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

Short title:

Heated film of alginate-chicken stock

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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.
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 of 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 $\alpha$-L-guluronic and $\beta$-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
reduce the absorption of fats in foods subjected to frying (Albert and Mittal 2002) or to improve quality attributes of microwaveable chicken nuggets (Albert and others 2012). On the other hand, edible films can be made by drying a thin layer of wet gel. Since fragility can limit their potential for wrapping foods, flexibility, workability and distensibility of these products can be improved by the presence of plasticizer compounds, such as polyols and lipids (Viera and others, 2011). In a recent paper, our group analyzed the physicochemical characteristics of calcium alginate which underwent heat treatment (different times at 180 °C) and observed an ochre color development and an increased brittleness of the material. Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) studies suggested that heating produced a dehydration of films, following by dehydration and degradation of alginate macromolecules. A potential application of heat treated films to enhance crispness and appearance of foodstuffs was an interesting result of our investigations. The roasted chicken skin is quite crispy and fragrant, however, since roasted skin contains a great deal of fat (~ 40% (w/w)), and cancer-causing compounds, many people remove it for health reasons. On the other hand, chicken stock is made by extraction from bones (with some residual chicken meat attached), vegetables, herbs, and spices. The components extracted from the ingredients (proteins, free amino acids, minerals, volatile compounds, fat, etc.) contribute to a chicken stock overall flavor. In home-prepared chicken stock, fat represents less than 1.2% (w/w) of total components. In this work, it was intended to develop a heat treated calcium alginate edible film prepared with chicken stock, to be used as an alternative to roasted chicken skin in order 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
Chicken stock proximate composition was determined in triplicate according to the Association of Official Agricultural Chemists International methods (AOAC 2002): humidity (method 925.10); proteins (method 920.87, factor 6.25), fat (method 932.06), ash (method 923.03). Total carbohydrates were determined by difference. Calcium content was measured by atomic absorption spectroscopy using an Unicam Solaar equipment (Model 969, Unicam Ltd., Cambridge, UK). Glucose concentration in the chicken stock was determined by the methodology proposed by Trinder (1969).

Peptide content of chicken stock and bovine gelatin was determined by high-performance liquid chromatography (HPLC) (Shimadzu LC-10, Kyoto, Japan) using a calibrated size exclusion HPLC column Bio SEC-3000 (Phenomenex, Torrance, CA, USA) (300 mm X 7.8 mm). The chromatography conditions were: eluent, buffer phosphate 100 mM, 100 mM NaCl, pH 6.8; flow rate, 0.8 mL min\(^{-1}\); injection volume, 20 \(\mu\)L; peptide concentration, 5 mg mL\(^{-1}\); temperature, 25 °C and wavelength detection, 280 nm.

**Preparation of dry films**

Solid SA was gently added to chicken stock in order to give a final SA concentration of 0.9 % (w/v). The mixture was stirred for 12 h until homogeneity was obtained. After that, the solution was heated at 60 °C, degassed and 88 g of it were poured into plastic Petri plates of 13.5 cm in diameter. The plates were left to rest at 25 °C for 3 h and then introduced into an oven for 3 h at 50 °C. The dry films were withdrawn from Petri dishes and stored in plastic containers. These calcium alginate-chicken stock dry films were named Alg-St.

To study the possible plasticizing effect of fat from chicken stock on Alg-St films, a portion of chicken stock was defatted by extraction with petroleum ether. Using this
defatted chicken stock, dry films were prepared in accordance with the methodology described above. These films were called Alg-DSt. The fat content of Alg-St and Alg-DSt samples were determined by the same method used in the chemical analysis of chicken stock.

Dry films without stock, but with the same final calcium concentration were also prepared (Alg-Ca). The source of gelling cation was a calcium gluconolactate solution (Soazo and others 2015a). Final SA and calcium concentrations were the same for all the films assayed in this work.

To characterize the importance of different chicken stock components, such as proteins and carbohydrates, on FTIR analysis and appearance of films of Alg-St, some dry films were made by adding to Alg-Ca formulations either gelatin (Alg-Ca-Gel) or gelatin/glucose (Alg-Ca-Gel-Glu). Gelatin and glucose concentration in these samples were equal to total protein and total carbohydrate content in the chicken stock used. These values were taken from the chemical analysis of chicken stock.

Dry films with physical defects such as air bubbles, holes and cracks were discarded. The selected films were stored at 25 °C and 55 % RH for 24 h until testing.

**Fourier transform infrared (FTIR) spectroscopy**

FTIR spectra of Al-Ca, Alg-St, Alg-Ca-Gel and Alg-Ca-Gel-Glu were determined using an IR-Prestge-21 spectrophotometer (Shimadzu, Kyoto, Japan) (Soazo and others 2015a).

**Heat treatment of calcium alginate-chicken stock dry films**

Alg-St dry film samples were put into a Teflon™ coated cooking vessel. These films 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 2.5d, 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 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:

$$\text{Opacity} = \frac{Abs_{600}}{l}$$  

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 $\Delta E$ (total color difference) were determined following the works of Yam and Papadakis (2004) and Soazo and others (2015a). It has been noted that $\Delta E$ 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
which more than 90% is water and 3–4% is dissolved meat components. The calcium content was enough to obtain optimal calcium alginate gels for forming dry films (Alg-St) without further aggregation of the cation (Soazo and others 2015a). Protein and peptides are the major structural component of animal stocks. It is well known that gelatin, resulting from the hydrolysis of collagen present in connective tissue, is the principal ingredient of this culinary preparation. In the chicken stock used in this work, the molecular weight of gelatin peptides, determined by HPLC, ranged from 0.35 to 63 kDa (Figure 1). Total lipid (fat) includes mainly emulsified triglycerides, free fatty acids, small amount of essential oils, and traces of cholesterol. As it can be seen in Table 1, total lipids were a minor component of the chicken stock used in this study.

On the other hand, commercial bovine gelatin used in chemical studies contained ~ 60\% of peptides between 0.35 and 63 kDa (Figure 1) and slightly higher lysine content than chicken gelatin (~ 1.04\%) (Norizah and others 2013).

**Fourier transform infrared (FTIR) spectroscopy**

Infrared spectrum of Alg-Ca (Figure 2) showed the characteristics peaks of alginate: OH stretching vibrations at approximately 3220 cm\(^{-1}\), and asymmetric COO\(^{-}\) and symmetric COO\(^{-}\) stretching peaks near 1590 and 1410 cm\(^{-1}\) (Soazo and others 2015a). Figure 2 also shows that Alg-Ca and Alg-St infrared spectra had similar patterns in the range from 2800 to 3600 cm\(^{-1}\). However, these spectra were quite different for wavenumbers between 800 and 1700 cm\(^{-1}\). In this range, Alg-St spectrum resembled Alg-Ca-Gel infrared spectrum, as it can be appreciated in Figure 2. For proteins, typical infrared features are amide I and amide II bands, located in the regions between 1600 and 1700 cm\(^{-1}\) and between 1500 and 1600 cm\(^{-1}\), respectively. Amide I band is 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 $\sim 0.7\%$ (w/w) for Alg-St and $\sim 0.02\%$ (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
polymer chains and thus improving the chain mobility and flexibility. Therefore, in this study, fat acted as a plasticizer, reducing TS and increasing E of dry films, Table 2.

It has been noted that the values of TS, E and EM for Alg-Ca were similar to those reported previously (Rhim 2004; Soazo and others 2015a) and were similar to the values corresponding to cellophane, a quite stiff material. The presence of fat in alginate films reduced more than 300 times the stiffness of the material. As a result of these effects, Alg-St films obtained in this work possessed adequate flexibility and manageability properties to be used as food wrapping.

Mechanical properties of heat treated films

Soazo and others (2015a) observed that calcium alginate dry films tolerate heating up to 180 °C for 24 min. However, the heating of Alg-St at higher temperatures produced the immediate calcination of the sample. After several tests were performed, a temperature of 130 °C was chosen to avoid this problem. Thickness of Alg-St was not significantly modified by heating at 130 °C up to 15 min, Table 3. Soazo and others (2015a) reported that thickness of heated calcium alginate films (Alg-Ca) dramatically decreased due to a dehydration process, but this is not the case for heated Alg-St. Therefore, other processes must be taken into account.

On the other hand, Table 3 shows that the increase in heating time led to an increase in TS. Although heat treatment rapidly diminished E values, a greater increase in the time of treatment did not modify significantly film deformation. Heat treatment had a significant effect on film stiffness as EM values show. All the changes observed in mechanical properties in function of heating time produced an appreciable increase in brittleness (and therefore in crispness) of Alg-St. For heating times longer than 15 min, 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. $\Delta E$ 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.
Cross-section of Alg-Ca heated at 130 °C for 15 min showed a reduced thickness produced by dehydration of the untreated film (Soazoa and others 2015a), Figure 6(a,1) and Figure 6(a,2). Instead of this, the cross section of heated Alg-St did not vary significantly in thickness with treatment, Figure 6(b,1) and Figure 6(b,2). In addition, untreated Alg-St presented an increased thickness in comparison with untreated Alg-Ca. Similar result was presented in Table 2. SEM micrographs revealed that the internal microstructure of dry films was affected by the presence of fat (Benavides and others 2012). In that sense, Alg-St showed a more heterogeneous structure when compared with Alg-Ca, presenting a laminar type matrix.

7. Conclusions

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

8. Acknowledgements

The chicken stock used in this work was prepared by Chef Damian Delorenzi.
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


Table 1. Chemical composition of St.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results (mean ± SD) (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>96.8 ± 2.7</td>
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<tr>
<td>Ash</td>
<td>0.500 ± 0.015</td>
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<tr>
<td>Fat</td>
<td>0.0300 ± 0.0009</td>
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<tr>
<td>Protein</td>
<td>2.0 ± 0.3</td>
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<tr>
<td>Carbohydrate</td>
<td>0.60 ± 0.03</td>
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<tr>
<td>Glucose</td>
<td>0.126 ± 0.003</td>
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<tr>
<td>Calcium</td>
<td>0.0130 ± 0.0004</td>
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Table 2. Thickness and mechanical properties of different dry film samples assayed in this work.

<table>
<thead>
<tr>
<th>Dry film sample</th>
<th>Thickness (μm)</th>
<th>TS (MPa)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alg-Ca</td>
<td>50 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.2 ± 3.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.7 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alg-St</td>
<td>189 ± 12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.0 ± 3.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Alg-DSt</td>
<td>112 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.1 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.7 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup> 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.

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Thickness (μm)</th>
<th>TS (MPa)</th>
<th>E (%)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>189 ± 12\textsuperscript{a}</td>
<td>4.6 ± 0.8\textsuperscript{a}</td>
<td>66.0 ± 3.0\textsuperscript{b}</td>
</tr>
<tr>
<td>5</td>
<td>177 ± 35\textsuperscript{a}</td>
<td>6.0 ± 1.2\textsuperscript{ab}</td>
<td>54.1 ± 4.7\textsuperscript{a}</td>
</tr>
<tr>
<td>10</td>
<td>177 ± 19\textsuperscript{a}</td>
<td>5.8 ± 1.2\textsuperscript{ab}</td>
<td>52.5 ± 4.6\textsuperscript{a}</td>
</tr>
<tr>
<td>15</td>
<td>185 ± 10\textsuperscript{a}</td>
<td>6.4 ± 0.4\textsuperscript{b}</td>
<td>48.2 ± 2.6\textsuperscript{a}</td>
</tr>
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\textsuperscript{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.

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

\textsuperscript{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.