

1 **Heat treatment of calcium alginate films obtained by ultrasonic atomizing:**
2 **physicochemical characterization**

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15

16 **Abstract**

17 Planar films of calcium alginate were obtained using an ultrasonic atomizing device.
18 Sodium alginate solutions of 0.6% and 0.9% (w/v) were nebulized with calcium
19 gluconolactate solutions (gelling agent) of 0, 1, 2 and 3% (w/v) at a flow rate of 0.3 mL
20 min⁻¹ for 20 min. After drying, thickness and mechanical properties were determined.
21 In view of the results of mechanical properties, manageability and flexibility, calcium
22 alginate films obtained using 0.9% sodium alginate and 2% calcium gluconolactate
23 were selected as “optimum dry film” samples. These samples were cut into rectangular
24 pieces and heated at 180°C for 0, 4, 8, 12, 20 and 24 min. Thickness, mechanical and
25 optical properties, differential scanning calorimetry (DSC) thermograms, Fourier
26 transform infrared spectroscopy (FTIR) spectra, and scanning electron microscopy
27 (SEM) micrographs were analyzed in order to characterize the physicochemical
28 properties of heat-treated samples. The heat treatment produced thickness reduction, a
29 yellow ochre color development and an increase in the brittleness of the films. DSC,
30 FTIR and SEM studies suggested that heat treatment produced further dehydration of
31 dry films and thermal dehydration-degradation of alginate macromolecules.

32

33 **Key words:** Calcium alginate films, Ultrasonic atomizing, Heat treatment,
34 Physicochemical characterization

35

36 **1. Introduction**

37 Sodium alginate is one of the most important polysaccharides used for hydrogels
38 preparation (Draget, 2000). The hydrogel properties are typically controlled by alginate
39 chemical microstructure determined by α -L-guluronic and β -D-mannuronic present in
40 varying proportions and sequences, type of gelling ions and gelling conditions. Alginate
41 gelation occurs when divalent cations (usually Ca^{2+}) interact with blocks of guluronic
42 residues. According to the “egg-box” model (Grant, Morris, Rees, & Smith, 1973), two
43 contiguous, diaxially linked guluronic residues form a cavity that acts as a binding site
44 for calcium ions. This binding induces chain-chain associations forming stable junction
45 zones of dimers and lateral interactions between these dimers. As a result, the gel is
46 formed and mechanical properties are directly related to the number of “egg-box” sites.
47 Thus, the increase in network crosslink density results in a higher fracture stress.

48 The procedure of introducing gelling ions is an additional parameter influencing the
49 properties of alginate hydrogels (Draget, 2000). The external gelling method consists in
50 exposing alginate solution directly to the gelling ions solution and alginate hydrogel is
51 irreversible formed due to ion diffusion. The second method, called internal gelling, is
52 based on mixing an insoluble source of gelling ions with the alginate solution followed
53 by releasing the gelling ions by lowering the pH value after addition of organic acids or
54 by hydrolyzing lactones (Papajová, Bujdoš, Chorvát, Stach, & Lacík, 2012). When the
55 external gelling method is used for preparation of planar alginate hydrogels, the almost
56 instantly gelation of alginate produces a heterogeneous dispersion of gel lumps. In view
57 of this problem, preparation of planar alginate hydrogels by external gelling requires
58 slow rate of exposure of alginate solution to gelling ions in order to control gelling and
59 hydrogel properties. This issue was tackled by exposing solution of sodium alginate to
60 an aerosolized spray of Ca^{2+} solution (Cathell & Schauer, 2007; Papajová, Bujdoš,

61 Chorvát, Stach, & Lacík, 2012). However, these authors obtained small planar alginate
62 hydrogels. One objective of this research was to develop an ultrasonic atomizing device
63 that allows the preparation of calcium alginate films of larger surface areas, suitable for
64 being used as edible films.

65 On the other hand, although gelation kinetics is altered by the source of calcium, neither
66 the final alginate gel strength nor the resistance to calcium diffusion are modified (Lee,
67 & Rogers, 2012). CaCl_2 reaches a gel strength plateau fastest, followed by calcium
68 lactate and calcium gluconate. CaCl_2 is the most usual source of calcium when the bitter
69 taste can be masked and a fast throughput is required, while calcium organic salts may
70 have an advantage when the membrane thickness/hardness needs to be manipulated.
71 Calcium gluconolactate, a commonly food additive, is calcium gluconate mixed with
72 calcium lactate. Other objective of the present work was to use calcium gluconolactate
73 as the gelling agent for the formation of dry calcium alginate edible films.

74 In contrast to most gelling polysaccharides, alginate gels have the particular feature of
75 being cold setting and are heat stable. In practice, this means alginate gels can be heat
76 treated without melting. This is the reason why alginates are used in baking creams
77 (Smidsrød & Draget, 2004) as an edible barrier to reduce fat uptake in fried foods
78 (Albert & Mittal, 2002) and as an edible coating that improve the quality of
79 microwaveable chicken nuggets (Albert, Salvador, & Fiszman, 2012). The last objective
80 of this research was to study the physicochemical characteristics of dry calcium alginate
81 films subjected to heat treatment.

82

83 **2. Materials and Methods**

84

85 **2.1. Materials**

86 Sodium alginate (SA) from brown algae (medium viscosity), calcium lactate hydrate
87 (CLH) and calcium gluconate anhydrous (CGA) were purchased from Sigma-Aldrich
88 (St. Louis, MO, USA). SA had an approximate mannuronic/ guluronic ratio of 1.56, a
89 degree of polymerization range of 400-600, and a molecular weight of 80000-120000.
90 Solid CLH and CGA were mixed at a weight ratio of 4:1. This mixture was called
91 calcium gluconolactate, CG. All other reagents were of analytical grade.

92

93 **2.2. Preparation of dry alginate films by external gelling method**

94 Solid SA and distilled water were mixed in order to obtain solutions of 0.6% and 0.9%
95 (w/v). The mixtures were magnetically stirred until a homogeneous phase was obtained.
96 Both solutions were degassed by sonication. Following degassing, plastic Petri plates
97 (13.5 cm in diameter) were filled with the different SA solutions assayed (88 g/plate).
98 Then, SA solutions were concentrated by evaporation in an oven (Tecno Dalvo, Santa
99 Fe, Argentina) at 50 °C for 3h. CG solutions were prepared at different concentrations:
100 0 (for control sample), 1, 2 and 3% (w/v). The concentrations of SA and CG solutions
101 used in this work were selected based on preliminary experiments in which hardness of
102 wet gel measured by a compression test was the critical parameter for selection (Chen &
103 Opara, 2013). In that sense, gels with relative high resistance to deformation without
104 rupture were chosen for the subsequent dried process. The setup used for the formation
105 of alginate hydrogels by external gelling method is shown in Figure 1. The Petri plates
106 were placed on a circular base and covered by a modified plastic dome. The system was
107 driven by an electric engine rotating at 7 rpm. A transparent plastic tube (I.D. 6 mm)
108 connected an ultrasound nebulizer Aspen NU400 (Aspen, Buenos Aires, Argentina)
109 with a diffuser inside the dome. The nebulizer reservoir was filled with the different CG
110 solutions. The aerosol of each gelling solution was introduced into the dome at a flow

111 rate of 0.3 mL min⁻¹ for 20 min. After that, the plates were withdrawn from the system,
112 left to stand at room temperature for 3 h and then put into the oven for 3 h at 50 °C. The
113 dry films were then removed from the Petri dishes and stored in hermetic plastic
114 containers at room temperature. The films used in the different tests were selected based
115 on the lack of physical defects such as cracks, bubbles and holes and on their
116 manageability and flexibility.

117

118 **2.2.1 Dry film thickness**

119 The thickness of three replicates of each film formulation were measured with an
120 electronic digital disk micrometer (Schwyz[®], China) at nine locations on the film to the
121 nearest 1 µm.

122

123 **2.2.2 Mechanical properties of dry films**

124 Tensile test was carried out using a motorized test frame (Mecmesin Multitest 2.5*d*,
125 Mecmesin, Sterling, VA, USA) equipped with a 100 N digital force gauge. Three
126 sample strips (7 x 60 mm) of each formulation were cut and clamped between tensile
127 grips. The initial distance between grips was 30 mm and the crosshead speed was 0.05
128 mm s⁻¹. From stress-strain curves, tensile strength (TS) and elongation (E) were
129 determined. TS was calculated by dividing the peak load by the cross sectional area
130 (thickness of film x 7 mm) of the initial film and E was calculated as the percentile of
131 the change in the length of specimen respect to the original distance between the grips
132 (30 mm). TS of the films is a measure of the maximal force per original cross-sectional
133 area that the film could sustain before breaking, and E measures the capacity of the film
134 to extend before breaking (Silva, Bierhalz, & Kieckbusch, 2009).

135 Mechanical properties, manageability and flexibility were the parameters considered to
136 select the optimum dry film (ODF) to be used in heat treatment studies.

137

138 **2.3 Heat treatment of dry films**

139 ODF samples were cut into seven rectangular pieces. Each one of the pieces was put
140 between two microscope slides. The end of the microscope slides were then clamped
141 together using bulldog clips. The samples were heated (ODFH) in a forced oven at 180
142 °C (Industrias Brafh, Rosario, Argentina). To study the effect of heating, each piece was
143 withdrawn at different heating times (0, 4, 8, 12, 16, 20 and 24 min). This experiment
144 was repeated three times.

145

146 **2.3.1 Thickness and mechanical properties of heat treated samples**

147 The thickness and mechanical properties of ODFH samples were measured in
148 accordance with section 2.2.1 and section 2.2.2.

149

150 **2.3.2 Optical properties**

151

152 **2.3.2.1 Opacity**

153 Opacity of ODFH samples was evaluated according the method of Siripatrawan and
154 Harte (2010) with modifications. ODFH samples were cut into rectangular pieces (10 x
155 30 mm), heat treated in agreement with section 2.3 and placed on the internal side of a
156 spectrophotometer cell (Jasco V-550, Tokyo, Japan). Light absorbance of the film
157 samples was measured at 600 nm and the opacity was calculated using the following
158 equation:

159

160 $Opacity = Abs_{600} / l$ (1)

161

162 where Abs_{600} is the value of absorbance at 600 nm and l is the film thickness in mm.

163

164 **2.3.2.2 Color measurements**

165 ODFH samples were used to obtain the digital images. A wooden box according to the

166 design described in Mendoza and Aguilera (2004), with some modifications, was used.

167 Samples were illuminated using four fluorescent lamps (Osram, Biolux, Natural

168 Daylight, 18W/965, Munich, Germany) with a color temperature of 6500 K (D_{65} ,

169 standard light source commonly used in food research) and a color-rendering index Ra

170 of 95%. Additionally, electronic ballast and an acrylic light diffuser ensured uniform

171 illumination system. Samples were photographed employing a digital camera (Nikon P

172 7100, Nikon, Jakarta, Indonesia) on a matte white background using the following

173 camera settings: manual mode with lens aperture at $f = 8$ and time of exposition 1/200,

174 no flash, ISO sensibility 400, maximum resolution (3648 x 2736 pixels), and storage in

175 RAW format.

176 An IT8 calibration card (Wolf Faust, Germany) was photographed under the same

177 conditions than heat treated films and was used to obtain the International Colour

178 Consortium (ICC) profile employing the Lprof software (Free Software Foundation,

179 Inc., Boston, MA, USA). This profile was applied to sample images using Photoshop

180 (Adobe Systems, Inc., USA). L , a , and b average values (considering the whole sample)

181 were obtained from histogram window and then were converted to L^* (lightness), a^*

182 (red-green), and b^* (yellow-blue) following the work of Yam and Papadakis (2004).

183 Total colour differences (ΔE) were calculated as follows:

184

185
$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$
 (2)

186

187
$$\Delta L = L^* - L_0^*$$
 (3)

188

189
$$\Delta a = a^* - a_0^*$$
 (4)

190

191
$$\Delta b = b^* - b_0^*$$
 (5)

192

193 where L_0^* , a_0^* and b_0^* are the standard values of the standard white plate and L^* , a^* and
194 b^* are the measured values of the sample.

195

196 **2.3.3 Differential scanning calorimetry (DSC)**

197 Thermal properties of ODF, SA, CLH and CGA were measured using a differential
198 scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan). Aliquots of approximately 10
199 mg of dried samples were placed into aluminum pans, sealed and scanned over the
200 range 30-350 °C with a heating rate of 10° C min⁻¹. The empty aluminum pan was used
201 as a reference. Each sample was run in duplicate.

202

203 **2.3.4 Fourier transform infrared (FTIR) spectroscopy**

204 The spectra of ODF and ODFH samples were determined using FTIR with an IR-
205 Prestige-21 spectrophotometer (Shimadzu, Kyoto, Japan) under attenuated total
206 reflectance (ATR) mode. The spectra were recorded in absorbance mode from 600 to
207 4000 cm⁻¹ using 20 scans at 4 cm⁻¹ resolution.

208

209 **2.3.5 Scanning electron microscopy (SEM)**

210 In order to study the influence of heat treatment on ODFH microstructure, SEM
211 experiments were carried out. Film samples were cryo-fractured by immersion in liquid
212 nitrogen and mounted on bronze stubs perpendicularly to their surface. The portions
213 were coated with gold during 15 min at 70-80 mTorr. Micrographs of films cross-
214 section were taken with a scanning electron microscope (AMR 1000, Leitz, Wetzlar,
215 Germany) using an accelerating voltage of 20 kV. Magnification of 500 was used in this
216 work.

217

218 **2.4 Statistical analysis**

219 Statistical analysis was performed using Statgraphics Plus for Windows (Manugistics
220 Inc, Rockville, MA, USA). Analysis of variance (ANOVA) was used and when the
221 effect of the factors was significant ($p < 0.05$), the test of multiple ranks honestly
222 significant difference (HSD) of Tukey was applied (95% of confidence level).

223

224 **3. Results and Discussion**

225

226 **3.1. Appearance and thickness of the films**

227 Calcium alginate films obtained by the methodology described in Section 2.2. were
228 visually homogeneous without brittle areas or bubbles. In addition, the films were easily
229 manageable and flexible.

230 Figure 2 shows the thickness of the films prepared from SA solutions of 0.6 and 0.9%
231 (w/v) in presence of different CG concentrations in the nebulizer reservoir. Film
232 thickness increased as the concentration of SA increased for all the CG concentrations
233 assayed ($p < 0.05$), Figure 2. As can also be seen in this Figure, the presence of CG

234 increased film thickness at both SA concentrations assayed ($p < 0.05$). However, no
235 significant difference was found between CG concentrations at each SA concentration
236 studied ($p < 0.05$). These results were similar to those reported previously by Rhim
237 (2004) who prepared calcium alginate films by direct addition of CaCl_2 into sodium
238 alginate solutions (mixing films).

239

240 **3.2 Mechanical properties**

241 Tensile strength of films prepared from SA solutions of 0.6 and 0.9% (w/v) in presence
242 of different CG concentrations in the nebulizer reservoir is shown in Figure 3. Calcium
243 alginate films were strong as indicated by high values of TS. TS of the films
244 significantly increased as the concentration of SA increased for all the CG
245 concentrations in the nebulizer reservoir assayed ($p < 0.05$). On the other hand, as CG
246 concentration increased first a strengthening and then a weakening of films was
247 observed for both SA concentrations studied ($p < 0.05$). TS increased up to 2% CG
248 concentration while beyond this value a decrease in this mechanical property was
249 observed. A similar pattern was observed by Cuadros, Skurtys and Aguilera (2012) for
250 calcium alginate fibers produced with a microfluidic device. These authors suggested
251 that the “egg-box” model used to describe ionotropic gelation of alginate only partly
252 explains the relation with microstructural and mechanical properties of the gelled
253 material and proposed that the decrease in gel strength that was observed after the
254 maximum was achieved, was due to the reversion of the system to the formation of
255 dimers with no association between them.

256 Figure 4 shows that E of the films significantly increased as the concentration of SA
257 increased for all the CG concentrations ($p < 0.05$). On the other hand, E significantly
258 increased when 1% CG concentration was used, while beyond 2% a decrease in this

259 mechanical property was observed. E is related to the ability of a material to resist
260 changes of shape without cracking. The E values obtained in the present work for
261 calcium alginate films were similar to those reported by Rhim (2004).

262 In view of the results of mechanical properties, manageability and flexibility, calcium
263 alginate films obtained using 0.9% SA concentration and 2% CG concentration in the
264 nebulizer reservoir were selected as the optimum dry film (ODF) to be used in heat
265 treatment studies.

266

267 **3.3 Characterization of ODFH samples**

268 While the visual aspect of ODFH samples is shown in Figure 5, Table 1 presents their
269 thickness and optical parameters. Thickness of ODFH samples dramatically decreased
270 up to 8 min of heat treatment, possibly due to a dehydration process.

271 Opacity is an established measurement of the transparency of a film. A higher value of
272 opacity means a lesser transparency (Pereda, Amica, Rácz, & Marcovich, 2011). The
273 tendency of opacity to increase with the length of heating may be attributed to two
274 factors: an intense color development and the decrease in the thickness of the film
275 (Equation 1). The color properties of ODFH samples summarized in Table 1 reinforce
276 this suggestion. ODFH samples changed their appearance from transparent yellow to
277 opaque yellow ochre in function of heating time. In that sense, after 24 min of heat
278 treatment, ΔE increased approximately 14 times. This change was principally promoted
279 by redness (a^*), while yellowness (b^*) and changes in lightness (L^*) played a minor
280 role. These changes in color parameters can be assigned to chemical degradation of the
281 components of the system.

282 Mechanical properties of ODFH samples are presented in Figure 6 and Figure 7. Figure
283 6 shows that heat treatment for 4 min and 8 min at 180 °C produced a significant

284 increase in TS values compared to the sample without any treatment. However, heating
285 for longer times promoted a decrease in TS of ODFH samples. Despite this decrease in
286 TS, the values obtained in this range remained relatively high (Rhim, 2004). On the
287 other hand, E decreased gradually with the intensity of heat treatment (Figure 7). In that
288 sense, E reduced approximately 3 times in comparison to the sample without treatment
289 after 24 min of heating. As a result of these changes, an increase in the brittleness of
290 ODFH with the severity of heat treatment was observed. It can be considered that
291 dehydration process was a determining factor in mechanical properties modifications.

292 Figure 8 shows DSC thermograms of the different samples assayed in this work. ODF
293 samples presented an endothermic peak at 99 °C that corresponds to dehydration of the
294 cross-linked gel matrix (alginate-calcium cation) (Taha, Nasser, Ardakani, & Al Khatib,
295 2008). The exothermic peaks observed at temperatures between 175 and 275 °C result
296 from degradation of alginate due to dehydration and depolymerisation of the protonated
297 carboxylic groups and oxidation reactions of the macromolecule (Sarmiento, Ferreira,
298 Veiga, & Ribeiro, 2006). SA thermograms were consistent with those reported
299 previously (Al-Remawi, 2012). In that sense, SA presented similar thermogram patterns
300 as ODF samples, with an endothermic band due to dehydration and a dehydration-
301 degradation band at higher temperatures. CLH showed a dehydration peak at 100 °C
302 although did not show degradation peaks at temperatures below 300 °C (Sakata,
303 Shiraushi, & Otsuka, 2005). In contrast, CGA only showed a degradation peak near 250
304 °C. These last results strengthen the hypothesis that the observed changes in thickness
305 and mechanical properties could be assigned to dehydration of the alginate-calcium ion
306 matrix, while early stages of degradation of alginate were the cause of color changes in
307 ODFH samples (Figure 5).

308 FTIR spectra of ODF samples (Figure 9) showed characteristics peaks of alginate:
309 hydroxyl group at $\approx 3220 \text{ cm}^{-1}$ (stretching) and carboxylate peaks at ≈ 1590 and 1410
310 cm^{-1} (asymmetric and symmetric stretching) (Campañone, Bruno, & Martino, 2014).
311 The presence of large amount of water in alginate solutions resulted in the saturation of
312 signal for O-H stretching band around $3000\text{-}3600 \text{ cm}^{-1}$ wavenumber (Xiao, Gu, & Tan,
313 2014). However, the drying process applied in this work to calcium alginate gels
314 drastically reduced the intensity of this band. The further decrease in the intensity of this
315 band promoted by heat treatment (ODFH samples) may be explained by the release of
316 additional water molecules retained in the film matrix. Figure 9 also shows that as the
317 heat treatment progressed, the intensity of the band around 1590 and 1410 cm^{-1}
318 increased. Similar phenomenon has been reported by Xiao et al. (2014) for the drying
319 process of sodium alginate films. These authors attributed this behavior to the
320 evaporation of water.

321 SEM micrographs of fractured surface revealed that heat treatment affected the internal
322 microstructure of films, Figure 10. ODF showed a more homogeneous structure when
323 compared with ODFH sample. Cross-section of ODFH heated for 24 min at $180 \text{ }^\circ\text{C}$
324 showed a reduced thickness and a laminar structure produced by strong dehydration.
325 This heterogeneous matrix of ODFH is an indicator of the loss of structural integrity,
326 and consequently higher mechanical brittleness.

327

328 **4. Conclusions**

329 Dry calcium alginate films of adequate size and mechanical properties, suitable to be
330 used as edible films, were obtained using a novel device in which a sodium alginate
331 solution was nebulised with a calcium gluconolactate solution as gelling agent. The dry
332 films obtained were then subjected to heat treatment at $180 \text{ }^\circ\text{C}$ for different times and

333 physicochemical characteristics of the resultant products were analyzed. Heat treatment
334 produced thickness reduction, a yellow ochre color development and an increase in the
335 brittleness of the dry films. DSC, FTIR and SEM studies suggested that the changes
336 observed may be attributed to further dehydration of dry films and to the first steps of
337 thermal degradation of alginate macromolecules. These studies point to a potential
338 application of these heat-treated films as enhancers of crisp texture and optical
339 properties of foodstuffs.

340

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Figure captions

Figure 1. Experimental setup for formation of planar alginate hydrogels by external gelling method.

Figure 2. Thickness of films prepared with different sodium alginate concentrations in presence of different calcium gluconolactate concentrations in the nebulizer reservoir. Each value is the mean of three replicates. Error bars indicate standard deviations. Different letters above columns indicate significant differences ($p < 0.05$).

Figure 3. Tensile strength of films prepared with different sodium alginate concentrations in presence of different calcium gluconolactate concentrations in the nebulizer reservoir. Each value is the mean of three replicates. Error bars indicate standard deviations. Lower case letters indicate significant differences between sodium alginate concentrations for each calcium gluconolactate solution assayed ($p < 0.05$). Upper case letters indicate significant differences among calcium gluconolactate concentrations for each sodium alginate solution used ($p < 0.05$).

Figure 4. Elongation of films prepared with different sodium alginate concentrations in presence of different calcium gluconolactate concentrations in the nebulizer reservoir. Each value is the mean of three replicates. Error bars indicate standard deviations. Lower case letters indicates significant differences between sodium alginate concentrations for each calcium gluconolactate solution assayed ($p < 0.05$). Upper case letters indicates significant differences among calcium gluconolactate concentrations for each sodium alginate solution used ($p < 0.05$).

Figure 5. Photographs of heated optimum dry film samples.

Figure 6. Tensile strength of heated optimum dry film samples. Each value is the mean of three replicates. Error bars indicate standard deviations. Different letters above columns indicate significant differences ($p < 0.05$).

Figure 7. Elongation of heated optimum dry film samples. Each value is the mean of three replicates. Error bars indicate standard deviations. Different letters above columns indicate significant differences ($p < 0.05$).

Figure 8. Differential scanning calorimetry thermograms of (a) optimum dry film, (b) sodium alginate, (c) calcium lactate hydrate and (d) calcium gluconate anhydrous samples.

Figure 9. Fourier transform infrared spectra of optimum dry film: (—) and optimum dry film heated for 24 min at 180 °C: (—•—).

Figure 10. Scanning electron microscopy micrographs of (a) optimum dry film and (b) optimum dry film heated for 24 min at 180 °C.

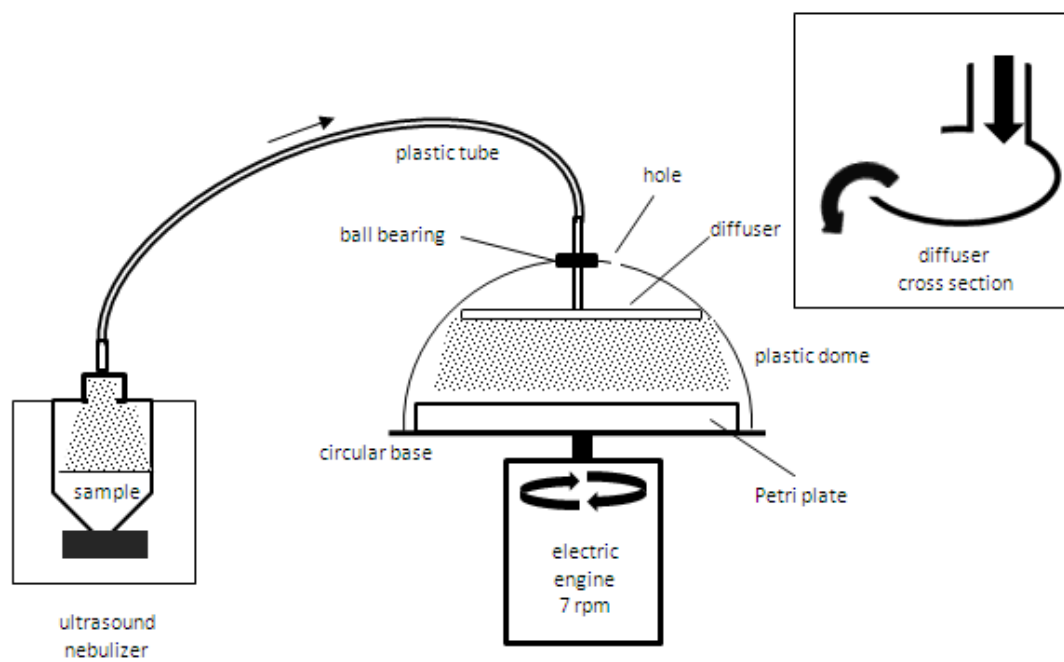


Figure 1

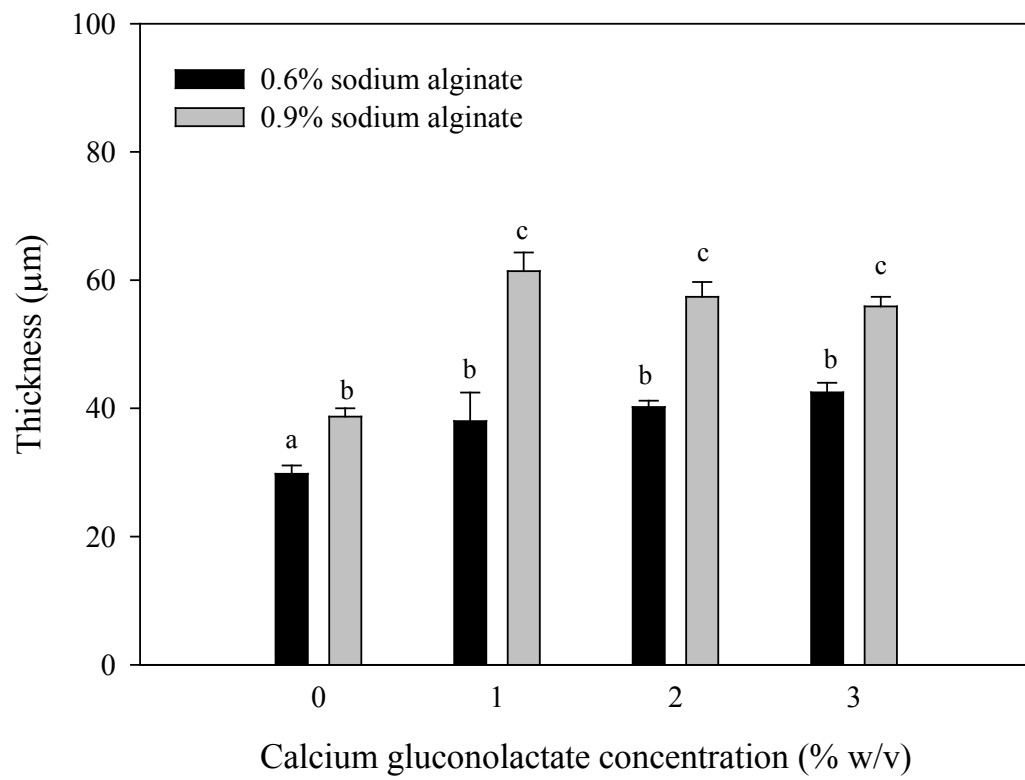


Figure 2

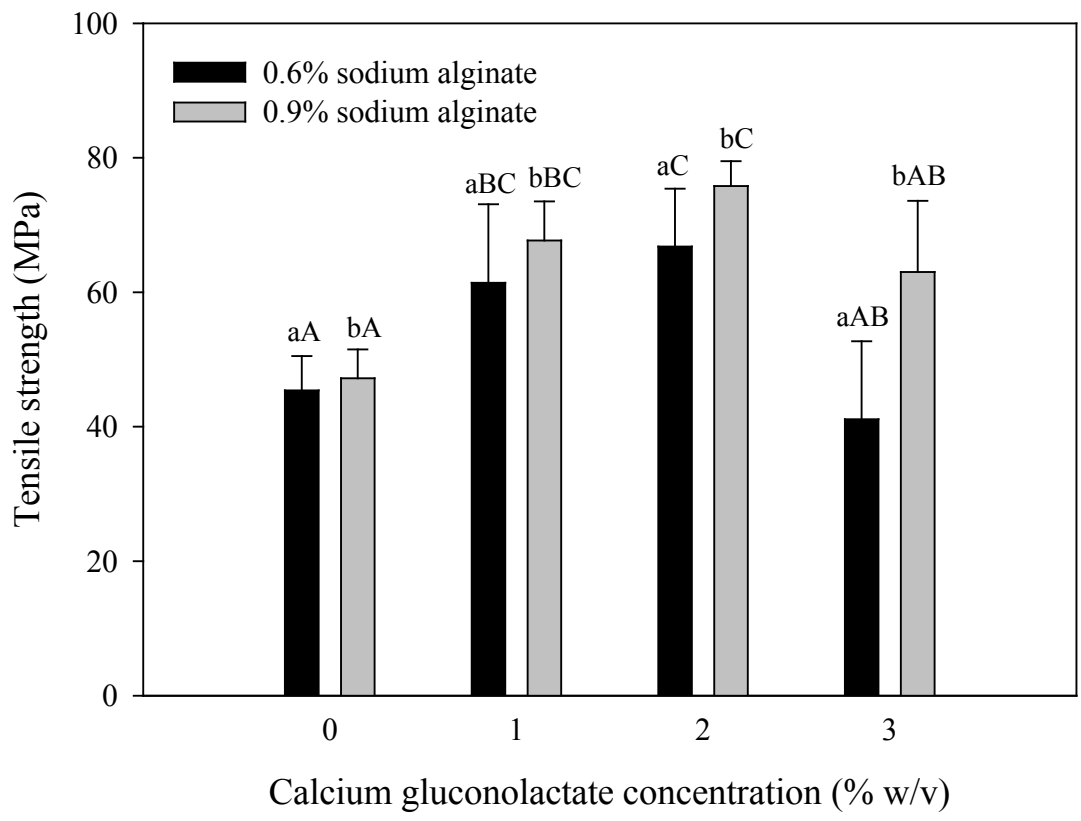


Figure 3

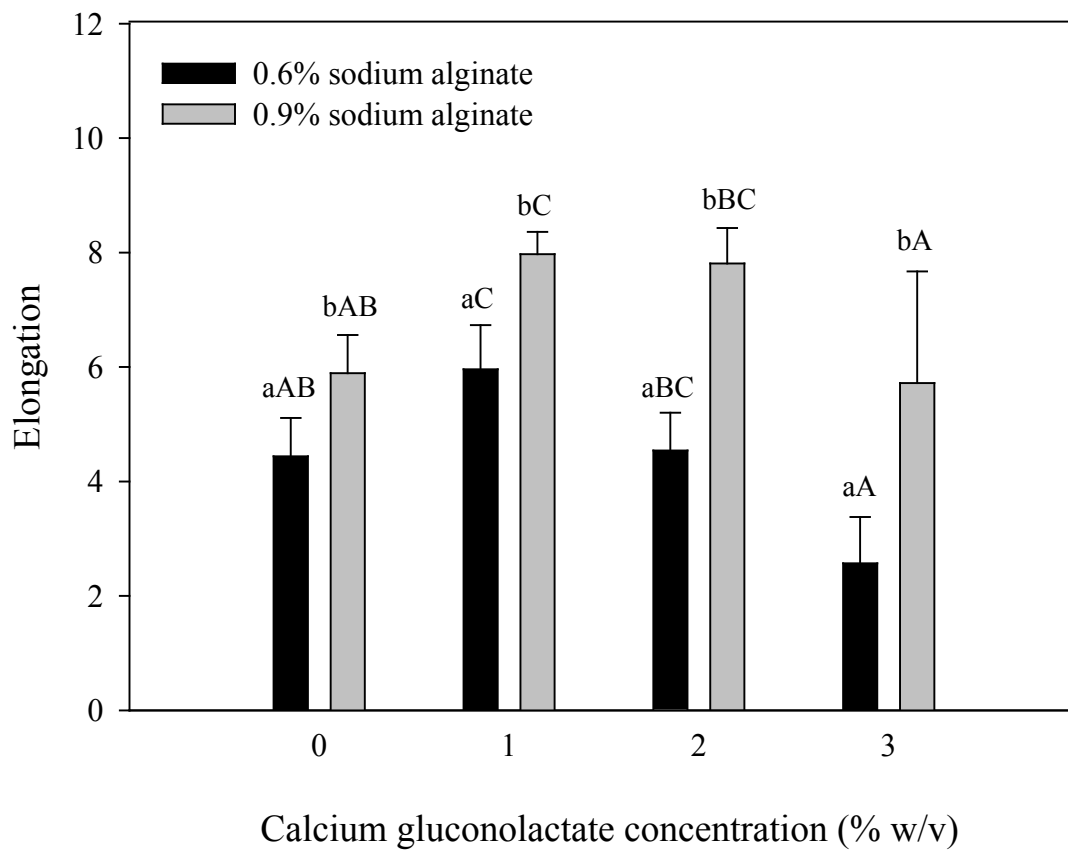


Figure 4

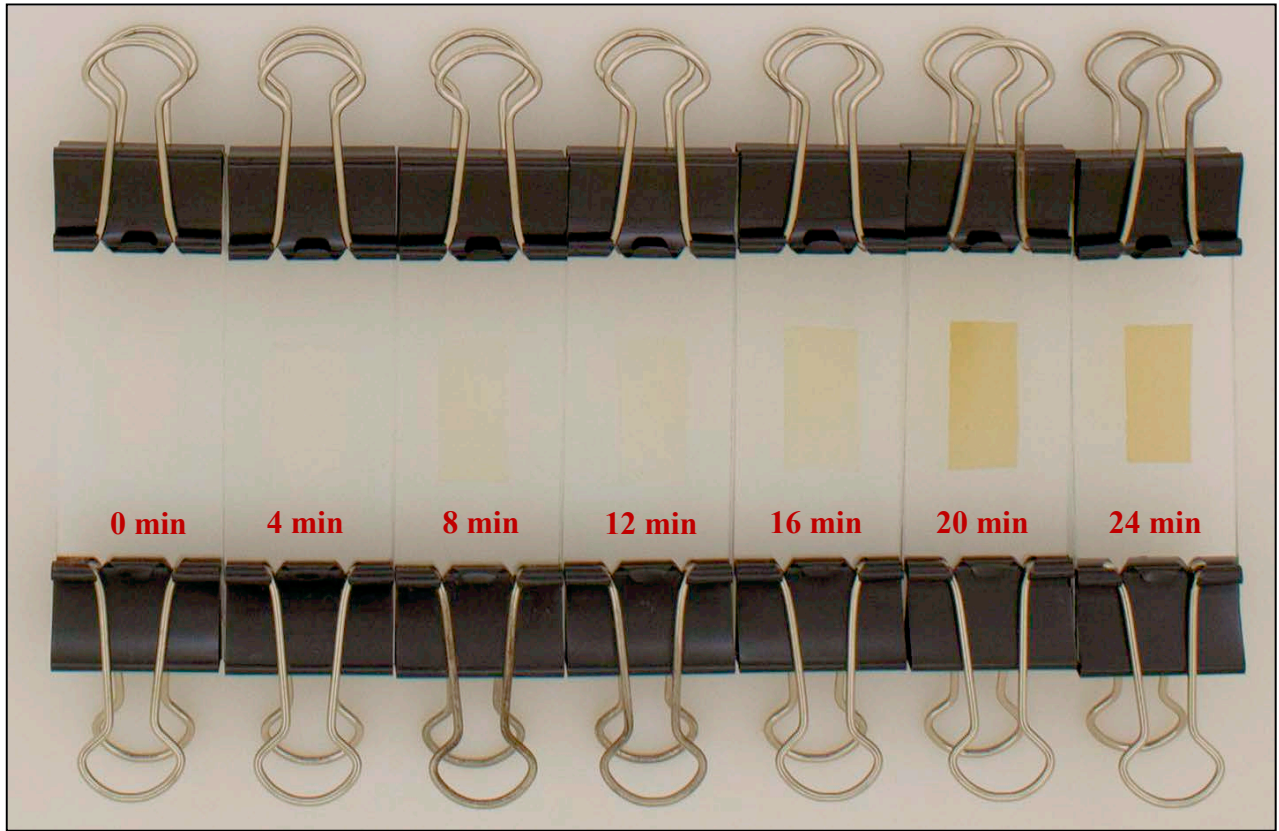


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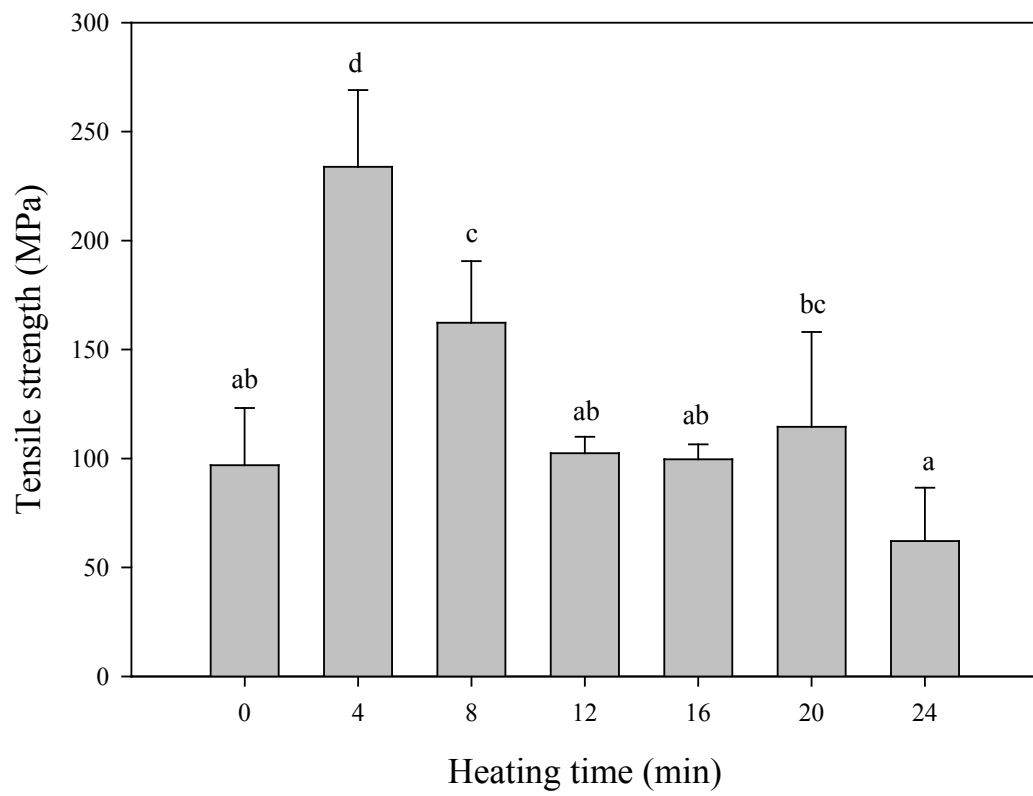


Figure 6

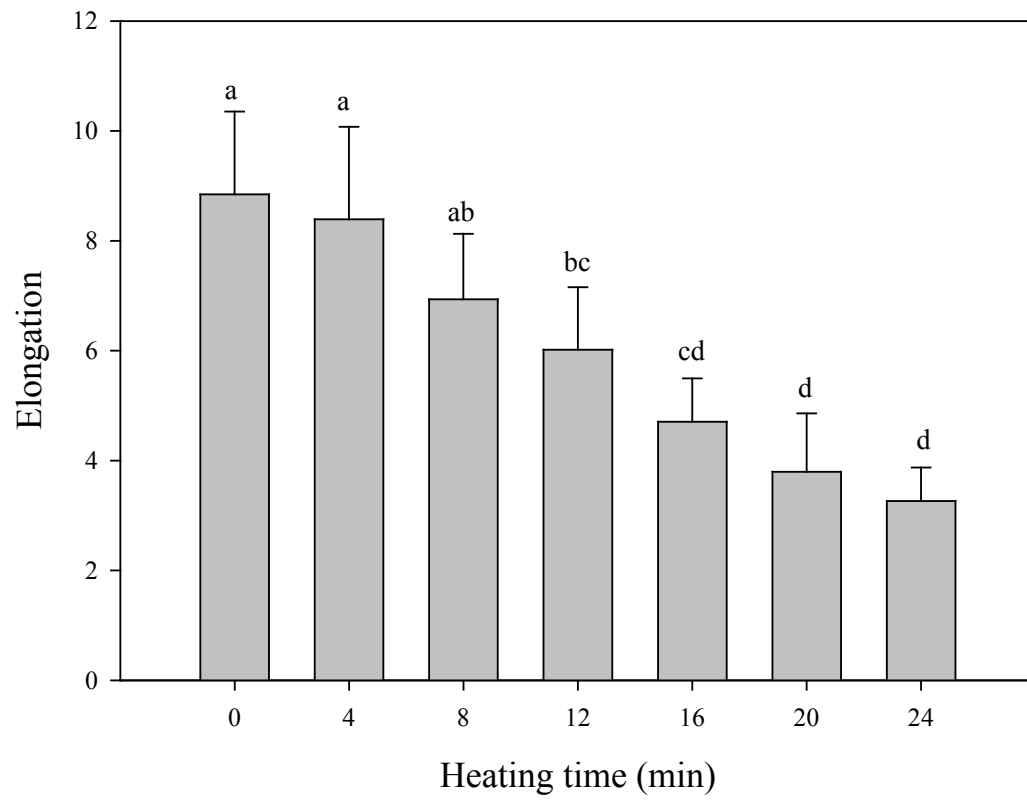


Figure 7

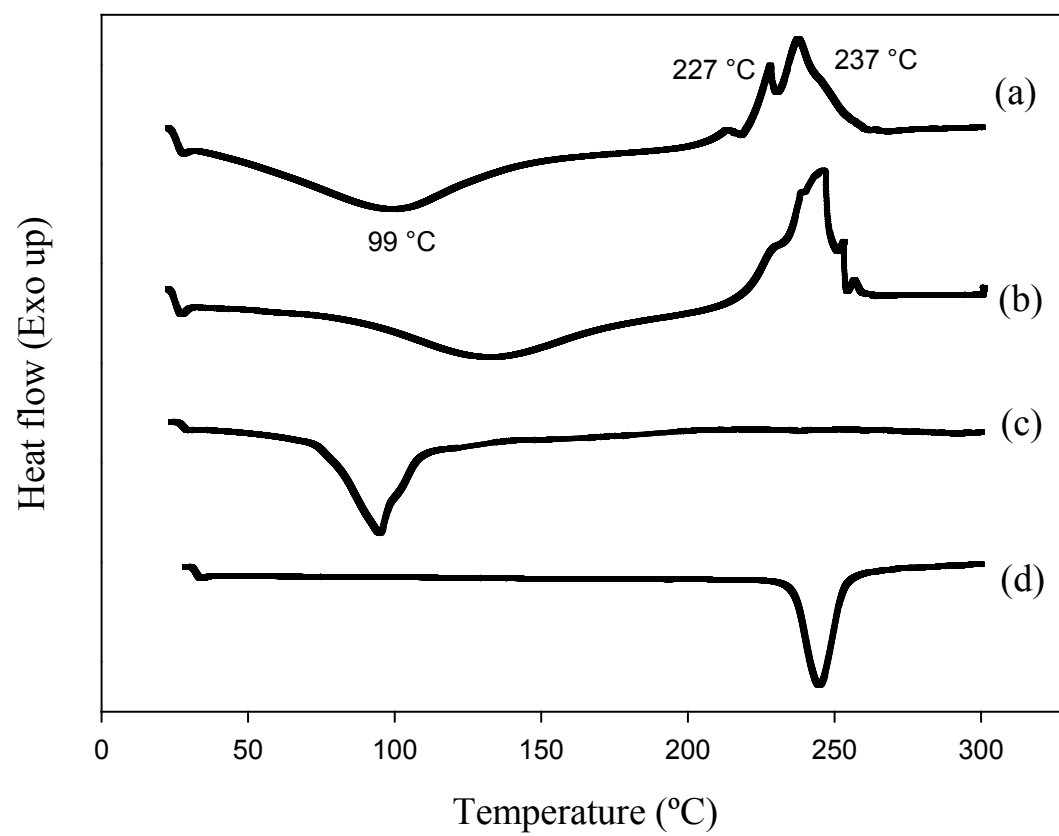


Figure 8

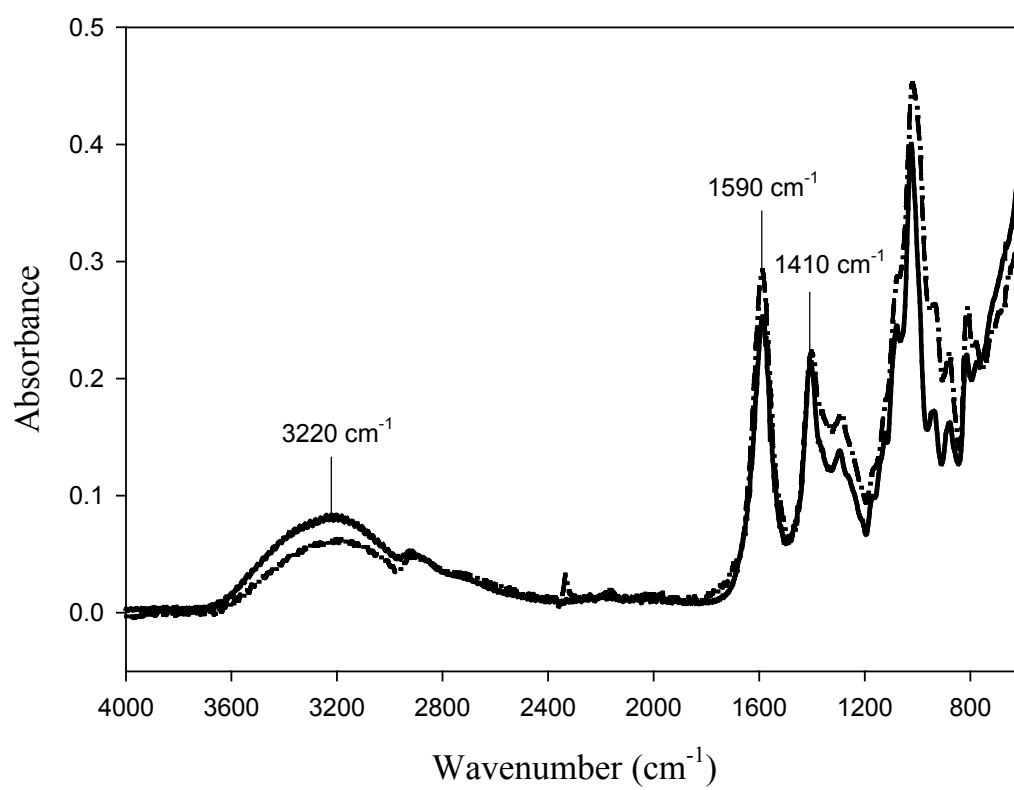


Figure 9