, 1700 and 1640 cm^{-1} corresponding to C=C, C=O and C=C-C=C vibrations, respectively (Chen *et al.,* 2006). The presence of the CH bending vibration of the *cis*-alkene cluster at 2970 cm^{-1}, confirms the presence of polyunsaturated fatty acid (omega-3) (Moomand & Lim, 2014). The FTIR spectra of EPI gels presented in Figure 2 revealed the presence of distinctive bands of both EPI and EO. This finding proved the effective EO loading in the EPIGs matrices.

3.4.2 Thermogravimetric Analysis of EPIGs

Thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses were conducted to evaluate the thermal stability and decomposition profiles of the raw materials and the formulated gels (Figure 3). The primary thermal degradation stage for pure EPI occurred between 316°C and 325°C. In contrast, the decomposition of pure EO as established in previous work was situated within the 397 – 400°C range (Taktak *et al.*, 2019).

The DTG profiles for the EPIGs reflected the degradation related patterns of both constituents, signifying the effective entrapment of the oil within the gel matrix. Indeed, the peak temperature for EO degradation within the gels ranged from 375°C to 382°C. A marginal elevation in the onset of thermal degradation was observed as EO content increased, with values rising from 375°C (EPIG1:4H) to 382 °C

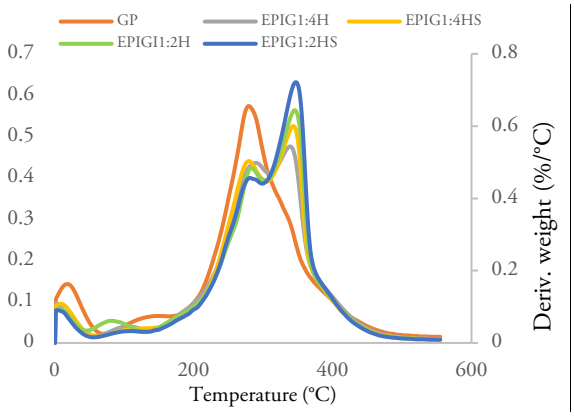

Figure 3. Thermal properties of unloaded protein isolate gel (GP) and emulsion protein isolate based gel EPIG1:4H, EPIG1:4HS, EPIG1:2H and EPIG1:2HS which were obtained by the gelification of European eel: European protein isolate (EO: EPI) emulsion at different ratios 1: 4 and 1: 2 (w/w), with the homogenization using Ultra-Turrax (H) or Ultra-Turrax followed by ultrasonication treatment (HS), respectively.

(EPIG1:2H). Additionally, ultrasonication treatment exerted a stabilizing effect, delaying thermal decomposition. For instance, the maximum degradation temperature (T_{max}) increased from 375°C to 380°C for the EPIG1:2H and EPIG1:2HS samples, respectively. This enhancement is likely attributable to the efficacy of the homogenization-ultrasonication (HS) process in stabilizing the protein-oil interface, particularly at higher lipid loads. The presence of the characteristic thermal peak degradation of eel oil in the EPIGs as well as the FTIR spectra analysis demonstrated the higher loading ability of the EPIGs to retain oil in the gel matrices.

3.4.3 Textural Properties

Visual inspection of the EPIGs, documented in Supplementary Data, revealed an increase in opacity and whiteness compared to the control (unloaded) gels, a phenomenon associated with light scattering by the emulsified oil droplets. Macroscopic observations indicated that EPI possesses a robust capacity to form rigid, elastic gels, suggesting that the protein-stabilized oil droplets effectively participate in the formation of a cohesive, three-dimensional (3D) network (Li *et al.*, 2023).

These observations were quantified through texture profile analysis, as summarized in Table 3. The mechanical properties of the gels (hardness, elasticity, adhesiveness, and cohesiveness), were significantly modulated by the incorporation of EO. Specifically, hardness and adhesiveness were attenuated following the emulsification procedure. While the textural properties showed identical characteristics of different EPIGs. Therefore, several parameters could affect the textural properties of protein gel which is the mechanical action related to the emulsification treatments employed to prepare the emulsions. Moreover, textural analysis is also related to the chemical bonds which enhance the connection of myofibrillar protein molecules during the heat-induced gelation by the ionic bonds, hydrogen bonds, hydrophobic interactions, and covalent bonds. Thus, the thermal treatment could affect chemical interactions involved in the protein network formation, which exhibits significant effect on gel texture (Yan *et al.*, 2020).

Interestingly, lipid incorporation facilitated a marginal increase in gel elasticity ranging from 12.93 mm to 13.19 mm. This reinforcement of the elastic component is likely due to the active role of EO droplets as "active fillers" within the EPI matrix, which strengthens the structural connectivity and enhances the resilience of the resulting network (Line *et al.*, 2005).

3.4.4 Microstructural Analysis

SEM was utilized to elucidate the internal architecture of the formulated gels (Figure 4). The micrographs revealed a distinctly porous microstructure in the EPIG samples compared to the control (unloaded) gels. This porosity could be attributed to protein characteristic structure employed as biopolymer for EO-loading. In contrast, the unloaded protein gels exhibited a sufficiently, firm, cohesive, and homogenous matrix, associated to better gel characteristic previously reported by the textural analysis.

Table 3. Textural Properties of Unloaded Protein Isolate Gel (GP) and Emulsion Protein Isolate-Based Gels; EPIG1:4H, EPIG1:4HS, EPIG1:2H, and EPIG1:2HS, respectively

Samples	Hardness (N)	Cohesion	Elasticity (mm)	Adhesion (N)	Masticability (Nmm)	Breaking Strength (N)	Force adhesivity (N)
GP	0.95 ± 0.01 ^{a}	0.40 ± 0.06 ^{b}	12.43 ± 0.96 ^{a}	0.38 ± 0.05 ^{a}	4.70 ± 1.01 ^{a}	0.88 ± 0.11 ^{a}	0.98 ± 0.56 ^{a}
EPIG1: 4H	0.58 ± 0.06 ^{bc}	0.58 ± 0.01 ^{a}	13.04 ± 0.76 ^{a}	0.34 ± 0.04 ^{a}	4.41 ± 0.80 ^{a}	0.58 ± 0.06 ^{ab}	0.23 ± 0.01 ^{b}
EPIG1: 4HS	0.58 ± 0.01 ^{bc}	0.63 ± 0.05 ^{a}	13.01 ± 0.50 ^{a}	0.37 ± 0.02 ^{a}	4.81 ± 0.45 ^{a}	0.47 ± 0.18 ^{b}	0.27 ± 0.01 ^{b}
EPIG1: 2H	0.66 ± 0.02 ^{b}	0.57 ± 0.01 ^{ab}	13.19 ± 0.44 ^{a}	0.38 ± 0.01 ^{a}	4.96 ± 0.16 ^{a}	0.52 ± 0.19 ^{b}	0.24 ± 0.05 ^{b}
EPIG1: 2HS	0.54 ± 0.01 ^{c}	0.66 ± 0.136 ^{a}	12.93 ± 0.67 ^{a}	0.36 ± 0.07 ^{a}	4.62 ± 1.12 ^{a}	0.54 ± 0.01 ^{ab}	0.22 ± 0.01 ^{b}

Data are provided as mean ± SD. $^{a-c}$ different letters in the same column indicate significant differences between sample ($p < 0.05$).

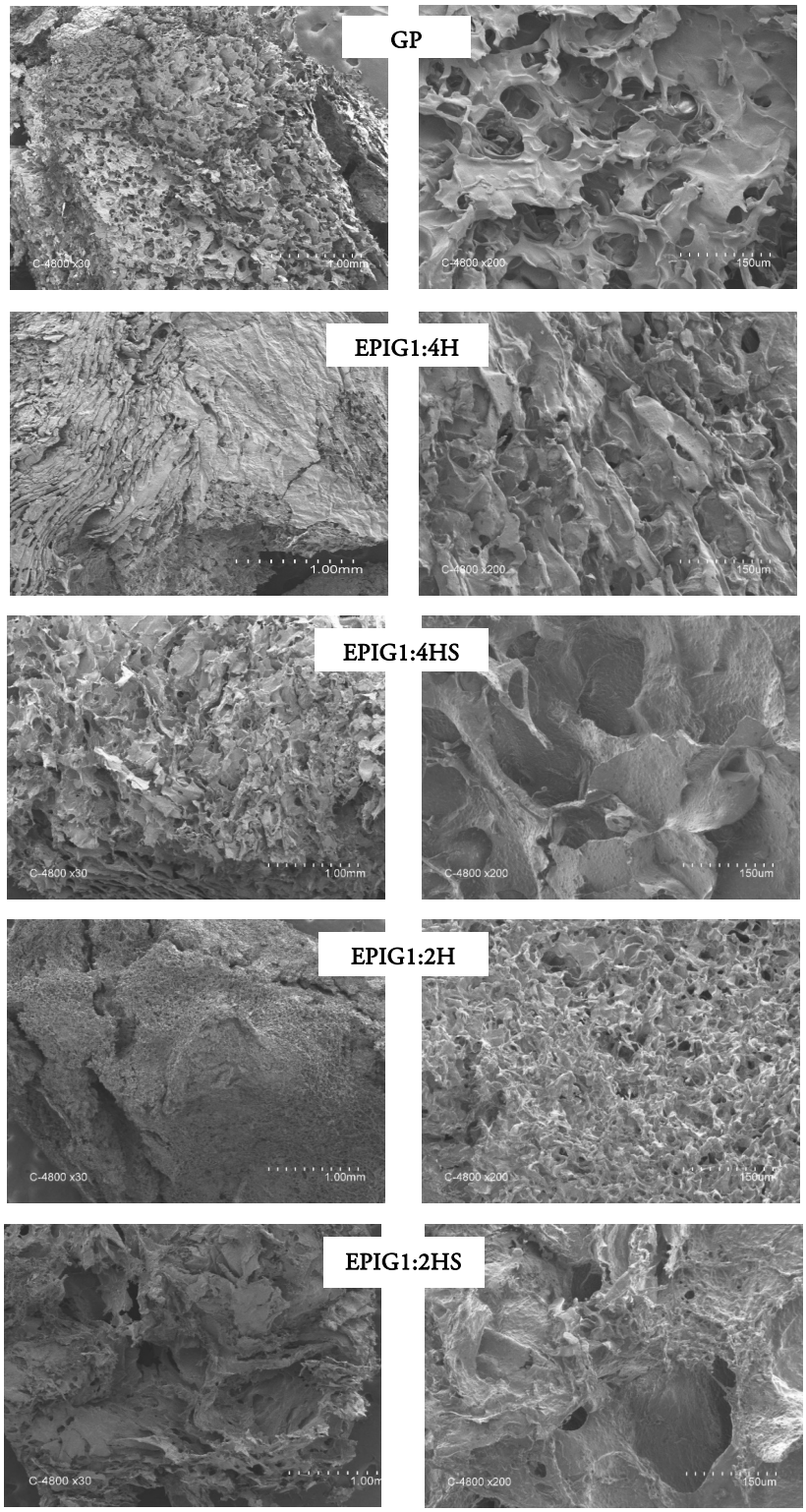

Figure 4. Microstructural Analysis of Unloaded Protein Isolate Gel (GP) and Emulsion Protein Isolate Based Gel EPIG1:4H, EPIG1:4HS, EPIG1:2H and EPIG1:2HS Obtained through Gelation of EO: EPI Emulsion at Various Ratios 1: 4 and 1: 2 (w/w)

Among the fortified samples, an increase in the oil-to-protein ratio (EPIG1:2) was associated with the formation of significantly larger and more irregular pores. This morphological shift aligns with the textural analysis suggesting that higher lipid concentrations modulate the cross-linking density of the proteinaceous network. Conversely, the EPIG1:4 formulation—characterized by a lower oil content—displayed a more compact and less porous architecture. This structural density may be attributed to a higher interfacial protein concentration relative to the lipid phase, facilitating a more uniform droplet distribution and a more robust continuous phase.

These findings are in accordance with the findings of Li *et al.* (2023), who reported that increasing lipid fraction can lead to droplet aggregation within the gel matrix, thereby altering its microscopic continuity. Furthermore, the application of combined homogenization and ultrasonication (HS) treatment appeared to enhance the interfacial affinity between the EPI and EO. This synergistic processing approach effectively reduced the porosity in the EPIG1:2HS samples compared to those subjected to homogenization alone (EPIG1:2H) (Anvari & Joyner, 2017).

3 Conclusions

The present study evaluated the efficacy of EPI as a novel biopolymer for the fabrication of emulsion-based gels fortified with EO. The results demonstrate that a 4% (w/v) EPI solution solubilized in acetic acid possesses a high gelation point, forming a thermo-irreversible, stable matrix upon controlled thermal treatment.

The resulting EO:EPI emulsions exhibited exceptional physical stability, as evidenced by high zeta-potential values and resistance to creaming. Detailed characterization via FTIR and thermogravimetric analysis confirmed the successful and efficient loading of the bioactive eel oil within the gelled protein architecture. Textural and microstructural assessments revealed that while the incorporation of EO introduces a degree of porosity, it also enhances the elastic properties of the matrix, particularly when processed using combined homogenization and ultrasonication.

In conclusion, EPI-based emulsion gels represent a promising and functional delivery system for lipid-based bioactive. These findings provide a robust foundation for the development of high-protein, nutrient-fortified food products and offer a versatile template for future applications in food science and nutraceutical encapsulation.

Source of funding: Financial assistance (equipment) for this study was provided by the Ministry of Higher Education and Scientific Research, Tunisia (grant no.18PJEC08-01).

Previous submissions: The manuscript was not presented as part at a meeting previously.

Authors' Contribution: Taktak W.: Conceptualization, Data curation, Writing Original draft preparation; **Affes S. and Hamdi M.**: Investigation and Methodology; **Li S.**: Conceptualization, Funding acquisition; **Nasri M., Nasri R., and Karra-Chaabouni M.**: Editing, Manuscript review and Validation.

Conflicts of Interest: The authors report no declarations of interest.

Preprint deposit: No

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