Section: Food Engineering and Materials Science

1. Title page:

Physicochemical characterization of a heat treated calcium alginate dry film prepared with chicken stock

Short title:

Heated film of alginate-chicken stock

Germán D. Báez[a,b], Gisela N. Piccirilli[c,d], Griselda A. Ballerini[e], Agustín Frattini[f], Pablo A. Busti[a], Roxana A. Verdini[c,d], and Néstor J. Delorenzi[a]*

[a]Área Tecnología de los Alimentos, Departamento de Tecnología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina

[b]Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, CONICET, Suipacha 531, 2000 Rosario, Argentina

[c]Área Bromatología y Nutrición, Departamento de Ciencias de los Alimentos y del Medio Ambiente, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina

[d]Instituto de Química Rosario (UNR-CONICET), Suipacha 570, 2000 Rosario, Argentina

[e]Centro de Investigaciones y Desarrollo en Tecnología de los Alimentos, Universidad Tecnológica Nacional, Facultad Regional Rosario, Estanislao Zeballos 1341, 2000 Rosario, Argentina

[f]Área Física, Departamento de Química-Física, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina

* Corresponding author. Tel/Fax: 54-341-4804598

E-mail address: ndeloren@fbioyf.unr.edu.ar

2. Abstract

Solid sodium alginate was dissolved into chicken stock in order to give a final alginate concentration of 0.9% (w/v). Calcium ions present in chicken stock were enough to induce ionic gelation. After drying, Fourier transform infrared spectroscopy, thickness and mechanical properties of films obtained were determined. Calcium alginate-chicken stock films were heated at 130 °C for different times between 0 and 15 min. Mechanical and optical studies, differential scanning calorimetry, visual aspect and scanning electron microscopy were carried out to describe physicochemical properties of heat treated films. Heating developed a maroon ochre color and increased the brittleness (crispness) of the films related to the intensity of the treatment. Differential scanning thermometry and study on appearance of the films suggested that Maillard reactions may be responsible for the observed changes. Maillard reactions mainly occurred between reducing sugar monomers and free amino groups of gelatin peptides present in the chicken stock, and between alginate and gelatin peptides to a lesser extent. In addition, the plasticizing effect of fat added with chicken stock was also studied. These studies suggest a potential use of heat treated chicken stock films as a substitute of roasted chicken skin.

Key Words: edible film, calcium alginate, chicken stock, heat treatment, physicochemical characterization.

3. Practical Application

Crisp texture and optical properties of heat treated calcium alginate-chicken stock films obtained in this work were similar to roasted chicken skin. Therefore, this information can be used by product developers, culinary scientists and professional chefs in designing food products in which these kinds of films are employed to wrap chicken meat pieces that are then subjected to cooking. This study provides the basis for the preparation of healthier alternatives to traditional roasted skin through reduction dangerous components, such as fat (including cholesterol) and carcinogenic compounds, without loss of overall flavor intensity.

4. Introduction

Among the great variety of polysaccharides used for the preparation of hydrogels, one of the most important is sodium alginate (Draget 2000). The proportion and sequence of α-L-guluronic and β-D-mannuronic acids, the gelling ions present in the environment and the conditions of gelation determine the microstructure that controls the physicochemical properties of alginate gels. When divalent cations, mainly Ca^{2+}, interact with blocks of guluronic acid residues, alginate gelation occurs. The microstructure of the gel is well described by the "egg-box" model (Grant and others 1973).

Alginate gels, unlike most polysaccharides gels, can be heated without melting. The production of cold setting gels and the stability to heat treatment allow the use of alginate in baking creams (Smidsrød and Draget 2004), in edible coatings either to

75 reduce the absorption of fats in foods subjected to frying (Albert and Mittal 2002) or to
76 improve quality attributes of microwaveable chicken nuggets (Albert and others 2012).
77 On the other hand, edible films can be made by drying a thin layer of wet gel. Since
78 fragility can limit their potential for wrapping foods, flexibility, workability and
79 distensibility of these products can be improved by the presence of plasticizer
80 compounds, such as polyols and lipids (Viera and others, 2011).
81 In a recent paper, our group analyzed the physicochemical characteristics of calcium
82 alginate which underwent heat treatment (different times at 180 °C) and observed an
83 ochre color development and an increased brittleness of the material. Differential
84 scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), and
85 scanning electron microscopy (SEM) studies suggested that heating produced a
86 dehydration of films, following by dehydration and degradation of alginate
87 macromolecules. A potential application of heat treated films to enhance crispness and
88 appearance of foodstuffs was an interesting result of our investigations.
89 The roasted chicken skin is quite crispy and fragrant, however, since roasted skin
90 contains a great deal of fat (~ 40% (w/w)), and cancer-causing compounds, many
91 people remove it for health reasons. On the other hand, chicken stock is made by
92 extraction from bones (with some residual chicken meat attached), vegetables, herbs,
93 and spices. The components extracted from the ingredients (proteins, free amino acids,
94 minerals, volatile compounds, fat, etc.) contribute to a chicken stock overall flavor. In
95 home-prepared chicken stock, fat represents less than 1.2% (w/w) of total components.
96 In this work, it was intended to develop a heat treated calcium alginate edible film
97 prepared with chicken stock, to be used as an alternative to roasted chicken skin in order
98 to reduce previously described disadvantages. The principal aim of the present work

was to study the physicochemical characteristics, such as mechanical, optical and structural properties of the developed films.

5. Materials and Methods

Materials

Sodium alginate (SA) from brown algae (mannuronic/guluronic ratio of ~ 1.56, degree of polymerization range of 400-600, molecular weight of 80000-120000 and medium viscosity), calcium gluconate anhydrous and calcium lactate hydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solid calcium lactate hydrate and calcium gluconate anhydrous were mixed at a weight ratio of 4:1 (this mixture was named calcium gluconolactate). Commercial bovine gelatin (Low Bloom) was kindly provided by PB Leiner (Santa Fe, Argentina). Glucose was obtained from Cicarelli (Rosario, Argentina). Other reagents used in this work were of analytical grade.

Chicken stock recipe

Chicken stock was prepared using 2 kg of roasted chicken carcass and a *mirepoix* consisting of 250 g of onion, 150 g of carrot, 150 g of celery and 150 g of leek. All the ingredients were placed in a stock pot and covered with cold water. The mixture was brought to the boil and then, 1 g of thyme, 1 g of rosemary, 1 g of parsley and 0.8 g of black peppercorn were added. After simmered very gently for 6 hours, the chicken stock was filtered and clarified using egg white. Finally chicken stock was filtered again, aliquoted and stored at – 20 °C.

Chemical analysis of chicken stock

123 Chicken stock proximate composition was determined in triplicate according to the
124 Association of Official Agricultural Chemists International methods (AOAC 2002):
125 humidity (method 925.10); proteins (method 920.87, factor 6.25), fat (method 932.06),
126 ash (method 923.03). Total carbohydrates were determined by difference. Calcium
127 content was measured by atomic absorption spectroscopy using an Unicam Solaar
128 equipment (Model 969, Unicam Ltd., Cambridge, UK). Glucose concentration in the
129 chicken stock was determined by the methodology proposed by Trinder (1969).
130 Peptide content of chicken stock and bovine gelatin was determined by high-
131 performance liquid chromatography (HPLC) (Shimadzu LC-10, Kyoto, Japan) using a
132 calibrated size exclusion HPLC column Bio SEC-3000 (Phenomenex, Torrance, CA,
133 USA) (300 mm X 7.8 mm). The chromatography conditions were: eluent, buffer
134 phosphate 100 mM, 100 mM NaCl, pH 6.8; flow rate, 0.8 mL min^{-1}; injection volume,
135 20 μL; peptide concentration, 5 mg mL^{-1}; temperature, 25 °C and wavelength detection,
136 280 nm.
137
138 **Preparation of dry films**
139 Solid SA was gently added to chicken stock in order to give a final SA concentration of
140 0.9 % (w/v). The mixture was stirred for 12 h until homogeneity was obtained. After
141 that, the solution was heated at 60 °C, degassed and 88 g of it were poured into plastic
142 Petri plates of 13.5 cm in diameter. The plates were left to rest at 25 °C for 3 h and then
143 introduced into an oven for 3 h at 50 °C. The dry films were withdrawn from Petri
144 dishes and stored in plastic containers. These calcium alginate-chicken stock dry films
145 were named Alg-St.
146 To study the possible plasticizing effect of fat from chicken stock on Alg-St films, a
147 portion of chicken stock was defatted by extraction with petroleum ether. Using this

148 defatted chicken stock, dry films were prepared in accordance with the methodology
149 described above. These films were called Alg-DSt. The fat content of Alg-St and Alg-
150 DSt samples were determined by the same method used in the chemical analysis of
151 chicken stock.
152 Dry films without stock, but with the same final calcium concentration were also
153 prepared (Alg-Ca). The source of gelling cation was a calcium gluconolactate solution
154 (Soazo and others 2015a). Final SA and calcium concentrations were the same for all
155 the films assayed in this work.
156 To characterize the importance of different chicken stock components, such as proteins
157 and carbohydrates, on FTIR analysis and appearance of films of Alg-St, some dry films
158 were made by adding to Alg-Ca formulations either gelatin (Alg-Ca-Gel) or
159 gelatin/glucose (Alg-Ca-Gel-Glu). Gelatin and glucose concentration in these samples
160 were equal to total protein and total carbohydrate content in the chicken stock used.
161 These values were taken from the chemical analysis of chicken stock.
162 Dry films with physical defects such as air bubbles, holes and cracks were discarded.
163 The selected films were stored at 25 °C and 55 % RH for 24 h until testing.
164
165 **Fourier transform infrared (FTIR) spectroscopy**
166 FTIR spectra of Al-Ca, Alg-St, Alg-Ca-Gel and Alg-Ca-Gel-Glu were determined using
167 an IR-Prestge-21 spectrophotometer (Shimadzu, Kyoto, Japan) (Soazo and others
168 2015a).
169
170 **Heat treatment of calcium alginate-chicken stock dry films**
171 Alg-St dry film samples were put into a Teflon™ coated cooking vessel. These films
172 were heated in an electric convection oven (Zonda, Rosario, Argentina) (2700 W, 50

Hz, 220 V) at 130 °C in order to study the effect of heating. Different samples were removed at different times of heating (0, 5, 10 y 15 min).

Thickness of films

The thickness of three replicates of films assayed in this work (Alg-St, Alg-DSt, Alg-Ca and heated Alg-St) was measured using an electronic digital disk micrometer (Schwyz™, China) at nine locations of each film.

Mechanical properties of films

Tensile test of films assayed in this work (Alg-St, Alg-DSt, Alg-Ca and heated Alg-St) was fulfilled using a texturometer (Mecmesin Multitest 2.5*d*, Mecmesin, Sterling, VA, USA) provided with a 100 N digital force gauge. Strips (7 x 60 mm) of each sample were clamped between tensile grips in triplicate. Distance between grips was 30 mm and crosshead speed was 0.05 mm s^{-1}. Mechanical parameters determined from stress-strain plots were: tensile strength (TS), elongation (E) and elastic modulus (EM). TS was calculated by dividing the peak load by the cross sectional area (film thickness x 7 mm) of the initial film. E was determined as the percentage of change in the length of each sample respect to the initial distance between grips. EM was determined from the slope at the origin of stress-strain plots. While TS is a measure of the maximal force per original cross-sectional area that the film could support before breaking, E estimates the capacity of the film to extend before breaking (Da Silva and others 2009) and EM measures the stiffness of the material.

Opacity

Opacity of heat treated Alg-St sample was determined following the methodology proposed by of Siripatrawan and Harte (2010). Rectangular pieces of film samples (10 x 30 mm) were located on the internal side of a spectrophotometer cell (Jasco V-550, Tokyo, Japan). Light absorbance of the film samples was measured at 600 nm (Abs_{600}). Opacity was calculated using the equation:

$$\text{Opacity} = Abs_{600} \ / \ l \qquad (1)$$

where l is the film thickness in mm.

Color measurements of heat treated films

The design described by Mendoza and Aguilera (2004), with some modifications (Soazo and others 2015b), was used to measure heat treated Alg-St color. L^* (lightness), a^* (red-green), b^* (yellow-blue) and ΔE (total color difference) were determined following the works of Yam and Papadakis (2004) and Soazo and others (2015a). It has been noted that ΔE quantifies the difference between sample color parameters and a white plate standard**.**

Differential scanning calorimetry studies

Thermal properties of Alg-Ca and Alg-St were determined using a differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan). Approximately, 10 mg of each dried films were sealed into aluminum pans and scanned over a range between 30 and 350 °C with a heating rate of 10 °C min^{-1}, while an empty aluminum pan was used as reference. Each one of the samples was run in triplicate.

Appearance of heat treated films

Alg-Ca, Alg-Ca-Gel and Alg-Ca-Gel-Glu dry films were subjected to heat treatment at 130 °C for 15 min. After cooling to room temperature, a portion of each sample was photographed by a digital camera. These studies were made in order to infer the influence of different chicken stock components on Alg-St heating process.

Scanning electron microscopy studies

To study the influence of heat treatment on Alg-Ca and Alg-St microstructures, SEM experiments were carried out. Film samples were cryo-fractured, fixed on bronze stubs at an angle of 90° to the surface and gold coated in vacuum. Micrographs of films cross-section were taken with a scanning electron microscope (AMR 1000, Leitz, Wetzlar, Germany). Photographs were taken at an accelerating voltage of 20 kV and at a magnification of 500X.

Statistical analysis

Statgraphics Plus for Windows (Manugistics Inc, Rockville, MA, USA) was used to perform statistical analysis of the data obtained. The test of multiple ranks honestly significant difference (HSD) of Tukey was applied (95% of confidence level) when the effect of the factors obtained by the analysis of variance (ANOVA) was significant ($p<0.05$).

6. Results and Discussion

Chemical composition of chicken stock and gelatin

Table 1 shows the chemical composition of chicken stock prepared in the present work. The composition was similar to that called as initial chicken stock by McGee (2004), in

247 which more than 90% is water and 3–4% is dissolved meat components. The calcium
248 content was enough to obtain optimal calcium alginate gels for forming dry films (Alg-
249 St) without further aggregation of the cation (Soazo and others 2015a). Protein and
250 peptides are the major structural component of animal stocks. It is well known that
251 gelatin, resulting from the hydrolysis of collagen present in connective tissue, is the
252 principal ingredient of this culinary preparation. In the chicken stock used in this work,
253 the molecular weight of gelatin peptides, determined by HPLC, ranged from 0.35 to 63
254 kDa (Figure 1). Total lipid (fat) includes mainly emulsified triglycerides, free fatty
255 acids, small amount of essential oils, and traces of cholesterol. As it can be seen in
256 Table 1, total lipids were a minor component of the chicken stock used in this study.
257 On the other hand, commercial bovine gelatin used in chemical studies contained ~ 60
258 % of peptides between 0.35 and 63 kDa (Figure 1) and slightly higher lysine content
259 than chicken gelatin (~ 1.04 %) (Norizah and others 2013).
260
261 **Fourier transform infrared (FTIR) spectroscopy**
262 Infrared spectrum of Alg-Ca (Figure 2) showed the characteristics peaks of alginate: OH
263 stretching vibrations at approximately 3220 cm^{-1}, and asymmetric COO^- and symmetric
264 COO^- stretching peaks near 1590 and 1410 cm^{-1} (Soazo and others 2015a).
265 Figure 2 also shows that Alg-Ca and Alg-St infrared spectra had similar patterns in the
266 range from 2800 to 3600 cm^{-1}. However, these spectra were quite different for
267 wavenumbers between 800 and 1700 cm^{-1}. In this range, Alg-St spectrum resembled
268 Alg-Ca-Gel infrared spectrum, as it can be appreciated in Figure 2. For proteins, typical
269 infrared features are amide I and amide II bands, located in the regions between 1600
270 and 1700 cm^{-1} and between 1500 and 1600 cm^{-1}, respectively. Amide I band is
271 primarily due to CO stretching vibrations, and NH bending is the principal responsible

for amide II band. It is evident that amide I and amide II gelatin bands (peaks at 1643 and 1561 cm^{-1}, respectively) overlap alginate bands in the range from 800 to 1700 cm^{-1}. In addition, Alg-Ca-Gel-Glu and Al-Ca-Gel spectra were similar (results not shown). Therefore, spectra comparisons emphasized the relevant importance of gelatin in determining the infrared spectrum of Alg-St.

Thickness and mechanical properties of films

The determined fat content in dry films was ~ 0.7% (w/w) for Alg-St and ~ 0.02% (w/w) for Alg-DSt. Besides this, Table 2 shows the thickness of the dry films assayed in this work. This property varied significantly in the following order: Alg-St > Alg-DSt > Alg-Ca, indicating that the presence of fat may play a determining role in the physical properties of these samples. Moreover, the remaining amount of fat present after solvent extraction could be responsible for the intermediate behavior of Alg-DSt. In addition to the effect on thickness, TS of dry films significantly decreased as fat concentration increased, while E significantly increased as fat concentration increased (Table 2). Similar effects were obtained by Benavides and others (2012) studying the addition of oregano essential oil in alginate films. The trend observed is also consistent with other results obtained previously by Cagri and others (2001), Pranoto and others (2005) and Rojas-Graü and others (2007). These authors concluded that the addition of oils usually reduces TS as a result of the discontinuities in film structure developed by their presence. Moreover, because oils present in chicken stock are liquid at room temperature, oil droplets can easily be deformed, enhancing in this way the extensibility of films (Fabra and others 2008). Calcium alginate films present a high internal ionic interaction between alginate and calcium ions. The presence of fat in the film-forming dispersion may interfere the crosslinking, reducing intermolecular forces between

297 polymer chains and thus improving the chain mobility and flexibility. Therefore, in this
298 study, fat acted as a plasticizer, reducing TS and increasing E of dry films, Table 2.
299 It has been noted that the values of TS, E and EM for Alg-Ca were similar to those
300 reported previously (Rhim 2004; Soazo and others 2015a) and were similar to the
301 values corresponding to cellophane, a quite stiff material. The presence of fat in alginate
302 films reduced more than 300 times the stiffness of the material. As a result of these
303 effects, Alg-St films obtained in this work possessed adequate flexibility and
304 manageability properties to be used as food wrapping.
305
306 **Mechanical properties of heat treated films**
307 Soazo and others (2015a) observed that calcium alginate dry films tolerate heating up to
308 180 °C for 24 min. However, the heating of Alg-St at higher temperatures produced the
309 immediate calcination of the sample. After several tests were performed, a temperature
310 of 130 °C was chosen to avoid this problem. Thickness of Alg-St was not significantly
311 modified by heating at 130 °C up to 15 min, Table 3. Soazo and others (2015a) reported
312 that thickness of heated calcium alginate films (Alg-Ca) dramatically decreased due to a
313 dehydration process, but this is not the case for heated Alg-St. Therefore, other
314 processes must be taken into account.
315 On the other hand, Table 3 shows that the increase in heating time led to an increase in
316 TS. Although heat treatment rapidly diminished E values, a greater increase in the time
317 of treatment did not modify significantly film deformation. Heat treatment had a
318 significant effect on film stiffness as EM values show. All the changes observed in
319 mechanical properties in function of heating time produced an appreciable increase in
320 brittleness (and therefore in crispness) of Alg-St. For heating times longer than 15 min,
321 Alg-St sample was calcined.

Optical properties of heat treated films

The visual aspect of heat treated Alg-St sample is shown in Figure 3 and optical parameters are presented in Table 4. Opacity is a measurement of film transparency. The higher the value of opacity, the lesser is transparency of the film (Pereda and others 2011). The increase in opacity with heating time may be attributed to the increase in light absorption, since thickness was not modified significantly (Table 3 and Table 4). The treated sample changed their appearance from a brown color with a moderated opacity to an intense opaque maroon color in function of the time of heating. ΔE increased approximately twice for 15 min of heating. This color change was principally promoted by lightness (L^*) and redness (a*), while yellowness (b*) played a minor role.

Differential scanning calorimetry of films

The endothermic peak observed in Alg-Ca films near 100 °C corresponds to the cross-linked gel matrix dehydration (Soazo and others 2015a; Taha and others 2008), Figure 4. The exothermic peak observed at temperatures near 235 °C results from alginate degradation due to dehydration and depolymerization of the protonated carboxylic groups and oxidation reactions of carbohydrate macromolecules (Soazo and others 2015a; Sarmento and others 2006). However, Alg-St thermogram was quite different from that obtained for Alg-Ca. The dehydration zone around 100 °C disappeared, while an endothermic peak between 120 and 175 °C was generated. In addition, the sharp exothermic peak observed at 236 °C, related to the degradation of alginate, was drastically reduced and the maximum was shifted to 229 °C. These results indicated that alginate molecules are involved in different types of processes that determine the dissimilarity between Alg-St and Alg-Ca thermograms. A similar finding was reported

by Liang and others (2014) in the formation of ε-polylysine-chitosan. The authors suggested that this conjugate implies a Maillard reaction between the reducing end of chitosan and the amino groups of ε-polylysine. They also observed that in the final stages of this reaction a characteristic browning of the system was produced. In the case of Alg-St samples, it is possibly that the reducing end of alginate reacted with gelatin amino groups. In addition, Oates and Ledward (1990) reported that at temperatures lower than 140 °C a moderate depolymerization of alginate macromolecules take place. This depolymerization results in an increased reactivity as more end groups are made available.

On the order hand, it should also be noted that the presence of reducing sugar monomers in Alg-St, such as glucose, can react with free amino groups of gelatin peptides to form conjugates via Maillard reaction. This reaction could also be included in the endothermic peak of Alg-St thermogram.

Appearance of heat treated films

Heat treated Alg-Ca is practically a transparent and colorless material, Figure 5 (a). Heated Alg-Ca-Gel showed a mild browning color supporting the presence of a moderate alginate/gelatin Maillard reaction, Figure 5 (b). However, it can be seen in Figure 5 (c) that heated Alg-Ca-Gel-Glu exhibited an intense ochre color due to glucose/gelatin reaction.

The results presented in this section strengthen the hypothesis that the reactions that occur during heating of Alg-St dry films at 130 °C correspond to Maillard reactions, mainly between reducing sugar monomers and free amino groups of gelatin peptides, and between alginate and gelatin peptides in a lesser degree.

372 **Scanning electron microscopy of films**

373 Cross-section of Alg-Ca heated at 130 °C for 15 min showed a reduced thickness
374 produced by dehydration of the untreated film (Soazo and others 2015a), Figure 6(a_1)
375 and Figure 6(a_2). Instead of this, the cross section of heated Alg-St did not vary
376 significantly in thickness with treatment, Figure 6(b_1) and Figure 6(b_2). In addition,
377 untreated Alg-St presented an increased thickness in comparison with untreated Alg-Ca.
378 Similar result was presented in Table 2. SEM micrographs revealed that the internal
379 microstructure of dry films was affected by the presence of fat (Benavides and others
380 2012). In that sense, Alg-St showed a more heterogeneous structure when compared
381 with Alg-Ca, presenting a laminar type matrix.

382

383 **7. Conclusions**

384 In this work, a heat treated calcium alginate dry film prepared with chicken stock was
385 developed. Heat treatment of the dry film at 130 °C for 15 min produced the
386 development of an opaque maroon color and an increased brittleness of films. DSC and
387 chemical studies suggested that the observed changes may be attributed to Maillard
388 reactions, principally between glucose and gelatin peptides, and between alginate and
389 gelatin peptides in a lesser extent. These studies suggest a potential use of heat treated-
390 chicken stock films as a substitute of roasted chicken skin since crisp texture and optical
391 properties were alike. However, sensory studies are needed before the product can be
392 recommended.

393

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396

9. Author Contributions

Germán D. Báez, First author

Gisela N. Piccirilli, execution

Griselda A. Ballerini, HPLC measurements

Agustín Frattini, DSC measurements

Pablo A. Busti, interpretation and writing

Roxana A. Verdini, interpretation and writing

Néstor J. Delorenzi, Corresponding author

10. References

Albert S, Mittal GS. 2002. Comparative evaluation of edible coatings to reduce fat uptake in a deep-fried cereal product. Food Res. Int., 35(5): 445-458.

Albert A, Salvador A, Fiszman SM. 2012. A film of alginate plus salt as an edible susceptor in microwaveable food. Food Hydrocoll., 27(2): 421-426.

AOAC. 2002. Official Methods of Analyses of Association of Analytical Chemist. 17th ed. Washington DC, USA.

Benavides S, Villalobos-Carbajal R, Reyes JE. 2012. Physical, mechanical and antibacterial properties of alginate film: effect of the crosslinking degree and oregano essential oil concentration. J. Food Eng., 110(2): 232-239.

420 Cagri A, Ustunol Z, Ryser ET. 2001. Antimicrobial, mechanical and moisture barrier
421 properties of low pH whey protein-based edible films containing p-aminobenzoic or
422 sorbic acids. J. Food Sci., 66(6): 865-870.
423
424 Draget KI. 2000. Alginates. In: Phillips GO, Williams PA, editors. Handbook of
425 hydrocolloids. Boca Raton: CRC Press. p. 379-395.
426
427 Da Silva MA, Bierhalz ACK, Kieckbusch TG. 2009. Alginate and pectin composite
428 films crosslinked with Ca^{2+} ions: effect on the plasticizer concentration. Carbohyd.
429 Polym., 77(4): 736-742.
430
431 Fabra MJ, Talens P, Chiralt A. 2008. Tensile properties and water vapor permeability of
432 sodium caseinate films containing oleic acid-beeswax mixtures. J. Food Eng., 85(3):
433 393-400.
434
435 Grant GT, Morris ER, Rees DA, Smith PJC. 1973. Biological interactions between
436 polysaccharides and divalent cations: the egg-box model. FEBS Lett., 32(1): 195-198.
437
438 Liang C, Yuan F, Liu F, Wang Y, Gao Y. 2014. Structure and antimicrobial mechanism
439 of ε-polylysine-chitosan conjugates through Maillard reaction. *International* J. Biol.
440 Macromol., 70: 427-434.
441
442 McGee H. 2004. On food and cooking: the science and lore of the kitchen. 2nd ed. New
443 York, NY: Scribner. p. 601-602.
444

445 Mendoza F, Aguilera JM. 2004. Application of image analysis for classification of
446 ripening bananas. J. Food Sci., 69(9): 474-477.
447
448 Norizah MS, Badii F, Howell NK. 2013. Preparation and characterisation of chicken
449 skin gelatin as an alternative to mammalian gelatin. Food Hydrocoll., 30(1): 143-151.
450
451 Oates CG, Ledward DA. 1990. Studies on the effect of heat on alginates. Food
452 Hydrocoll., 4(3): 215-220.
453
454 Pereda M, Amica G, Rácz I, Marcovich NE. 2011. Structure and properties of
455 nanocomposite films based on sodium caseinate and nanocellulose. J. Food Eng.,
456 103(1): 76-83.
457
458 Pranoto Y, Salokhe VM, Rakshit SK. 2005. Physical and antibacterial properties of
459 alginate-based film incorporated with garlic oil. Food Res. Int., 38(3): 267-272.
460
461 Rhim JW. 2004. Physical and mechanical properties of water resistant sodium alginate
462 fims. Lebensm. Wiss. Technol., 37(3): 323-330.
463
464 Rojas-Graü MA, Avena-Bustillos RJ, Olsen C, Friedman M, Henika OR, Martín-
465 Belloso O, Pan Z, McHugh TH. 2007. Effects of plant essential oils and oils compounds
466 on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films.
467 J. Food Eng., 81(3): 634-641.
468

469 Sarmento B, Ferreira D, Veiga F, Ribeiro A. 2006. Characterization of insulin-loaded
470 alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR
471 studies. Carbohyd. Polym., 66(1): 1-7.
472
473 Siripatrawan U, Harte BR. 2010. Physical properties and antioxidant activity of an
474 active film from chitosan incorporated with green tea extract. Food Hydrocoll., 24(8):
475 770-775.
476
477 Smidsrød O, Draget KI. 2004. Alginate gelation technologies. In: Dickinson E,
478 Bergenstahl B, editors. Food colloids. Protein, lipids and polysaccharides. Abington
479 Hall: Woodhead Publishing Ltd. p. 279-293.
480
481 Soazo M, Báez G, Barboza A, Busti PA, Rubiolo A, Verdini R, Delorenzi NJ. 2015a.
482 Heat treatment of calcium alginate films obtained by ultrasonic atomizing:
483 physicochemical characterization. Food Hydrocoll., 51: 193-199.
484
485 Soazo M, Pérez LM, Rubiolo AC, Verdini RA. 2015b. Prefreezing application of whey
486 protein-based edible coating to maintain quality attributes of strawberries. Int. J. Food
487 Sci. Tech., 50(3): 605-611.
488
489 Taha MO, Nasser W, Ardakani A, Al Khatib HS. 2008. Sodium lauryl sulfate impedes
490 drug release from zink-crosslinked alginate beads: switching from enteric coating
491 release into biphasic profiles. Int. J. Pharm., 350 (1-2): 291-300.
492

493 Trinder P. 1969. Determination of blood glucose using an oxidase-peroxidase system
494 with a non-carcinogenic chromogen. Ann. Clin. Biochem., 6: 24-32.
495
496 Viera MGA, da Silva MA, dos Santos LO, Beppu MM. 2011. Natural-based plasticizers
497 and biopolymer films: a review. Eur. Polym. J., 47(3): 254-263.
498
499 Yam KL, Papadakis SE. 2004. A simple digital imaging method for measuring and
500 analyzing color of food surfaces. J. Food Eng., 61(1): 137-142.

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Table 1. Chemical composition of St.

Parameters	Results (mean ± SD) (g/100g)
Water	96.8 ± 2.7
Ash	0.500 ± 0.015
Fat	0.0300 ± 0.0009
Protein	2.0 ± 0.3
Carbohydrate	0.60 ± 0.03
Glucose	0.126 ± 0.003
Calcium	0.0130 ± 0.0004

515 Table 2. Thickness and mechanical properties of different dry film
516 samples assayed in this work [a].

517 Dry film sample	Thickness (μm)	TS (MPa)	E (%)
518 Alg-Ca	50 ± 5^{a}	90.2 ± 3.3^{c}	8.7 ± 2.2^{a}
519 Alg-St	189 ± 12^{c}	4.6 ± 0.8^{a}	66.0 ± 3.0^{c}
520 Alg-DSt	112 ± 13^{b}	8.1 ± 1.2^{b}	41.7 ± 2.5^{b}

[a] Values with different letters in the same column are significantly different ($p<0.05$).

Table 3. Mechanical properties of heat treated Alg-St at 130 °C a.

Heating time (min)	Thickness (μm)	TS (MPa)	E (%)
0	189 ± 12^{a}	4.6 ± 0.8^{a}	66.0 ± 3.0^{b}
5	177 ± 35^{a}	6.0 ± 1.2^{ab}	54.1 ± 4.7^{a}
10	177 ± 19^{a}	5.8 ± 1.2^{ab}	52.5 ± 4.6^{a}
15	185 ± 10^{a}	6.4 ± 0.4^{b}	48.2 ± 2.6^{a}

[a] Values with different letters in the same column are significantly different ($p<0.05$).

Table 4. Optical properties of heat treated Alg-St at 130 °Ca.

Heating time (min)	Opacity	L*	a*	b*	E*
0	4.4 ± 0.5^{a}	64.6 ± 1.0^{d}	10.2 ± 1.9^{a}	38.6 ± 2.2^{b}	33.6 ± 2.5^{a}
5	4.2 ± 0.5^{a}	52.7 ± 1.6^{c}	25.9 ± 1.6^{b}	55.3 ± 1.9^{c}	57.9 ± 2.3^{b}
10	6.0 ± 0.5^{b}	37.1 ± 2.5^{b}	37.8 ± 0.6^{c}	54.3 ± 3.8^{c}	70.7 ± 1.0^{c}
15	7.5 ± 0.8^{c}	23.8 ± 1.0^{a}	36.6 ± 0.4^{c}	34.9 ± 1.3^{a}	69.4 ± 0.5^{c}

a Values with different letters in the same column are significantly different ($p<0.05$).

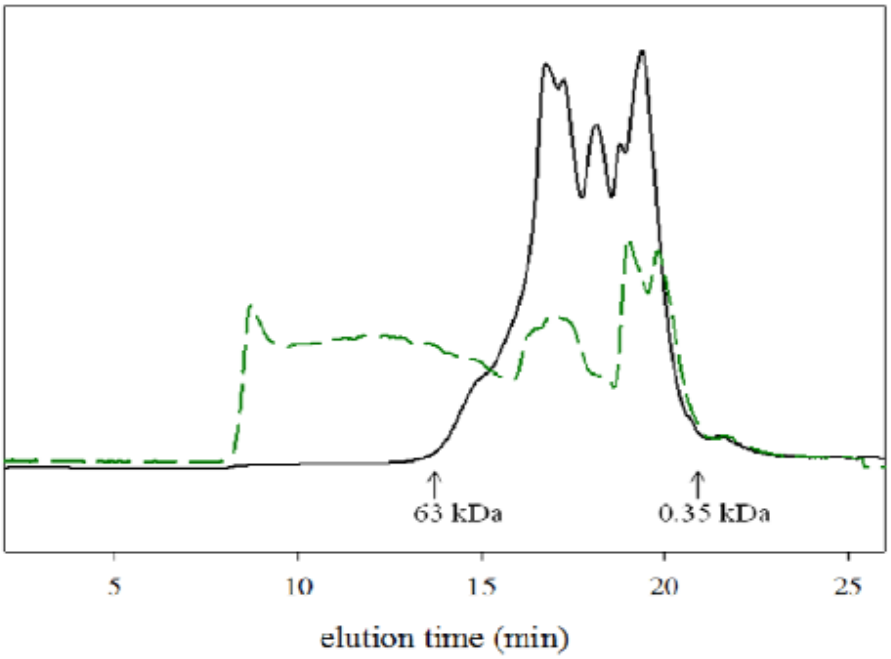

Figure 1. HPLC peptide profiles of (—) St and (----) commercial bovine gelatin.

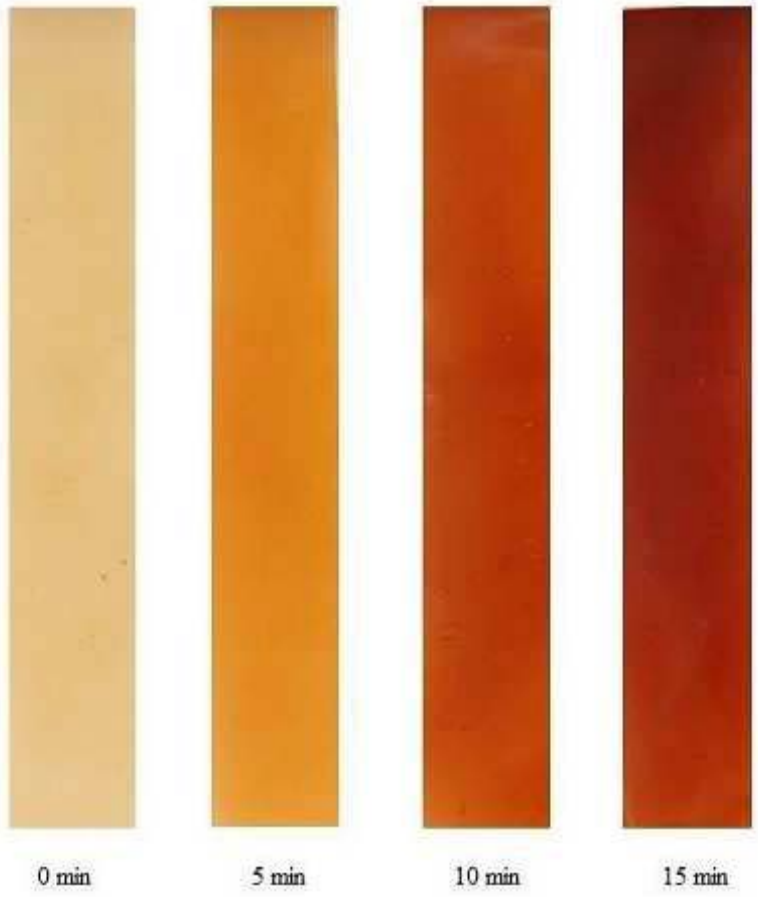

Figure 2. Photographs of heated Alg-St.

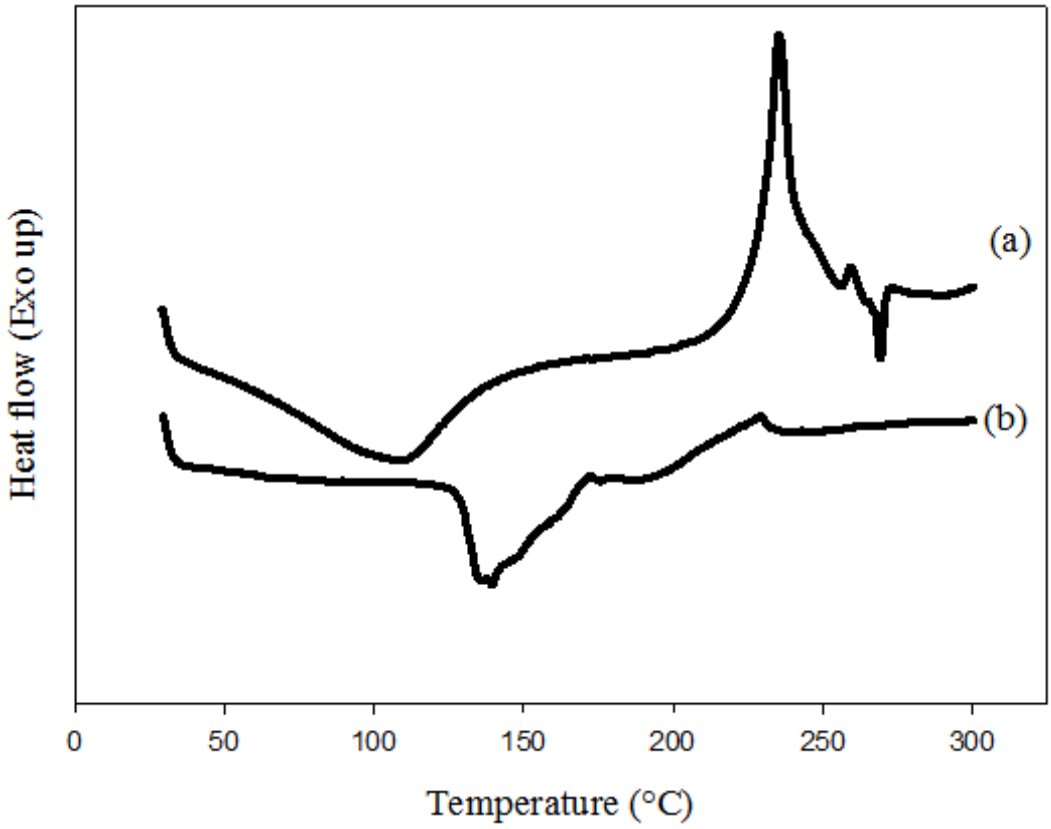

Figure 3. Differential scanning calorimetry thermograms of (a) Alg-Ca and (b) Alg-St.

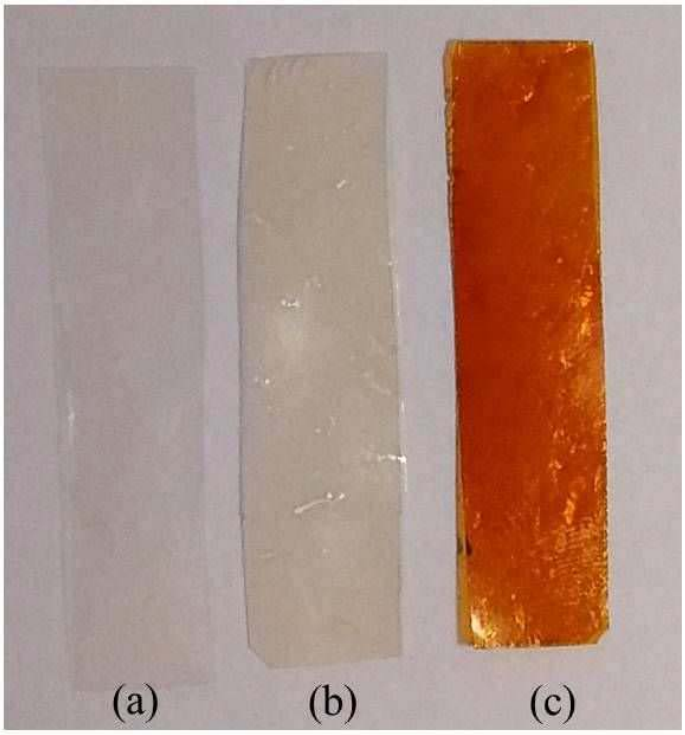

Figure 4. Photographs of heated (a) Alg-Ca, (b) Alg-Ca-Gel and (c) Alg-Ca-Gel-Glu.

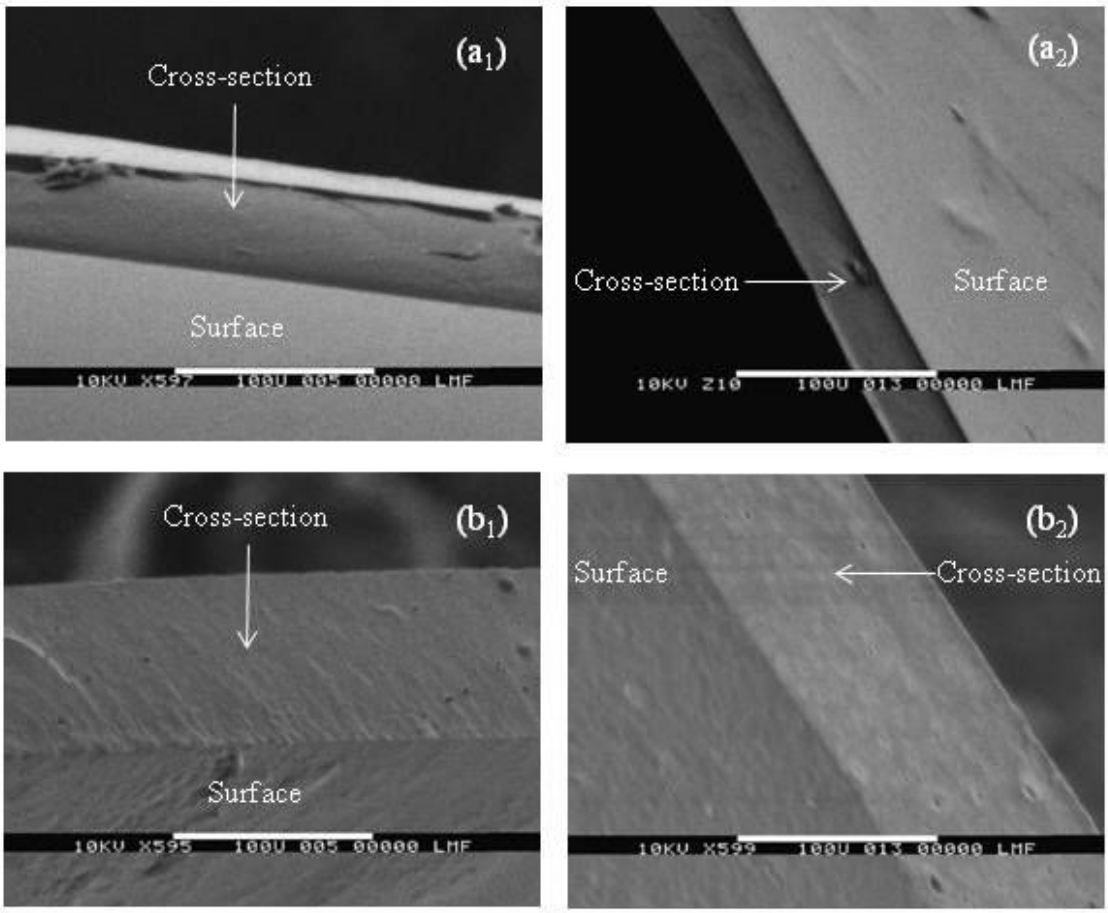

Figure 5. Scanning electron microscopy micrographs of (a1) untreated Alg-Ca, (a2) heat treated Alg-Ca, (b1) untreated Alg-St and (b2) heat treated Alg-St.