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

3
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12
13 **ABSTRACT**

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

31
32 **Keywords:** pH-shift process, Gelation, Protein isolate gel, Textural and rheological
33 properties, Microstructure.

35 **1. Introduction**

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

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

56 Many studies focused on protein gelation in food, due to its interesting textural
57 properties and its capacity to entrap molecules of interest into gel networks (Yang, Wang,
58 Vasanthan, & Chen, 2014). Particularly, fish protein gels are complex and their properties as
59 well as the mechanism of their formation have been the subject of several research works.
60 Properties of protein gels are determined based on the type and number of protein-protein
61 interactions, aggregation and arrangement of unfolded proteins, which are affected by the pH,
62 ionic strength, protein concentration, heating and cooling rates (Xiong & Blacnchard,
63 1997).Gel formation requires structural and functional changes of the constituent myofibrillar
64 proteins. In addition, the rheological and biochemical characteristics of the gel can be affected
65 either by intrinsic factors (species, freshness, and degree of stress before harvest) or extrinsic
66 factors (protein concentration, pH, ionic strength, and temperature) (Yin & Park, 2014). One

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

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

73

74 **2. Materials and methods**

75 **2.1. Fish sample**

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

80 **2.2. Preparation of European eel protein isolate**

81 The alkaline solubilization process was used to prepare EPI as described by (Chaijan,
82 Panpipat & Benjakul, 2010). Fish mince was homogenized with cold water (4 °C) at a ratio of
83 1:9 (w/v). The homogenate was adjusted to pH 11.5 with 2 N NaOH solution, gently stirred
84 for 60 min and then centrifuged at 9,500 g for 20 min at 4 °C. The soluble proteins in the
85 supernatant were precipitated at their nominal isoelectric point (pH 5.5) using 2 N HCl. The
86 precipitate was collected by centrifugation (9.500 g for 20 min) and then resuspended in
87 distilled water, followed by pH adjustment to 7.0 using 2 N NaOH solution. European eel
88 protein isolate (EPI) was freeze-dried using freeze-dryer lab (Moduloyd-230, Thermo Fisher
89 Scientific, USA) at a temperature of -50 °C and a pressure of about 121 mbar.

90 **2.3. Preparation of European eel protein isolate gels**

91 To prepare the gels, thermal treatment is required to induce changes in physicochemical
92 properties of proteins. EPI solutions, with different protein concentrations (7 and 10% w/v),
93 were prepared in distilled water. Then, the pH of the mixtures was adjusted to the target
94 values (2.0 or 10.0) using 2 N NaOH or 2 N HCl solutions. Thereafter, samples, were poured
95 into vials (2.5 × 5 cm, diameter × height) with a sample height of approximately 15 mm,
96 heated at 90 °C for 10 min and then immediately cooled in ice. The gelling solutions were
97 kept at 4 °C for 24 h to induce gel maturation (Chaijan et al., 2010). Gels obtained with 7%
98 EPI at pH 2.0 and 10.0 were named, respectively, EPIG7-p2 and EPIG7-p10, whereas,
99 EPIG10-p2 and EPIG10-p10 were attributed to gels obtained with 10% EPI prepared at pH
100 2.0 and 10.0, respectively.

101 To study the effect of salt on EPIG properties, different concentrations of NaCl (2, 3 and
102 5% w/w) were incorporated into the EPI solutions before the heat treatment.

103 **2.4. Chemical analysis**

104 The moisture and ash contents in protein isolate were determined according to the
105 AOAC standard methods 930.15 and 942.05, respectively (AOAC, 2000). Total nitrogen
106 content was determined using the Kjeldahl method according to the AOAC method number
107 984.13 (AOAC, 2000). Crude protein was estimated by multiplying total nitrogen content by
108 the factor of 6.25. Fat content was determined gravimetrically after Soxhlet extraction of dried
109 samples with hexane for 2 h using Nahita Model 655, Navarra. All measurements were
110 performed in duplicate and results are the average of two values.

111 **2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of EPI**

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

116 **2.6. Solubility of European eel protein isolate**

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

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

125 Solubility analysis was carried out in triplicate.

126 **2.7. Physico-chemical characterization of EPIGs**

127 **2.7.1. Color**

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

132 duplicate from three dependent samples and whiteness (W^*), differences in color (ΔE^*) and
133 chroma (C^*) were calculated using the following equations:

$$W^* = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$
$$C^* = (a^{*2} + b^{*2})^{1/2}$$
$$\Delta E^* = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2}$$

134

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

137 **2.7.2. Total pigment content**

138 The total pigment content (TPC) was determined as described by Lee, Hendricks, &
139 Cornforth (1999). A gel sample (1 g) was mixed with 9 ml of an acid–acetone mixture
140 (90:8:2, acetone: deionized water: HCl). The mixture was stirred with a glass rod and kept
141 undisrupted for 1 h at room temperature (25 ± 2 °C). The extract was filtered with a Whatman
142 No. 1 filter paper and its absorbance was read at 640 nm against an acid-acetone tube used as
143 blank. The total pigment content was calculated using the following formula:

$$144 \text{ Total pigment content} = A_{640} \times 680,$$

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

147 **2.7.3. Moisture release content**

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

$$153 \text{ Moisture release content (\%)} = [(X - Y) / X] \times 100,$$

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

155 **2.7.4. TCA-soluble peptide content**

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

161

162 **2.7.5. Fourier Transform Infrared (FTIR) analysis**

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

170 **2.7.6. Texture profile analysis**

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

178 **2.7.7. Rheological measurements**

179 Dynamic oscillatory study of EPIGs was determined using Bohlin CVO-100
180 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK). The inner diameter of the cup was
181 28.5 mm and the diameter of the bob was 26.5 mm , giving a gap between the cup and the bob
182 of 1.0 mm . Heating from $10\text{ to }80\text{ }^{\circ}\text{C}$ and back to $10\text{ }^{\circ}\text{C}$ took place at a scan rate of 01
183 $^{\circ}\text{C/min}$, frequency of 1 Hz , and target strain of 1% . The elastic modulus (G' ; Pa) and viscous
184 modulus (G'' ; Pa) were plotted as a function of temperature. Frequency sweep was carried out
185 at $20\text{ }^{\circ}\text{C}$ by applying oscillation amplitude over the frequency range of $0.01\text{-}100\text{ Hz}$ and at a
186 fixed strain of 1% . Finally, the strain amplitude sweep measurements were conducted at a
187 fixed frequency of 1 Hz and temperature of $20\text{ }^{\circ}\text{C}$ with strains in the range from 0.1% to
188 1000% to characterize the large deformation rheological properties. The elastic modulus (G' ;
189 Pa) and viscous modulus (G'' ; Pa) were recorded during the above dynamic rheological
190 measurements.

191 **2.8. Scanning electron microscopy**

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

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

198 **2.9. Statistical analysis**

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

203 **3. Results and discussion**

204 **3.1. Chemical composition of EPI**

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

214 **3.2. Solubility of EPI at different pH conditions**

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

230 **3.3. SDS-PAGE protein profile of EPI**

231 The protein molecular weight distribution of EPI was qualitatively evaluated by SDS-
232 PAGE and the profile is shown in **Fig. 2**. Typical fish protein bands, including molecular
233 heavy chain proteins (> 175 kDa) and actin bands (~ 45 kDa), were present in the *A. Anguilla*
234 muscle. Proteins with lower molecular weights than actin were also observed, which may
235 correspond to tropomyosin chains (< 45 kDa). Likewise, bands with a smaller size than
236 myosin may be of the paramyosin type (Romero, Cordobés, Guerrero, & Puppo, 2011).
237 Generally, isolates with a high content of myosin and actin have a tendency to form a
238 viscoelastic network and were found to be largely responsible for consistent gels. The heavy
239 chain of myosin is assumed to be the main subunit involved in gelation. Xiong, & Blacnchard,
240 (1997) revealed in their study that isolated muscle proteins could be used to elucidate gelation
241 mechanism of complex mixtures of myofibrillar proteins.

242 **3.4. Physico-chemical and gelling properties of EPIGs**

243 **3.4.1. Color and total pigment content**

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

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

258 Moreover, the estimation of total pigment content (TPC) of EPIGs showed that TPC
259 was higher for alkaline-treated EPIGs than for those prepared under acidic conditions (**Table**
260 **2**). In fact, TPC significantly ($p < 0.05$) increased from 23.12 mg / 100 g gel for EPIG7-p2 to
261 35.36 mg/100 g gel for EPIG7-p10, respectively, which can be correlated to the results of
262 EPIGs whiteness. In contrast, no correlation was found between salt concentrations and TPC
263 values.

264 3.4.2. Moisture release content

265 The moisture release content (MRC) of EPIGs is illustrated in Table 2. Regardless salt
266 concentration, the MRC decreased with the increase of pH and EPI concentration values. Data
267 revealed as well that alkaline-treated gels (EPIG7-p10 and EPIG10-p10), prepared with or
268 without the addition of NaCl, had significantly lower MRC (ranging from 9.88 to 13.55 g/100
269 g gel) than that obtained with acid gels, where MRC varied from 14.93 to 19.89 g/100 g gel.
270 This is in line with previous works from Kristinsson, & Liang (2006) and Rawdkuen et al.
271 (2009) who found lower expressible moisture contents of 10.51% and 6.51% for Atlantic
272 croaker and tilapia protein gels obtained in alkaline conditions, respectively. In contrast,
273 Abdelhedi et al. (2016) found that smooth hound fish gel prepared under alkaline conditions
274 (pH 9.0) had higher MRC than that obtained with acid gel.

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

281 Furthermore, the addition of 2% salt to acid gel (EPIG7-p2) decreased the MRC from
282 19.89 to 16.20 g/100 g gel ($p<0.05$). In alkaline conditions, NaCl addition slightly affected
283 the MRC levels which decreased from 13.55 g/100 g gel for EPIG7-p10 (without salt
284 incorporation) to 12.24 g/100 g gel for EPIG7-p10 prepared at a concentration of 2% NaCl.
285 The decrease in MRC could be explained by the increase in water retention of gels with 2%
286 NaCl by solubilizing myofibrillar proteins (Xiong, & Blacnchard 1997).

287 3.4.3. TCA-soluble peptide content

288 TCA-soluble peptide content of gels could be a sign of proteolysis caused by
289 enzymatic hydrolysis (autolysis) or acid hydrolysis (Abdollahi, Razaei, Jafarpour, &
290 Undeland, 2017). The amount of TCA-soluble peptides was determined in acidic and
291 alkaline-treated gels and the results are presented in **Table 3**. Gels prepared at pH 2.0 (EPIG7-
292 p2 and EPIG10-p2) exhibited significantly ($p<0.05$) greater TCA-soluble peptide contents
293 (2.06 and 2.72 $\mu\text{mol tyr/g gel}$, respectively) than gels treated at pH 10.0 (EPIG7-p10 and
294 EPIG10-p10), where TCA-soluble peptide contents were 1.83 and 1.62 $\mu\text{mol tyr/g gel}$,
295 respectively. This is in line with the study of Abdollahi et al. (2017) who reported that gels
296 made from acid-produced isolates had the highest amount ($p<0.05$) of TCA-soluble peptides,

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

299 Additionally, TCA-soluble peptide contents of gels were found to be EPI
300 concentration dependent. In fact, in acid EPIGs, the gel prepared with 7% EPI exhibited lower
301 TCA-soluble peptide content (2.06 $\mu\text{mol tyr/g gel}$) than gel obtained with 10% of proteins
302 (2.72 $\mu\text{mol tyr/g gel}$). However, TCA-soluble peptide contents in alkaline-formed gels (PIG7-
303 p10 and EPIG10-p10) decreased from 1.83 to 1.62 $\mu\text{mol tyr/g gel}$ with increasing of EPI
304 concentration.

305 The evaluation of the effect of NaCl concentration on peptides level revealed that
306 adding 2% NaCl to the acidic gel preparation decreased the TCA peptide content to 1.21 and
307 1.60 $\mu\text{mol tyr/g gel}$ for EPIG7-p2 and EPIG10-p2, respectively ($p < 0.05$). Beyond this
308 concentration of salt, TCA-soluble peptide levels increased in both alkali and acid gels. These
309 results could be explained by the solubilization of the protein chains up to a certain amount of
310 salt (in the 2% wt.). Nevertheless, higher NaCl concentration led to a decrease in the quality
311 of the gel, being thus more susceptible to protein hydrolysis.

312 **3.5. Fourier transform infrared analysis of EPIGs**

313 Fourier transform infrared (FT-IR) spectra of protein isolate and gels prepared using
314 the different treatments are shown in **Fig. 3**. Distinctive absorption bands are observed at
315 3273 cm^{-1} (Amide A, N-H or O-H), 2935–2962 cm^{-1} (amide B), 1643 cm^{-1} (Amide I, C=O
316 and C=N), 1533 cm^{-1} (Amide II, C-N and N-H), 1398 cm^{-1} (C-H bending), and 1236 cm^{-1}
317 (Amide III) (Chen et al., 2014) in all protein samples (powder and gels). Among these peaks,
318 the band between 3000 and 3500 cm^{-1} related to Amide A and free hydroxyl groups can be
319 useful to evaluate the interactions of the protein molecules with water (Perisic, Afseth, Ofstad,
320 & Kohler, 2011). In fact, EPIGs prepared under alkaline conditions exhibited a marked
321 reduction of the amide A intensity, compared to that detected in EPIGs prepared at pH 2.0,
322 which might be due to the increase of hydrogen bonding interactions between molecules in
323 the protein matrix, leading to a greater degree of hydration in acidic conditions. This result
324 was found to be correlated with the creamy appearance of the acid gel compared to the alkali
325 gel showing a hard and elastic structure.

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

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

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

345 **3.6. Textural characteristics of EPIGs**

346 Regarding the textural properties of the different gels (hardness, elasticity, adhesion
347 and cohesion), results showed that textural parameters of EPIGs depended on pH and EPI
348 concentration levels (Table 4). At 7% EPI concentration, elasticity of EPIGs significantly
349 increased with pH increase, from 5.5 mm for EPIG7-p2 to 7.69 mm for EPIG7-p10 ($p>0.05$).
350 Similarly, at 10% EPI concentration, the elasticity of EPIGs increased ($p<0.05$) from 5.53 for
351 EPIG10-p2 to 9.26 mm for EPIG10-p10. Abdollahi, et al. (2017) reported that the elasticity of
352 a muscle protein gel is mainly related to the contribution of myofibrillar proteins, especially
353 actomyosin. Moreover, results showed that higher adhesion values were observed with gels
354 treated under alkaline conditions (0.75 N and 1.59 N for EPIG7-p10 and EPIG10-p10,
355 respectively) than those obtained at acidic pH (0.33 N and 1.21 N for EPIG7-p2 and EPIG10-
356 p10, respectively). Thus, gels obtained in alkaline conditions were harder than acid gels,
357 which were more fragile and having a soft texture. This could be explained by increased
358 protein-protein interactions in the case of alkaline-obtained gels, related to harder and more
359 elastic gels. However, the presence of too much water in the acid-obtained gels may hamper
360 these inter- and intramolecular protein interactions, giving rise to soft and fragile gels as
361 previously observed (Cortes-Ruiz, Pacheco-Aguilar, Garcia-Sanches, & Lugo-Sanches,
362 2001). In addition, greater adhesion of EPIGs was observed with increasing EPI concentration
363 (from 0.75 N for EPIG 7-p10 to 1.59 N for EPIG10-p10).

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

371 **3.7. Viscoelastic properties**

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

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

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

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

408 **3.8. Morphology and microstructure of protein isolate gels**

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

429 **Conclusion**

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

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

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519 Alaska pollock surimi. *Food Chemistry*, 150, 463-468.

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

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

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 527 **Data are given in mean ± SD. ^{a-b} different letters in the same column indicate significant**
 528 **differences (p<0.05). API: *A. anguilla* protein isolate.**

529
 530 **Table 2:** Effect of pH value, EPI and NaCl concentration on color analysis, total pigment
 531 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 ^{cC}	70,22 ± 0.04 ^{aB}	19,76 ± 0.07 ^{bB}	23.12 ± 0.96 ^{aC}	19.89 ± 0.69 ^{aA}
	2	3.57 ± 0.11 ^a	67.53 ± 0.04 ^b	21.07 ± 0.01 ^a	22.44 ± 1.92 ^a	16.20 ± 0.17 ^b
	3	1.56 ± 0.08 ^b	68.64 ± 0.01 ^b	19.60 ± 0.11 ^b	24.14 ± 0.48 ^a	16.80 ± 0.90 ^b
	5	1.60 ± 0.16 ^b	68.42 ± 0.05 ^b	19.59 ± 0.10 ^b	24.48 ± 0.96 ^a	19.60 ± 1.55 ^a
EPIG7-p10	0	7.99 ± 0.09 ^{aA}	63.76 ± 0.03 ^{cC}	21.14 ± 0.52 ^{aA}	35.36 ± 0.96 ^{aB}	13.55 ± 0.50 ^{aB}
	2	7.35 ± 0.01 ^b	64.18 ± 0.19 ^b	21.85 ± 1.02 ^a	36.38 ± 1.44 ^a	12.24 ± 0.33 ^b
	3	5.54 ± 0.16 ^c	66.21 ± 0.12 ^a	20.40 ± 0.02 ^a	33.66 ± 1.44 ^{ab}	12.95 ± 0.18 ^{a,b}
	5	7.39 ± 0.09 ^b	64.05 ± 0.12 ^b	22.00 ± 0.72 ^a	31.96 ± 1.92 ^b	13.08 ± 0.26 ^a
EPIG10-p2	0	4.75 ± 0.02 ^{aB}	73.51 ± 0.01 ^{aA}	13.84 ± 0.02 ^{cD}	24.48 ± 0.96 ^{cC}	14.93 ± 1.29 ^{bB}
	2	3.71 ± 0.01 ^b	67.90 ± 0.01 ^d	19.15 ± 0.03 ^a	23.80 ± 1.92 ^c	13.05 ± 0.25 ^c
	3	2.38 ± 0.10 ^c	71.82 ± 0.02 ^c	16.28 ± 2.28 ^b	26.18 ± 0.48 ^b	15.51 ± 1.11 ^b
	5	4.77 ± 0.21 ^a	72.74 ± 0.02 ^b	13.92 ± 0.21 ^c	28.22 ± 0.48 ^a	16.80 ± 0.05 ^a
EPIG10-p10	0	7.86 ± 0.04 ^{aA}	64.57 ± 0.02 ^{dD}	18.99 ± 0.04 ^{b,c,C}	41.14 ± 0.48 ^{aA}	9.88 ± 0.09 ^{a,b,C}
	2	5.87 ± 0.03 ^c	66.29 ± 0.04 ^a	18.90 ± 0.85 ^b	46.24 ± 1.92 ^a	10.37 ± 0.26 ^a
	3	6.77 ± 0.02 ^b	64.88 ± 0.02 ^c	20.40 ± 0.04 ^a	44.88 ± 0.96 ^a	10.66 ± 0.37 ^a
	5	6.80 ± 0.06 ^b	65.24 ± 0.04 ^b	19.41 ± 0.01 ^b	41.82 ± 0.48 ^c	9.34 ± 0.10 ^c

533
 534 EPI represents protein isolate extracted from European eel muscle. EPIG7-p2 and EPIG7-p10 represent
 535 gels prepared with 7% EPI and treated at pH 2.0 and 10.0, respectively. EPIG10-p2 and EPIG10-p10 were gels
 536 prepared with 10% EPI and treated at pH 2.0 and 10.0, respectively. Data are given in mean ± SD. ^{a-d} different
 537 letters in the same gel sample with different concentrations of salt indicate significant differences (p<0.05). ^{A-D}
 538 different capital letters in the same column indicate significant differences between gels prepared without salt
 539 addition (p<0.05).

540

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

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	NaCl	TCA-soluble peptides	543
	concentration (%)	($\mu\text{mol tyr/g gel}$)	
EPIG7-p2	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	$2.33 \pm 0.04^{\text{a}}$	
EPIG7-p10	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	$2.69 \pm 0.06^{\text{a}}$	
EPIG10-p2	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	$2.45 \pm 0.07^{\text{a}}$	
EPIG10-p10	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

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

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

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

573

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

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590 **Figure captions:**

591

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

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

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

597

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

602

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

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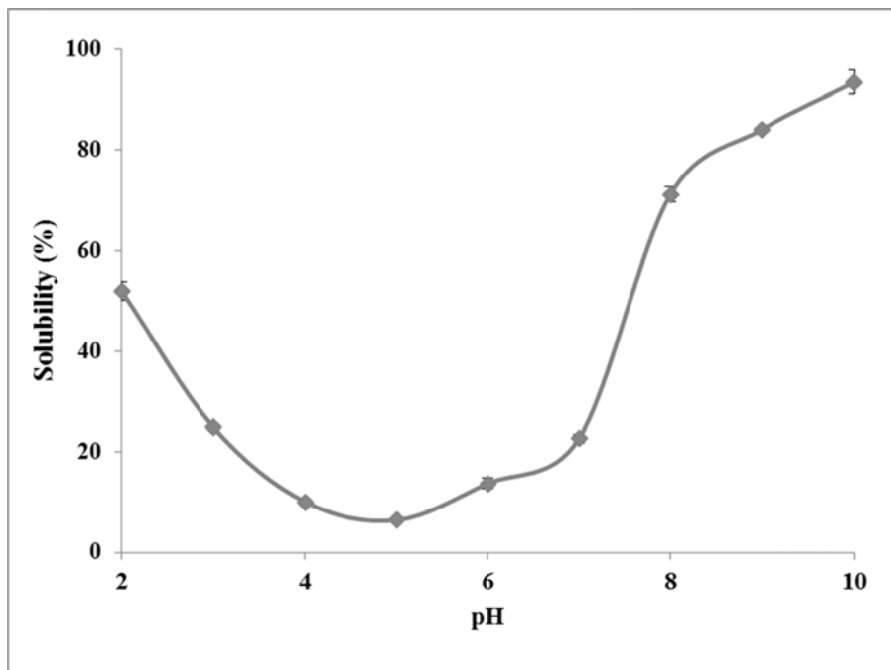
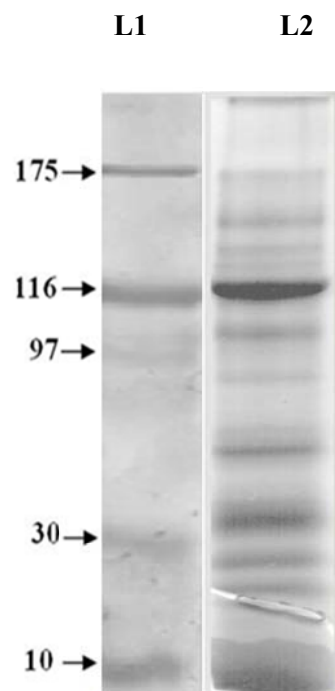


Fig. 1

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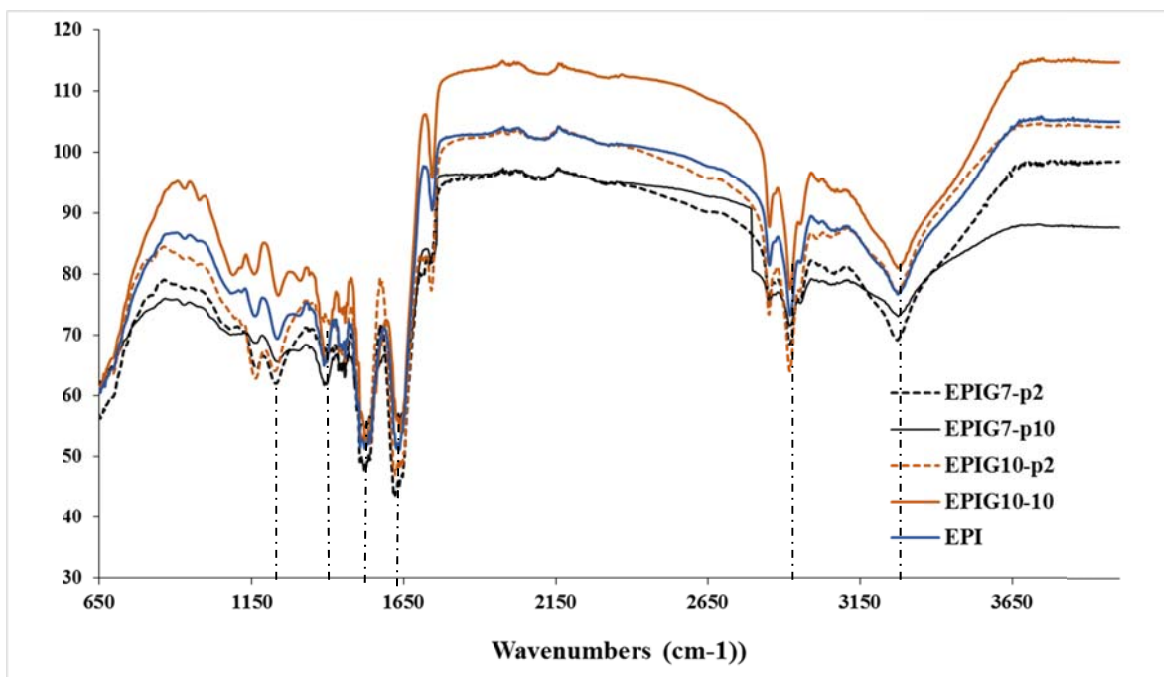
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Fig. 3

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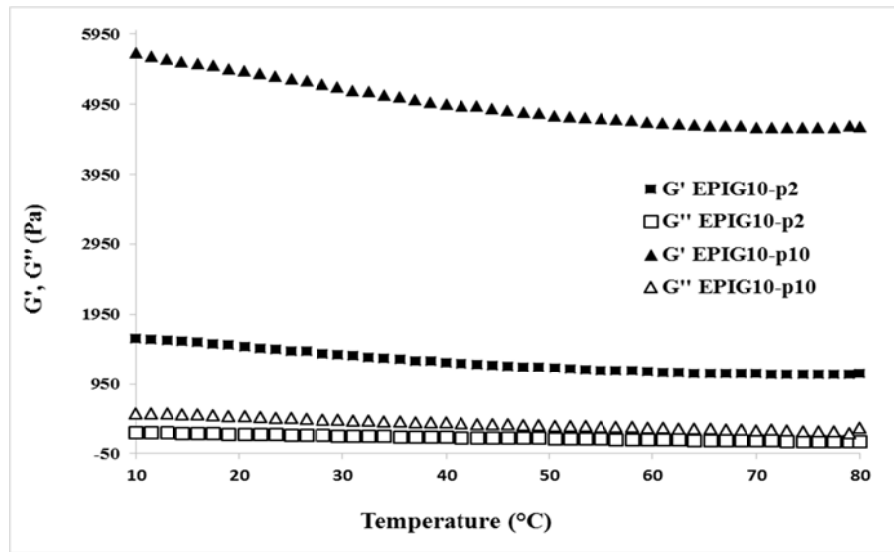
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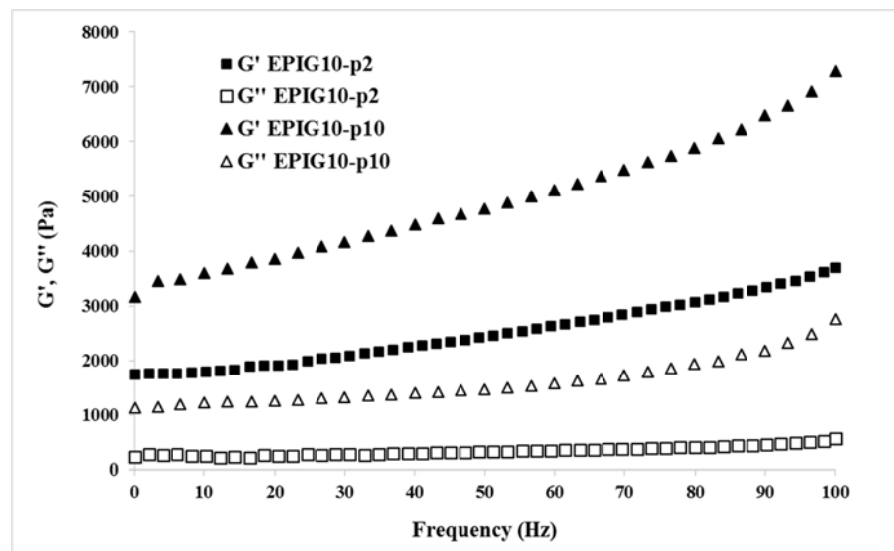
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(a)



(b)



(c)

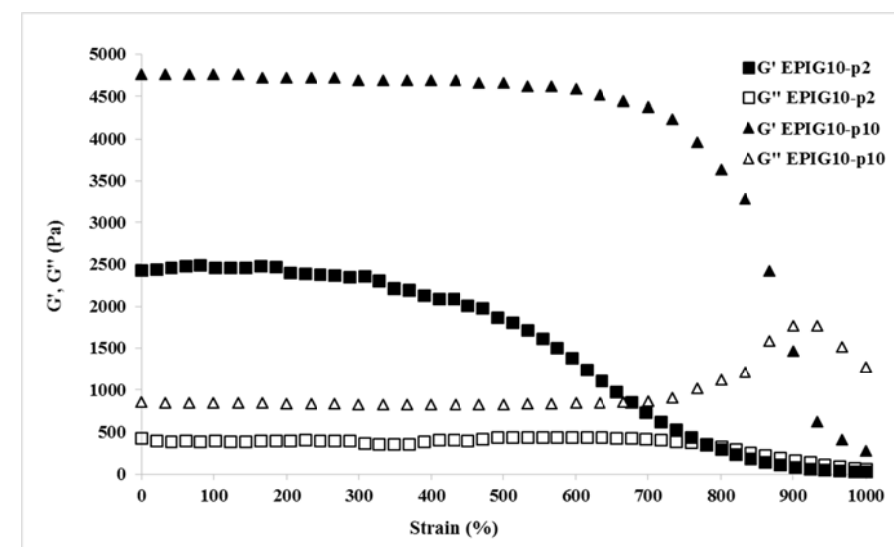
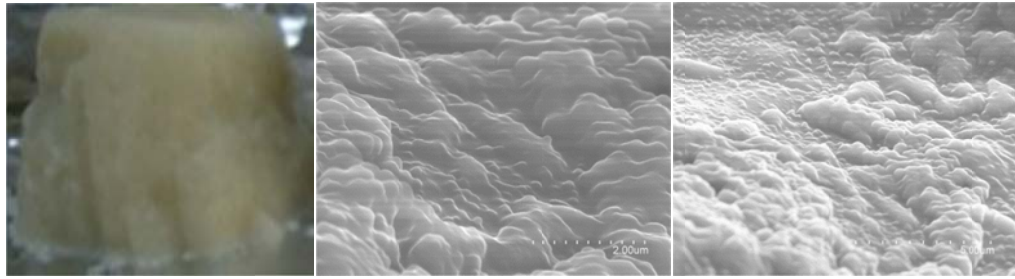


Fig. 4

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Fig. 5