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**Physicochemical, textural, rheological and microstructural properties of protein isolate gels produced from European eel (*Anguilla anguilla*) by heat-induced gelation process.**

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**ABSTRACT**

This study investigates the preparation and characterization of a protein isolate extracted from European eel (*Anguilla anguilla*) and its ability to generate protein isolate gel (EPIG). European eel protein isolate (EPI) was extracted from European eel muscle by a pH-shifting process. EPIGs were prepared by heat treatment of EPI with varying gelling parameters. The influence of pH value (2 and 10), EPI concentration (7 and 10%) and ionic strength on biochemical, textural, rheological and microstructural properties of EPIGs was studied. Textural and physicochemical analysis demonstrated a different gel forming mechanism for acidic and alkaline-treated gels. Results showed that whiteness of EPIGs increased with decreasing of EPI concentration and pH values. Moreover, EPIGs prepared under alkaline conditions were characterized by the lower soluble peptide level, higher water holding capacity and total pigment content. In addition, the acid EPIGs exhibited poorer gelling ability compared to alkali EPIGs. Thus, a more compacted gel-protein arrangement was formed under alkaline conditions. Furthermore, addition of salt slightly improved the elasticity of EPIGs at 2% wt. concentration. FT-IR analysis of EPIGs revealed more irregular secondary structure and higher hydration level in the acid EPIGs compared to alkaline EPIGs. Thus, this study shows the potential of protein isolate gels from *A. anguilla* as food texture enhancers.

**Keywords:** pH-shift process, Gelation, Protein isolate gel, Textural and rheological properties, Microstructure.

## 1. Introduction

Recently, several texture modifiers are used in food products, mainly based on globular proteins such as myofibrillar proteins extracted from meat and fish. Proteins constitute an interesting source of nutrients for humans and could be applied for the improvement of food products. In the fish industry, large quantities of proteins are generated by raw fish processing.

Fish protein isolate (FPI) can be extracted by solubilizing proteins at extreme pH values to separate soluble proteins, bone, skin, connective tissue, cellular membranes, and neutral lipids through a centrifugation step. After solubilization via the alkaline process, proteins are collected and recovered by isoelectric precipitation, giving rise to stable protein isolates (Kristinsson & Liang, 2006). Solubilization and precipitation can induce changes in the three dimensional (3D) structure of myosin and particularly myosin heavy chain (MHC), which have an effect on the functional properties of the proteins recovered and their miscibility during protein isolate production (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011). In addition, following the shifting pH treatment, the unique structure of protein produced is responsible for improved functional properties regarding gelation, emulsification and solubility. The partially unfolded/folded structure of proteins is more flexible, allowing the ability to form better protein networks on heating. Thus, during the pH-shifting process, structural changes (folding phenomena) cause an increase of surface hydrophobicity and reactive-SH groups in the produced proteins which may contribute to their technologically useful functionalities (Gehring et al., 2011; Nolsøe & Undeland, 2009).

Many studies focused on protein gelation in food, due to its interesting textural properties and its capacity to entrap molecules of interest into gel networks (Yang, Wang, Vasanthan, & Chen, 2014). Particularly, fish protein gels are complex and their properties as well as the mechanism of their formation have been the subject of several research works. Properties of protein gels are determined based on the type and number of protein-protein interactions, aggregation and arrangement of unfolded proteins, which are affected by the pH, ionic strength, protein concentration, heating and cooling rates (Xiong & Blacnchard, 1997). Gel formation requires structural and functional changes of the constituent myofibrillar proteins. In addition, the rheological and biochemical characteristics of the gel can be affected either by intrinsic factors (species, freshness, and degree of stress before harvest) or extrinsic factors (protein concentration, pH, ionic strength, and temperature) (Yin & Park, 2014). One

of the best methods to follow changes in physical characteristics related to molecular properties of a gel, is the heating and cooling cycle.

The aim of this study was to produce *A. anguilla* muscle protein isolate gels by applying a heat treatment. The effects of pH, protein isolate concentration, and NaCl addition on chemical, textural, rheological and microstructural properties of protein isolate gels, were evaluated.

## **2. Materials and methods**

### **2.1. Fish sample**

*A. anguilla* was purchased from the fish market of Sfax City, Tunisia. Samples were packed in polyethylene bags, placed in ice and then transported to the research laboratory within 30 min. Muscle was separated from viscera, rinsed with distilled water to remove salts and other contaminants and stored at -20 °C.

### **2.2. Preparation of European eel protein isolate**

The alkaline solubilization process was used to prepare EPI as described by (Chaijan, Panpipat & Benjakul, 2010). Fish mince was homogenized with cold water (4 °C) at a ratio of 1:9 (w/v). The homogenate was adjusted to pH 11.5 with 2 N NaOH solution, gently stirred for 60 min and then centrifuged at 9,500 g for 20 min at 4 °C. The soluble proteins in the supernatant were precipitated at their nominal isoelectric point (pH 5.5) using 2 N HCl. The precipitate was collected by centrifugation (9,500 g for 20 min) and then resuspended in distilled water, followed by pH adjustment to 7.0 using 2 N NaOH solution. European eel protein isolate (EPI) was freeze-dried using freeze-dryer lab (Moduloyd-230, Thermo Fisher Scientific, USA) at a temperature of -50 °C and a pressure of about 121 mbar.

### **2.3. Preparation of European eel protein isolate gels**

To prepare the gels, thermal treatment is required to induce changes in physicochemical properties of proteins. EPI solutions, with different protein concentrations (7 and 10% w/v), were prepared in distilled water. Then, the pH of the mixtures was adjusted to the target values (2.0 or 10.0) using 2 N NaOH or 2 N HCl solutions. Thereafter, samples, were poured into vials (2.5 × 5 cm, diameter × height) with a sample height of approximately 15 mm, heated at 90 °C for 10 min and then immediately cooled in ice. The gelling solutions were kept at 4 °C for 24 h to induce gel maturation (Chaijan et al., 2010). Gels obtained with 7% EPI at pH 2.0 and 10.0 were named, respectively, EPIG7-p2 and EPIG7-p10, whereas, EPIG10-p2 and EPIG10-p10 were attributed to gels obtained with 10% EPI prepared at pH 2.0 and 10.0, respectively.

To study the effect of salt on EPIG properties, different concentrations of NaCl (2, 3 and 5% w/w) were incorporated into the EPI solutions before the heat treatment.

## **2.4. Chemical analysis**

The moisture and ash contents in protein isolate were determined according to the AOAC standard methods 930.15 and 942.05, respectively (AOAC, 2000). Total nitrogen content was determined using the Kjeldahl method according to the AOAC method number 984.13 (AOAC, 2000). Crude protein was estimated by multiplying total nitrogen content by the factor of 6.25. Fat content was determined gravimetrically after Soxhlet extraction of dried samples with hexane for 2 h using Nahita Model 655, Navarra. All measurements were performed in duplicate and results are the average of two values.

## **2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of EPI**

Protein pattern of EPI was analyzed by SDS-PAGE according to the method of Laemmli (1970). 50 µg of protein sample were loaded onto the SDS gel and subjected to electrophoresis at 100V. Molecular weights were determined using a molecular weight marker (10–175 kDa).

## **2.6. Solubility of European eel protein isolate**

Solubility of protein isolate from *A. anguilla* was investigated at pH 2.0 and 10.0. Briefly, 200 mg of protein sample were suspended in 20 ml of distilled water and the pH of the mixtures was adjusted to the desired values with 6 N HCl or 6 N NaOH. The mixtures were stirred for 30 min at room temperature (25 ± 1 °C) and then centrifuged at 9.500 g for 15 min. After appropriate dilution, protein contents in the supernatants were determined according to the Lowry method (1951). Total protein content in the sample was determined by Kjeldahl method (AOAC, 2000). The solubility of samples, defined as the amount of soluble nitrogen from the total nitrogen, was calculated as follows:

$$\text{Nitrogen solubility (\%)} = \frac{\text{Protein content in the supernatant}}{\text{Total protein content in the sample}} \times 100$$

Solubility analysis was carried out in triplicate.

## **2.7. Physico-chemical characterization of EPIGs**

### **2.7.1. Color**

EPIGs were subjected to color evaluation on a Color Flex spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA). The illuminant C was used as the light source for the measurement of lightness (L\*), redness (a\*) and yellowness (b\*) degrees. A white plate was used to standardize the equipment. L\*, a\* and b\* values were determined in

duplicate from three dependent samples and whiteness ( $W^*$ ), differences in color ( $\Delta E^*$ ) and chroma ( $C^*$ ) were calculated using the following equations:

$$W^* = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$\Delta E^* = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2}$$

where  $L_0$ ,  $a_0$ , and  $b_0$  are the differences between the corresponding color parameters of the EPIGs and EPI.

### 2.7.2. Total pigment content

The total pigment content (TPC) was determined as described by Lee, Hendricks, & Cornforth (1999). A gel sample (1 g) was mixed with 9 ml of an acid–acetone mixture (90:8:2, acetone: deionized water: HCl). The mixture was stirred with a glass rod and kept undisturbed for 1 h at room temperature ( $25 \pm 2$  °C). The extract was filtered with a Whatman No. 1 filter paper and its absorbance was read at 640 nm against an acid-acetone tube used as blank. The total pigment content was calculated using the following formula:

$$\text{Total pigment content} = A_{640} \times 680,$$

where  $A_{640}$  is the absorbance of the samples at 640 nm and 680 is the conversion factor of hematin.

### 2.7.3. Moisture release content

Moisture release content (MRC) was measured by the method of Rawdkuen and Benjakul (2008). Briefly, gel samples were cut into small square pieces, weighed, and then placed between filter papers. An initial weight was placed on the top of the sample for 2 min at 4°C, and then the sample was removed from the papers and weighed again. Expressible moisture content was calculated using the following formula:

$$\text{Moisture release content (\%)} = [(X - Y) / X] \times 100,$$

where X and Y are the initial and final weights of the gel sample, respectively.

### 2.7.4. TCA-soluble peptide content

Gel samples (2 g) were homogenized with 5% of cold TCA (18 ml) for 2 min. The mixtures were incubated for 1 h at 4 °C, and then centrifuged at 9.500 g for 10 min. TCA-soluble peptide content in the supernatant was measured by the Lowry method (1951) using L-tyrosine as standard. The results are expressed in micromoles of tyrosine per gram of sample.

### **2.7.5. Fourier Transform Infrared (FTIR) analysis**

FTIR spectra of EPIGs and EPI were determined using a Perkin-Elmer infrared spectrometer (Spectrum 65, France) equipped with an attenuated total reflectance (ATR) accessory with a Zn Se crystal. A number of 32 scans were collected with  $4\text{ cm}^{-1}$  resolution in the wavelength range  $650\text{--}4000\text{ cm}^{-1}$ . Calibration was done using background spectrum recorded from the clean and empty cell at  $25\text{ }^{\circ}\text{C}$ . This analysis aimed to determine the modifications induced at the molecular scale between EPI and EPIGs. For FTIR data treatment, the Spectrum Suite ES software was used.

### **2.7.6. Texture profile analysis**

EPIGs were subjected to a texture profile analysis (TPA) using a Model TA-TX2 texture analyzer (Lloyd Instruments, Ltd., West Sussex, UK). Gel samples ( $15 \times 25\text{ mm}$ , height  $\times$  diameter) were placed between a cylindrical cell and a probe (12 mm in diameter). Then, the samples were compressed to 50% of their original height in a double cycle at a rate of  $30\text{ mm/min}$ . The texture profile parameters, including hardness (N), elasticity (mm), adhesion (N) and cohesiveness were computed from the resulting force-deformation curves (Bourne, Kenny, & Barnard, 1978).

### **2.7.7. Rheological measurements**

Dynamic oscillatory study of EPIGs was determined using Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK). The inner diameter of the cup was  $28.5\text{ mm}$  and the diameter of the bob was  $26.5\text{ mm}$ , giving a gap between the cup and the bob of  $1.0\text{ mm}$ . Heating from  $10\text{ }^{\circ}\text{C}$  to  $80\text{ }^{\circ}\text{C}$  and back to  $10\text{ }^{\circ}\text{C}$  took place at a scan rate of  $01\text{ }^{\circ}\text{C/min}$ , frequency of  $1\text{ Hz}$ , and target strain of  $1\%$ . The elastic modulus ( $G'$ ; Pa) and viscous modulus ( $G''$ ; Pa) were plotted as a function of temperature. Frequency sweep was carried out at  $20\text{ }^{\circ}\text{C}$  by applying oscillation amplitude over the frequency range of  $0.01\text{--}100\text{ Hz}$  and at a fixed strain of  $1\%$ . Finally, the strain amplitude sweep measurements were conducted at a fixed frequency of  $1\text{ Hz}$  and temperature of  $20\text{ }^{\circ}\text{C}$  with strains in the range from  $0.1\%$  to  $1000\%$  to characterize the large deformation rheological properties. The elastic modulus ( $G'$ ; Pa) and viscous modulus ( $G''$ ; Pa) were recorded during the above dynamic rheological measurements.

## **2.8. Scanning electron microscopy**

Microstructures of gels, prepared with  $10\%$  EPI concentration and at pH  $2.0$  and  $10.0$ , were visualized using a scanning electron microscope (SEM, Cambridge Scan-360 microscope) at an accelerating voltage of  $10.0\text{ kV}$ . The samples were frozen under liquid nitrogen. Before visualization, the samples were mounted on brass stub and sputtered with

gold to make it conductive. Samples were photographed at an angle of 90° to the surface to allow the observation of EPIG.

## **2.9. Statistical analysis**

All experiments and measurements were performed at least in triplicate. Data were expressed in mean  $\pm$  SD (standard deviation) and analyzed using SPSS package ver. 17.0 for Windows, professional edition (SPSS Inc., Chicago, USA) using ANOVA analysis. Differences were considered significant at  $p < 0.05$ .

## **3. Results and discussion**

### **3.1. Chemical composition of EPI**

Chemical compositions of fresh muscle and freeze-dried EPI are presented in Table 1. Results show that alkali-aided process followed by centrifugation lead to extract 95.14 % of EPI (based on the dry weight matter) and to eliminate about 91.24 % of lipid content compared to the fresh muscle. In fact, after the alkaline processing, soluble proteins were separated from lipids on the basis of density by centrifugation. Similar findings were reported by Wang, Wang, Chang, Gao, Li & Xue (2015) who found that Krill proteins were effectively recovered from defatted krill powder through an alkaline solubilization and an acidic precipitation. Thus, high amount of protein isolate extracted from *A. anguilla* muscle can be an adequate ingredient for food preparation intended for human consumption.

### **3.2. Solubility of EPI at different pH conditions**

The investigation of the EPI solubility at different pH values, is required to understand the functional properties of proteins including gelation and emulsification (Rawdkuen, Sai-Ut, Khamsorn, Chaijan, & Benjakul, 2009). To this end, the influence of different pH values (from 2.0 to 10.0) on the solubility of EPI was determined and is illustrated in **Fig. 1**. The highest solubility level of EPI was obtained at both alkaline (pH 10.0) and acidic pH levels (pH 2.0) reaching around 95% and 55%, respectively, thus being solubility much higher in alkaline conditions. In addition, a lower solubility was observed around pH 5.5, which is the isoelectric point of European eel protein isolate. These results are in line with those reported by Surasani, Khatkar, & Singh (2017) who found that the maximum solubility of protein isolate from pangas was obtained at extreme pHs, in both acidic and alkaline sides. Indeed, at extreme pH, the number of positive or negative charges is important, promoting the repulsion of protein molecules and their interaction with water molecules. However, at pH near the isoelectric point, the net charge of the protein is zero, which reduces the repulsion of protein molecules by electrostatic forces and promotes their self-aggregation by hydrophobic forces, thus contributing to the precipitation of proteins and their lower water solubility.



### 3.3. SDS-PAGE protein profile of EPI

The protein molecular weight distribution of EPI was qualitatively evaluated by SDS-PAGE and the profile is shown in **Fig. 2**. Typical fish protein bands, including molecular heavy chain proteins ( $> 175$  kDa) and actin bands ( $\sim 45$  kDa), were present in the *A. Anguilla* muscle. Proteins with lower molecular weights than actin were also observed, which may correspond to tropomyosin chains ( $< 45$  kDa). Likewise, bands with a smaller size than myosin may be of the paramyosin type (Romero, Cordobés, Guerrero, & Puppo, 2011). Generally, isolates with a high content of myosin and actin have a tendency to form a viscoelastic network and were found to be largely responsible for consistent gels. The heavy chain of myosin is assumed to be the main subunit involved in gelation. Xiong, & Blacnchard, (1997) revealed in their study that isolated muscle proteins could be used to elucidate gelation mechanism of complex mixtures of myofibrillar proteins.

### 3.4. Physico-chemical and gelling properties of EPIGs

#### 3.4.1. Color and total pigment content

The effects of pH variation, EPI concentration and salt addition on EPIGs color parameters, including whiteness ( $W^*$ ), chroma ( $C^*$ ) and color differences ( $\Delta E^*$ ), were studied. Results summarized in **Table 2** indicate that pH and EPI concentration values induced significant changes in gel color. In fact, whiteness ( $W^*$ ) of all EPIGs increased as the EPI concentrations increased. For acidic formed gels, whiteness ( $W^*$ ) significantly increased from 70.22 for EPIG7-p2 to 73.51 for EPIG10-p2 ( $p<0.05$ ). In addition, the acidic-treated gels prepared with 7% and 10% of EPI concentrations (70.22 and 73.51, respectively) exhibited higher  $W^*$  ( $p<0.05$ ) than those obtained with EPIG7-p10 and EPIG10-p10 (63.76 and 64.57, respectively).

In addition, the effect of salt concentration on  $W^*$  was further investigated (Table 2). Results revealed that the addition of NaCl significantly affected the color of the obtained gels ( $p<0.05$ ). Moreover,  $\Delta E^*$  values were inversely proportional to whiteness. Indeed,  $\Delta E^*$  levels of EPIG7-p2 and EPIG10-p2 without added salt (1.23 and 4.75, respectively) were lower than those of alkaline gels prepared with the same concentrations (7.99 and 7.86, respectively).

Moreover, the estimation of total pigment content (TPC) of EPIGs showed that TPC was higher for alkaline-treated EPIGs than for those prepared under acidic conditions (**Table 2**). In fact, TPC significantly ( $p<0.05$ ) increased from 23.12 mg / 100 g gel for EPIG7-p2 to 35.36 mg/100 g gel for EPIG7-p10, respectively, which can be correlated to the results of EPIGs whiteness. In contrast, no correlation was found between salt concentrations and TPC values.

### 3.4.2. Moisture release content

The moisture release content (MRC) of EPIGs is illustrated in Table 2. Regardless salt concentration, the MRC decreased with the increase of pH and EPI concentration values. Data revealed as well that alkaline-treated gels (EPIG7-p10 and EPIG10-p10), prepared with or without the addition of NaCl, had significantly lower MRC (ranging from 9.88 to 13.55 g/100 g gel) than that obtained with acid gels, where MRC varied from 14.93 to 19.89 g/100 g gel. This is in line with previous works from Kristinsson, & Liang (2006) and Rawdkuen et al. (2009) who found lower expressible moisture contents of 10.51% and 6.51% for Atlantic croaker and tilapia protein gels obtained in alkaline conditions, respectively. In contrast, Abdelhedi et al. (2016) found that smooth hound fish gel prepared under alkaline conditions (pH 9.0) had higher MRC than that obtained with acid gel.

In addition, EPI concentration is a crucial parameter which influences the moisture content in EPIG. In fact, MRC decreased from 13.55 g/100 g gel for EPIG7-p10 to 9.88 g/100 g gel for EPIG10-p10. In general, different expressible moisture indicates differences in the water holding capacity of the gel network in correlation with increasing breaking force. As a consequence, alkaline-aided gels, prepared with 10% EPI concentration, were characterized by the highest WHC and probably the hardest protein gel network.

Furthermore, the addition of 2% salt to acid gel (EPIG7-p2) decreased the MRC from 19.89 to 16.20 g/100 g gel ( $p < 0.05$ ). In alkaline conditions, NaCl addition slightly affected the MRC levels which decreased from 13.55 g/100 g gel for EPIG7-p10 (without salt incorporation) to 12.24 g/100 g gel for EPIG7-p10 prepared at a concentration of 2% NaCl. The decrease in MRC could be explained by the increase in water retention of gels with 2% NaCl by solubilizing myofibrillar proteins (Xiong, & Blanchard 1997).

### 3.4.3. TCA-soluble peptide content

TCA-soluble peptide content of gels could be a sign of proteolysis caused by enzymatic hydrolysis (autolysis) or acid hydrolysis (Abdollahi, Razaei, Jafarpour, & Undeland, 2017). The amount of TCA-soluble peptides was determined in acidic and alkaline-treated gels and the results are presented in **Table 3**. Gels prepared at pH 2.0 (EPIG7-p2 and EPIG10-p2) exhibited significantly ( $p < 0.05$ ) greater TCA-soluble peptide contents (2.06 and 2.72  $\mu\text{mol tyr/g gel}$ , respectively) than gels treated at pH 10.0 (EPIG7-p10 and EPIG10-p10), where TCA-soluble peptide contents were 1.83 and 1.62  $\mu\text{mol tyr/g gel}$ , respectively. This is in line with the study of Abdollahi et al. (2017) who reported that gels made from acid-produced isolates had the highest amount ( $p < 0.05$ ) of TCA-soluble peptides,

which might be related to the cleavage of some peptide bonds during the acidic treatments resulting in an increase in the soluble peptide content.

Additionally, TCA-soluble peptide contents of gels were found to be EPI concentration dependent. In fact, in acid EPIGs, the gel prepared with 7% EPI exhibited lower TCA-soluble peptide content (2.06  $\mu\text{mol tyr/g gel}$ ) than gel obtained with 10% of proteins (2.72  $\mu\text{mol tyr/g gel}$ ). However, TCA-soluble peptide contents in alkaline-formed gels (PIG7-p10 and EPIG10-p10) decreased from 1.83 to 1.62  $\mu\text{mol tyr/g gel}$  with increasing of EPI concentration.

The evaluation of the effect of NaCl concentration on peptides level revealed that adding 2% NaCl to the acidic gel preparation decreased the TCA peptide content to 1.21 and 1.60  $\mu\text{mol tyr/g gel}$  for EPIG7-p2 and EPIG10-p2, respectively ( $p < 0.05$ ). Beyond this concentration of salt, TCA-soluble peptide levels increased in both alkali and acid gels. These results could be explained by the solubilization of the protein chains up to a certain amount of salt (in the 2% wt.). Nevertheless, higher NaCl concentration led to a decrease in the quality of the gel, being thus more susceptible to protein hydrolysis.

### 3.5. Fourier transform infrared analysis of EPIGs

Fourier transform infrared (FT-IR) spectra of protein isolate and gels prepared using the different treatments are shown in **Fig. 3**. Distinctive absorption bands are observed at 3273  $\text{cm}^{-1}$  (Amide A, N-H or O-H), 2935–2962  $\text{cm}^{-1}$  (amide B), 1643  $\text{cm}^{-1}$  (Amide I, C=O and C=N), 1533  $\text{cm}^{-1}$  (Amide II, C-N and N-H), 1398  $\text{cm}^{-1}$  (C-H bending), and 1236  $\text{cm}^{-1}$  (Amide III) (Chen et al., 2014) in all protein samples (powder and gels). Among these peaks, the band between 3000 and 3500  $\text{cm}^{-1}$  related to Amide A and free hydroxyl groups can be useful to evaluate the interactions of the protein molecules with water (Perisic, Afseth, Ofstad, & Kohler, 2011). In fact, EPIGs prepared under alkaline conditions exhibited a marked reduction of the amide A intensity, compared to that detected in EPIGs prepared at pH 2.0, which might be due to the increase of hydrogen bonding interactions between molecules in the protein matrix, leading to a greater degree of hydration in acidic conditions. This result was found to be correlated with the creamy appearance of the acid gel compared to the alkali gel showing a hard and elastic structure.

Moreover, changes in the amide I band between 1600 and 1700  $\text{cm}^{-1}$  related to the secondary structure of proteins (Carbonaro & Nucara, 2010) related to mixed contributions from amide carbonyl C=O stretching vibrations, with smaller contributions of C–N stretching and N–H bending vibrations were observed upon gelling. Referring to the studies of Ojagh, Nunez-Flores, Lopez-Caballero, Montero, & Gomez-Guillen (2011), spectral changes, in the

amide I region, are associated with conformational changes, particularly in the secondary structure. Thus, the decrease in peak intensity in EPIG7-p2 might reflect the association of peptide fragments and indicated the loss of molecular order of intermolecular cross-links of protein, contrary to the alkaline prepared gel that showed a well-organized structure. Furthermore, in the acid-produced protein isolate gels, the Amide II band shifted to lower wavenumber ( $1533\text{ cm}^{-1}$ ) compared with alkali-EPIG ( $1535\text{ cm}^{-1}$ ). This might imply an increase in aggregation of  $\beta$  sheet structure of myosin in acid-produced protein isolate gel (Carbonaro & Nucara, 2010).

The amide III vibration mode has been mainly assigned to C-N stretching, N-H bending, and C-C stretching. Likewise, the amide III band centered at  $1260\text{--}1350\text{ cm}^{-1}$  can be correlated with  $\alpha$ -helix content. The values were slightly lower at 5% salt, since a random coil configuration may be promoted at high salt concentrations (Herrero, 2008). However, NaCl addition in EPIGs did not affect in general FTIR spectra of the proteins neither in alkaline nor in acid conditions.

### 3.6. Textural characteristics of EPIGs

Regarding the textural properties of the different gels (hardness, elasticity, adhesion and cohesion), results showed that textural parameters of EPIGs depended on pH and EPI concentration levels (Table 4). At 7% EPI concentration, elasticity of EPIGs significantly increased with pH increase, from 5.5 mm for EPIG7-p2 to 7.69 mm for EPIG7-p10 ( $p>0.05$ ). Similarly, at 10% EPI concentration, the elasticity of EPIGs increased ( $p<0.05$ ) from 5.53 for EPIG10-p2 to 9.26 mm for EPIG10-p10. Abdollahi, et al. (2017) reported that the elasticity of a muscle protein gel is mainly related to the contribution of myofibrillar proteins, especially actomyosin. Moreover, results showed that higher adhesion values were observed with gels treated under alkaline conditions (0.75 N and 1.59 N for EPIG7-p10 and EPIG10-p10, respectively) than those obtained at acidic pH (0.33 N and 1.21 N for EPIG7-p2 and EPIG10-p10, respectively). Thus, gels obtained in alkaline conditions were harder than acid gels, which were more fragile and having a soft texture. This could be explained by increased protein-protein interactions in the case of alkaline-obtained gels, related to harder and more elastic gels. However, the presence of too much water in the acid-obtained gels may hamper these inter- and intramolecular protein interactions, giving rise to soft and fragile gels as previously observed (Cortes-Ruiz, Pacheco-Aguilar, Garcia-Sanches, & Lugo-Sanches, 2001). In addition, greater adhesion of EPIGs was observed with increasing EPI concentration (from 0.75 N for EPIG 7-p10 to 1.59 N for EPIG10-p10).

The effect of NaCl on the textural properties of EPIGs was as well investigated (Table 4), and results revealed that NaCl addition decreased the hardness of EPIGs, except for the gel prepared with 10% of EPI at pH 10.0, where no change in hardness value was detected. Furthermore, the elasticity of EPIGs was slightly improved with salt addition. In fact, the elasticity of EPIG7-P2 increased upon addition of 5% wt. NaCl from 5.50 mm to 7.65 mm. These results could be explained by the formation of salt bridges and ionic interactions, which play a major role in cross-linking of proteins.

### 3.7. Viscoelastic properties

Rheological analysis can be used to determine the viscoelastic properties of gels. The rheological properties of EPIG10-p2 and EPIG10-p10 were studied as a function of temperature, frequency and strain. The changes in dynamic viscoelastic properties of gel samples as a function of temperature sweep are reflected by monitoring the storage modulus  $G'$  (elastic response) and loss modulus  $G''$  (viscous behavior) of the gelling system (**Fig. 1 (a)**). At all temperatures (from 10 to 80°C), the  $G'$  values were higher than  $G''$  values, indicating that both gels, EPIG10-p2 and EPIG10-p10, manifested gel-like behavior. In addition, elastic  $G'$  modulus of alkaline-treated gel was markedly higher than that of acid gel, by more than 5 fold, suggesting that EPIG10-10 can be considered as a strong gel (**Fig. 4 (b)**). This result is in agreement with those obtained previously in textural analysis, which again confirmed that EPIG10-10 was more resistant and elastic than the acidic-treated gel. Yang et al. (2014) found that the magnitude of  $G'$  increased as with increasing the solution pH and upon cooling. In fact, increasing the pH induces conformational changes on the protein structure, promoting more protein–protein interactions via hydrophobic associations and disulfide bridging.

Furthermore, frequency sweeps at a constant strain of 1% and a constant temperature of 10°C were performed to explore the viscoelastic behavior of European eel protein gels. As illustrated in **Fig. 4 (b)**, the mechanical spectra obtained of both gels revealed a gel-like behavior where elastic modulus ( $G'$ ) was remarkably greater than the loss modulus ( $G''$ ). Likewise, for acidic treated gel, the  $G'$  and  $G''$  moduli obtained from EPIG10-p2 manifested nearly a constant value in the range of frequency from 0.01 to 100 Hz, proving the stability of the cross-linked protein network. However, the elasticity of EPIG10-p10 gel ( $G'$ ) increased with the increase in frequency indicating that a higher gel strength for alkaline gel was obtained at higher frequency.

The viscoelastic modulus changes during strain sweep for EPIG10-p2 and EPIG10-p10 were also investigated and results are shown in **Fig. 4(c)**. In the linear viscoelastic region,

the loss and storage moduli were independent of strain with  $G'$  being higher than  $G''$ , suggesting a typical viscoelastic gel behavior. Beyond a critical strain, the increase of strain resulted in macroscopic gel fracture with a dramatic drop of  $G'$  values. In fact, at a cross-over point, the levels of  $G''$  became higher than those of  $G'$ , revealing a transition from gel to viscous liquid samples (**Fig. 4 (c)**). Therefore, the critical strain obtained with EPIG10-p2 was lower (410%) compared to that obtained with alkaline-treated (750%). The obtained result indicate that the alkaline treatment reinforces the protein-protein interactions and the proteins network formed in this gel are stronger and require a greater strain to disrupt it. This result is in line with the study of Kim et al. (2016) who found that the gel-protein arrangement is thought to have a highly compacted structure under alkaline conditions.

### 3.8. Morphology and microstructure of protein isolate gels

Morphological and microstructural investigation of EPIGs is crucial in the determination of its potential for biomedical or food engineering applications. The gel microstructures significantly differed and can be correlated with the previous physico-chemical characterization. Indeed, the EPIGs prepared at acid pH displayed a rougher surface, with scattered spherical protein aggregates, thus giving rise to a less consistent gels as previously observed from the texture analysis (**Fig. 5 (a)**). Conversely, the alkaline-formed gel (**Fig.5 (b)**) showed a more compact and uniform structure with smoother surface microstructure, which can be related to better gel characteristic, mainly higher elasticity and gel strength. Therefore, the observed homogenous and smooth structure of alkaline EPIG suggested that the alkaline treatment of protein isolate could remove interfering components for gelation. The improved protein network formed in alkaline EPIG, leading to a better gel microstructure, is additionally related to the better water holding capacity in this gel, as described by Li & Xia (2010). As a result, a fine, homogenous and organized protein gel microstructure would result in more absorptive capacity and better retention of water compared to a coarse gel structure with large pores. These findings could explain the difference in texture between the two obtained gels. Moreover, alkaline EPIG was more consistent and harder, whereas the acid gel showed a friable structure exhibiting a low resistance when applying a breaking force. Depending on the intended final application whether in the food or in the medical field, both acid and alkali EPIGs could be interesting and provide different textural properties.

### Conclusion

This work showed that protein isolate extracted from *A. anguilla* muscle by alkaline solubilization process can be a source of protein gels with good functional, textural and

microstructural properties. pH variation and concentration of protein isolate were found to significantly affect the properties of the produced protein isolate gels. In fact, gels prepared under the alkaline conditions and with high protein concentrations displayed better rheological and textural properties, the lowest peptide soluble content and the greatest water holding capacity. In contrast, the acidic conditions generated protein networks with higher water loss level and amount of short peptides. In conclusion, the protein isolate extracted from *A. anguilla* was able to produce hard gels without any additive and thereby might be a new gelling, texturing, and/or thickening ingredient of interest in both food and medicine applications.

#### Acknowledgements

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**Table1:** Chemical composition (% , in dry matter) of *A. anguilla* muscle and its protein isolate.

	Protein	Ash	Lipid
Muscle	46.56 ± 1.25 <sup>b</sup>	3.47 ± 0.26 <sup>a</sup>	42.94 ± 0.79 <sup>a</sup>
EPI	95.14 ± 0.86 <sup>a</sup>	2.22 ± 0.38 <sup>b</sup>	3.76 ± 0.50 <sup>b</sup>

Data are given in mean ± SD. <sup>a-b</sup> different letters in the same column indicate significant differences (p<0.05). API: *A. anguilla* protein isolate.

**Table 2:** Effect of pH value, EPI and NaCl concentration on color analysis, total pigment content (TPC) and water release content (WRC) of European eel protein gels (EPIGs).

Gel samples	NaCl concentration (%)	ΔE	W*	C*	TPC (mg/100 g gel)	WRC (g/100 g gel)
EPIG7-p2	0	1.23 ± 0.05 <sup>cC</sup>	70.22 ± 0.04 <sup>aB</sup>	19.76 ± 0.07 <sup>bB</sup>	23.12 ± 0.96 <sup>aC</sup>	19.89 ± 0.69 <sup>aA</sup>
	2	3.57 ± 0.11 <sup>a</sup>	67.53 ± 0.04 <sup>b</sup>	21.07 ± 0.01 <sup>a</sup>	22.44 ± 1.92 <sup>a</sup>	16.20 ± 0.17 <sup>b</sup>
	3	1.56 ± 0.08 <sup>b</sup>	68.64 ± 0.01 <sup>b</sup>	19.60 ± 0.11 <sup>b</sup>	24.14 ± 0.48 <sup>a</sup>	16.80 ± 0.90 <sup>b</sup>
	5	1.60 ± 0.16 <sup>b</sup>	68.42 ± 0.05 <sup>b</sup>	19.59 ± 0.10 <sup>b</sup>	24.48 ± 0.96 <sup>a</sup>	19.60 ± 1.55 <sup>a</sup>
EPIG7-p10	0	7.99 ± 0.09 <sup>aA</sup>	63.76 ± 0.03 <sup>cC</sup>	21.14 ± 0.52 <sup>aA</sup>	35.36 ± 0.96 <sup>aB</sup>	13.55 ± 0.50 <sup>aB</sup>
	2	7.35 ± 0.01 <sup>b</sup>	64.18 ± 0.19 <sup>b</sup>	21.85 ± 1.02 <sup>a</sup>	36.38 ± 1.44 <sup>a</sup>	12.24 ± 0.33 <sup>b</sup>
	3	5.54 ± 0.16 <sup>c</sup>	66.21 ± 0.12 <sup>a</sup>	20.40 ± 0.02 <sup>a</sup>	33.66 ± 1.44 <sup>ab</sup>	12.95 ± 0.18 <sup>a,b</sup>
	5	7.39 ± 0.09 <sup>b</sup>	64.05 ± 0.12 <sup>b</sup>	22.00 ± 0.72 <sup>a</sup>	31.96 ± 1.92 <sup>b</sup>	13.08 ± 0.26 <sup>a</sup>
EPIG10-p2	0	4.75 ± 0.02 <sup>aB</sup>	73.51 ± 0.01 <sup>aA</sup>	13.84 ± 0.02 <sup>cD</sup>	24.48 ± 0.96 <sup>cC</sup>	14.93 ± 1.29 <sup>bB</sup>
	2	3.71 ± 0.01 <sup>b</sup>	67.90 ± 0.01 <sup>d</sup>	19.15 ± 0.03 <sup>a</sup>	23.80 ± 1.92 <sup>c</sup>	13.05 ± 0.25 <sup>c</sup>
	3	2.38 ± 0.10 <sup>c</sup>	71.82 ± 0.02 <sup>c</sup>	16.28 ± 2.28 <sup>b</sup>	26.18 ± 0.48 <sup>b</sup>	15.51 ± 1.11 <sup>b</sup>
	5	4.77 ± 0.21 <sup>a</sup>	72.74 ± 0.02 <sup>b</sup>	13.92 ± 0.21 <sup>c</sup>	28.22 ± 0.48 <sup>a</sup>	16.80 ± 0.05 <sup>a</sup>
EPIG10-p10	0	7.86 ± 0.04 <sup>aA</sup>	64.57 ± 0.02 <sup>dD</sup>	18.99 ± 0.04 <sup>b,c,C</sup>	41.14 ± 0.48 <sup>cA</sup>	9.88 ± 0.09 <sup>a,b,C</sup>
	2	5.87 ± 0.03 <sup>c</sup>	66.29 ± 0.04 <sup>a</sup>	18.90 ± 0.85 <sup>b</sup>	46.24 ± 1.92 <sup>a</sup>	10.37 ± 0.26 <sup>a</sup>
	3	6.77 ± 0.02 <sup>b</sup>	64.88 ± 0.02 <sup>c</sup>	20.40 ± 0.04 <sup>a</sup>	44.88 ± 0.96 <sup>a</sup>	10.66 ± 0.37 <sup>a</sup>
	5	6.80 ± 0.06 <sup>b</sup>	65.24 ± 0.04 <sup>b</sup>	19.41 ± 0.01 <sup>b</sup>	41.82 ± 0.48 <sup>c</sup>	9.34 ± 0.10 <sup>c</sup>

EPI represents protein isolate extracted from European eel muscle. EPIG7-p2 and EPIG7-p10 represent gels prepared with 7% EPI and treated at pH 2.0 and 10.0, respectively. EPIG10-p2 and EPIG10-p10 were gels prepared with 10% EPI and treated at pH 2.0 and 10.0, respectively. Data are given in mean ± SD. <sup>a-d</sup> different letters in the same gel sample with different concentrations of salt indicate significant differences (p<0.05). <sup>A-D</sup> different capital letters in the same column indicate significant differences between gels prepared without salt addition (p<0.05).

**Table 3:** TCA-soluble peptides content in European eel protein gels (EPIGs).

	NaCl	TCA-soluble peptides	
	concentration (%)	( $\mu\text{mol tyr/g gel}$ )	
<b>EPIG7-p2</b>	0	$2.06 \pm 0.19^{\text{bB}}$	545
	2	$1.21 \pm 0.08^{\text{c}}$	546
	3	$2.25 \pm 0.15^{\text{a}}$	547
	5	$2.33 \pm 0.04^{\text{a}}$	
<b>EPIG7-p10</b>	0	$2.72 \pm 0.10^{\text{aA}}$	548
	2	$1.60 \pm 0.09^{\text{b}}$	549
	3	$2.56 \pm 0.22^{\text{a}}$	550
	5	$2.69 \pm 0.06^{\text{a}}$	
<b>EPIG10-p2</b>	0	$1.83 \pm 0.08^{\text{cC}}$	551
	2	$2.17 \pm 0.13^{\text{b}}$	552
	3	$2.60 \pm 0.15^{\text{a}}$	553
	5	$2.45 \pm 0.07^{\text{a}}$	
<b>EPIG10-p10</b>	0	$1.62 \pm 0.05^{\text{dD}}$	554
	2	$1.95 \pm 0.06^{\text{c}}$	555
	3	$2.50 \pm 0.07^{\text{b}}$	556
	5	$2.26 \pm 0.13^{\text{a}}$	
			557

EPI represents protein isolate extracted from European eel muscle. EPIG7-p2 and EPIG7-p10 represent gels prepared with 7% EPI and treated at pH 2.0 and 10.0, respectively. EPIG10-p2 and EPIG10-p10 were gels prepared with 10% EPI and treated at pH 2.0 and 10.0, respectively. Data are given in mean  $\pm$  SD. <sup>a-d</sup> different letters in the same gel sample with different concentrations of salt indicate significant differences ( $p < 0.05$ ). <sup>A-D</sup> different capital letters in the same column indicate significant differences between gels prepared without salt addition ( $p < 0.05$ ).

**Table 4:** Textural properties (hardness, adhesion, elasticity and cohesiveness) of European eel protein gels (EPIGs).

	NaCl concentration (%)	Hardness (N)	Cohesion	Elasticity (mm)	Adhesion (N)
<b>PIG7-p2</b>	0	0.82 ± 0.27 <sup>aB</sup>	0.37 ± 0.21 <sup>aB</sup>	5.50 ± 3.43 <sup>aB</sup>	0.33 ± 0.28 <sup>aB</sup>
	2	0.58 ± 0.01 <sup>a,b</sup>	0.35 ± 0.14 <sup>a</sup>	6.54 ± 2.19 <sup>a</sup>	0.20 ± 0.08 <sup>a</sup>
	3	0.56 ± 0.17 <sup>a</sup>	0.40 ± 0.22 <sup>a</sup>	5.63 ± 2.48 <sup>a</sup>	0.21 ± 0.05 <sup>a</sup>
	5	0.54 ± 0.01 <sup>a,b</sup>	0.50 ± 0.10 <sup>a</sup>	7.65 ± 2.22 <sup>a</sup>	0.27 ± 0.05 <sup>a</sup>
<b>PIG7-p10</b>	0	2.41 ± 1.99 <sup>aAB</sup>	0.34 ± 0.07 <sup>aB</sup>	7.69 ± 0.64 <sup>aA</sup>	0.75 ± 0.50 <sup>bA,B</sup>
	2	2.70 ± 1.91 <sup>a</sup>	0.38 ± 0.08 <sup>a</sup>	7.95 ± 0.42 <sup>a</sup>	1.11 ± 0.94 <sup>a,b</sup>
	3	2.60 ± 0.07 <sup>a</sup>	0.41 ± 0.04 <sup>a</sup>	7.35 ± 0.43 <sup>a</sup>	1.05 ± 0.07 <sup>a</sup>
	5	1.10 ± 0.12 <sup>b</sup>	0.30 ± 0.10 <sup>a</sup>	4.22 ± 1.66 <sup>b</sup>	0.32 ± 0.08 <sup>b</sup>
<b>PIG10-p2</b>	0	3.48 ± 0.77 <sup>aA</sup>	0.33 ± 0.19 <sup>aB</sup>	5.53 ± 2.81 <sup>a,bB</sup>	1.21 ± 0.92 <sup>aA</sup>
	2	0.99 ± 0.07 <sup>b</sup>	0.31 ± 0.21 <sup>a</sup>	4.43 ± 2.48 <sup>b</sup>	0.31 ± 0.23 <sup>b</sup>
	3	0.85 ± 0.14 <sup>b</sup>	0.41 ± 0.04 <sup>a</sup>	5.88 ± 1.04 <sup>b</sup>	0.35 ± 0.02 <sup>b</sup>
	5	3.72 ± 1.85 <sup>a</sup>	0.48 ± 0.12 <sup>a</sup>	7.76 ± 0.62 <sup>a</sup>	1.66 ± 0.43 <sup>a</sup>
<b>PIG10-p10</b>	0	2.61 ± 0.03 <sup>bA</sup>	0.61 ± 0.02 <sup>aA</sup>	9.26 ± 0.55 <sup>aA</sup>	1.59 ± 0.03 <sup>bA</sup>
	2	3.07 ± 0.01 <sup>a</sup>	0.55 ± 0.02 <sup>b</sup>	8.83 ± 0.33 <sup>a</sup>	1.70 ± 0.04 <sup>a</sup>
	3	2.93 ± 0.28 <sup>a</sup>	0.51 ± 0.01 <sup>b</sup>	8.91 ± 1.02 <sup>a</sup>	1.49 ± 0.13 <sup>b</sup>
	5	2.93 ± 0.28 <sup>a</sup>	0.51 ± 0.01 <sup>b</sup>	8.91 ± 1.02 <sup>a</sup>	1.49 ± 0.13 <sup>b</sup>

EPI represents protein isolate extracted from European eel muscle. EPIG7-p2 and EPIG7-p10 represent gels prepared with 7% EPI and treated at pH 2.0 and 10.0, respectively. EPIG10-p2 and EPIG10-p10 were gels prepared with 10% EPI and treated at pH 2.0 and 10.0, respectively. Data are given in mean ± SD. <sup>a-d</sup> different letters in the same gel sample with different concentrations of salt indicate significant differences (p<0.05). <sup>A-D</sup> different capital letters in the same column indicate significant differences between gels prepared without salt addition (p<0.05).

**Figure captions:**

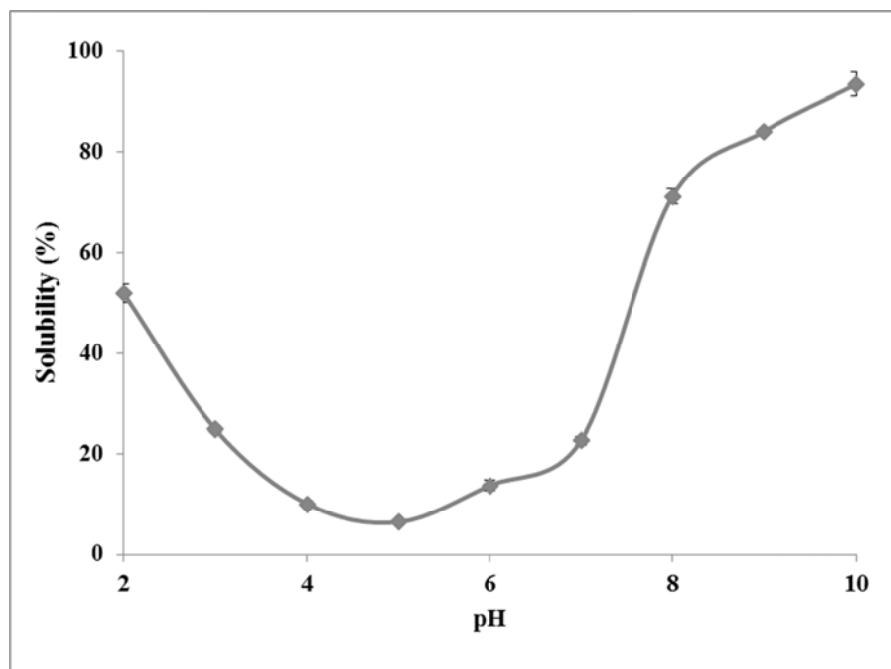
**Fig. 1.** Effect of pH on the solubility of European eel protein isolate (EPI).

**Fig. 2.** SDS-PAGE protein pattern of European eel protein isolate (EPI). Line 1: Standard proteins, L2: EPI.

**Fig. 3.** Fourier transform infrared (FT-IR) spectrum in the ranges of 650 - 4000  $\text{cm}^{-1}$  of European eel protein isolate (EPI) and European eel protein gels (EPIGs).

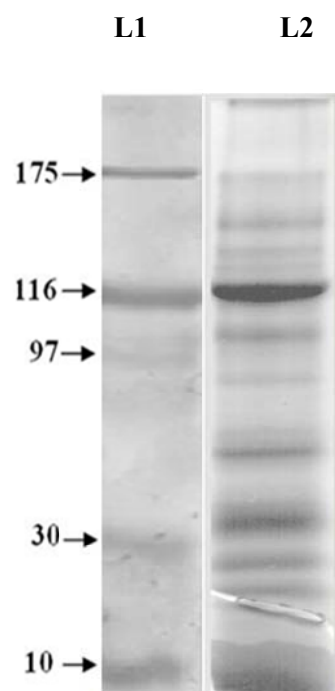
**Fig. 4.** Elastic modulus ( $G'$ : solid symbols) and loss modulus ( $G''$ : open symbols) of EPIG10 p2 and EPIG10 p10 as a function of temperature (a), frequency (b) and strain (c). EPIG10-p2 and EPIG10-p10 represent gels prepared with 10% EPI and treated at pH 2.0 and 10.0, respectively.

**Fig. 5.** Morphology and microstructure of EPIG10-p2 (a) and EPIG10-p10 (b). EPIG10-p2 and EPIG10-p10 represent gels prepared with 10% EPI and treated at pH 2.0 and 10.0, respectively.



**Fig. 1**

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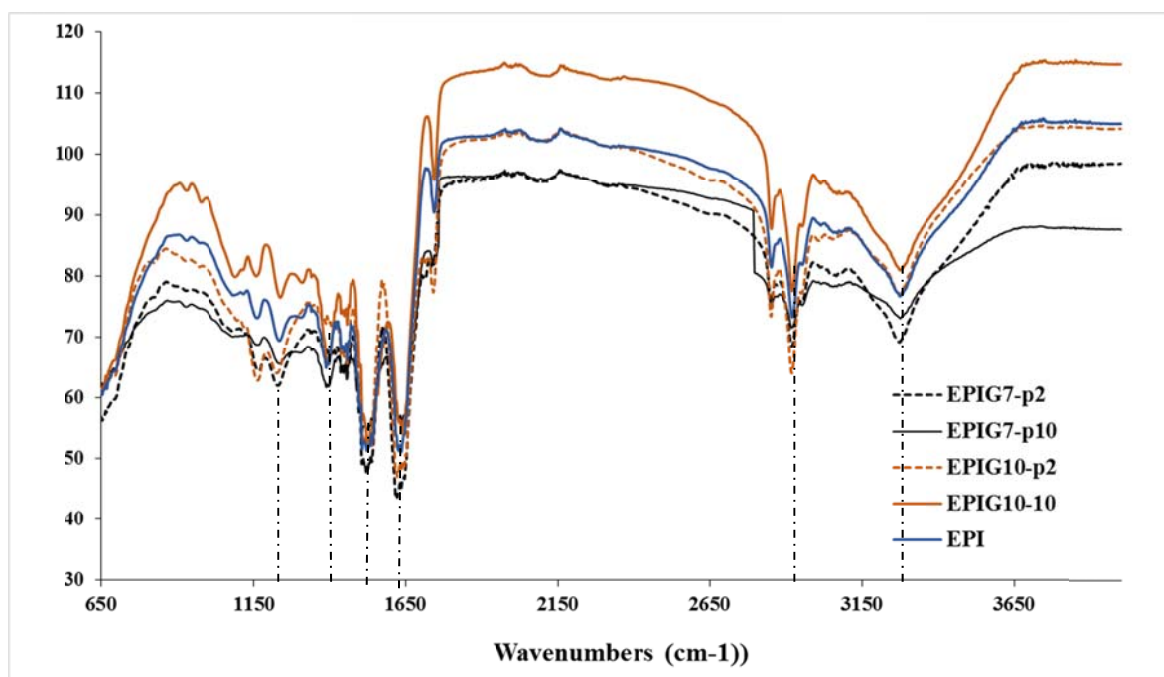


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**Fig.2**

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**Fig. 3**

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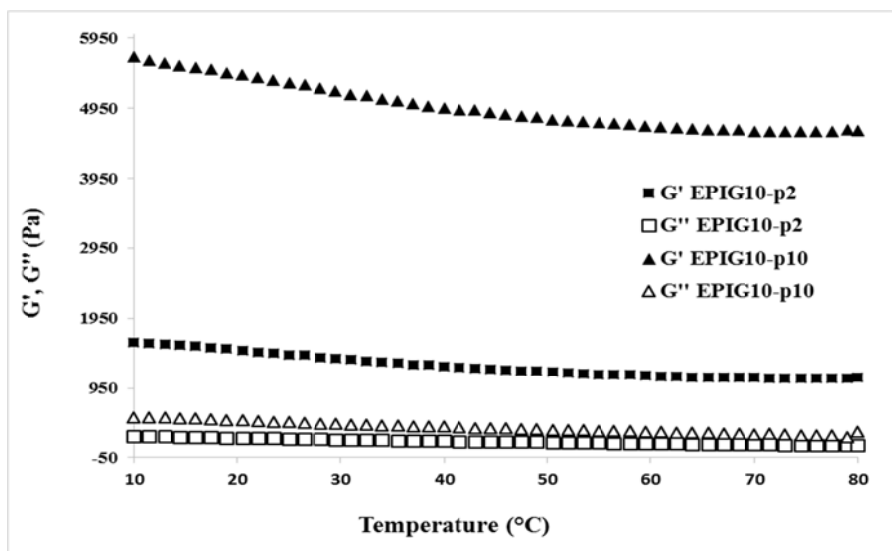
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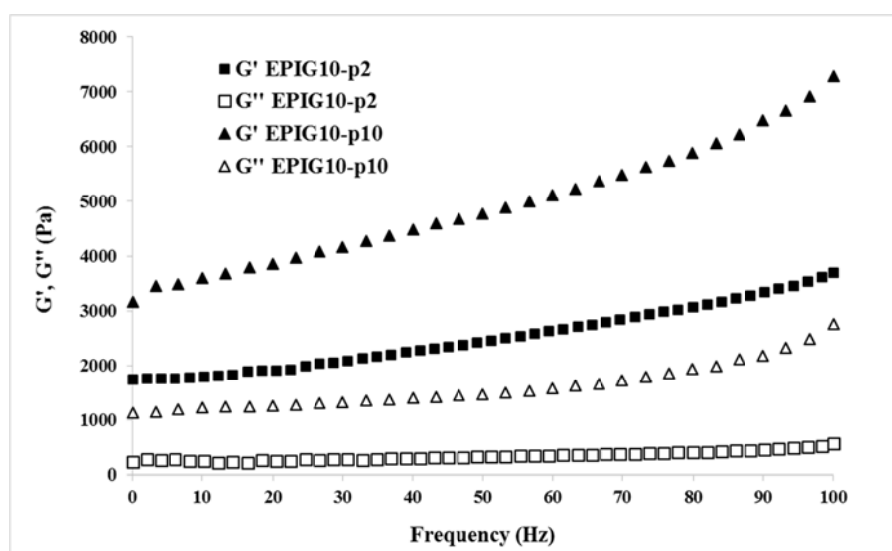
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(a)



(b)



(c)

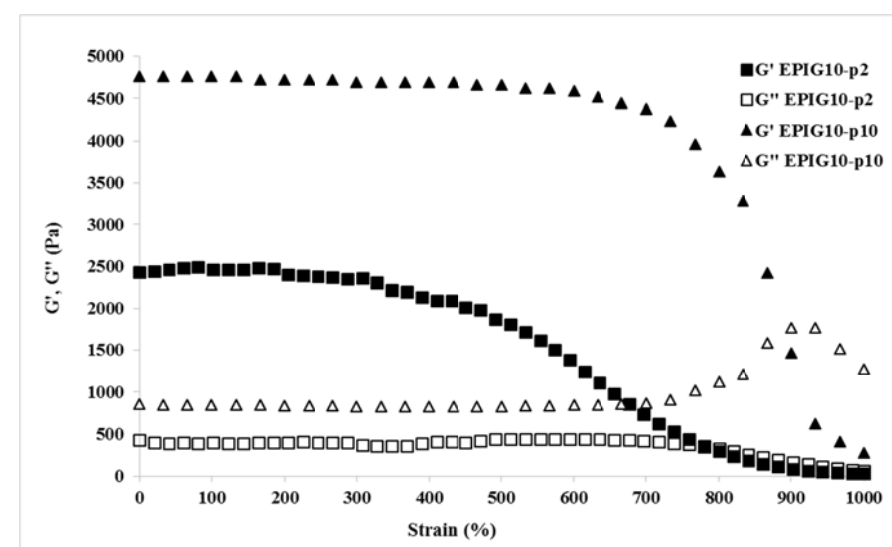
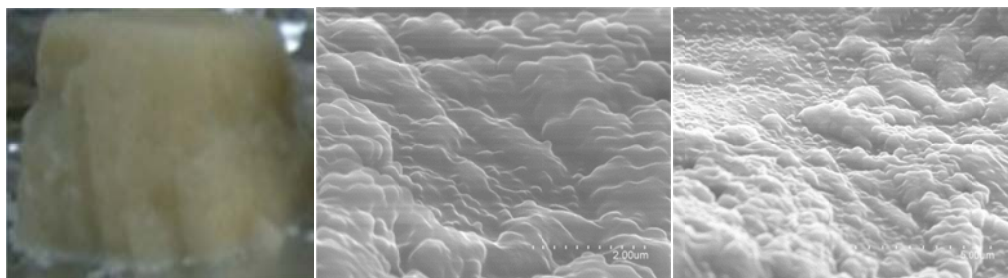


Fig. 4

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a)



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**Fig. 5**