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Alginate/gelatin crosslinked system through Maillard reaction: preparation, characterization and biological properties

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Abstract Maillard reaction (MR) was studied in aqueous model systems containing gelatin and sodium alginate, which were heat treated for different pH (7, 8, 9, 10 and 11) at three temperatures (70, 80 and 90 °C). Some indicators were used to evaluate this reaction:degree of crosslinking, release of gelatin, free amino groups and browning intensities. The results indicated that alginate/gelatin crosslinked by MR showed an increase of degree of crosslinking as the pH and temperatures were increasing while release of gelatin decreased. Furthermore, samples prepared at a high temperature exhibited stronger browning intensity owing to the formation of Maillard reaction products (MRPs). The obtained materials were analyzed by FTIR and XRD. The antioxidant ability by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and power reducing, as well as the anti-inflammatory activity were investigated.

Keywords Alginate · Gelatin · Maillard reaction and biological activity

Introduction

Sodium alginate is a water-soluble salt of alginic acid, a naturally occurring non-toxic polysaccharide found in all species of brown algae [1, 2]. It contains 1,4-linked-*D*-mannuronic acid and *L*-guluronic acid residues that are arranged in the polymer chain in blocks. These homogeneous blocks are separated by blocks made from random or alternating units of mannuronic and guluronic acids [3–5]. Although alginate is water-soluble in the neutral and alkaline pH regions, it is highly

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insoluble in acid pH, as well as in most organic solvents [6]. The biotechnological uses of alginates cover a wide range of applications in food and pharmaceutical industries owing to the consequences of the structural diversity biocompatibility, biodegradability, gelling properties, immunogenicity, non-toxicity, and relatively low cost [7, 8].

Gelatin, another natural and biodegradable polymer, is obtained by thermal denaturation and/or physical and chemical degradation of collagen [9]. This protein is widely used in food, pharmaceutical, cosmetic and photographic applications because of its functional, technological properties, biodegradability and biocompatibility [10, 11]. It is rarely used alone owing to its high brittleness, but is often modified through several methods (crosslinking, grafting and blending) [2, 12].

During this study, gelatin was crosslinked with alginate via Maillard reaction (MR). The latter also called a non-enzymatic browning reaction is related to the condensation between a carbonyl compound, especially a reducing sugar and an amine, which it is usually an amino acid, a peptide, or a protein [13].

MR is of interest in the following areas: nutrition, soil, aspect of textile, toxicology, among others [14]. It is a complex network of chemical reactions and is attributed to several changes in the sensory properties of foods, such as color, flavor, aroma and texture [15]. However, MR confers to proteins through Maillard reaction products (MRPS) functional properties, e.g. foam forming and emulsifying ability and it also gives to treated saccharides highly viscous solutions as well as gel forming [16]. In addition, MR is influenced by many factors as temperature, pH, water activity, and reactants types and concentrations. Moreover, MRPs like volatile compounds, non-volatile intermediates, brown melanoidins exhibit antioxidant properties [17–21].

The action mechanisms of MRPs are considered to involve radical chain-breaking activity [22], metal chelating ability, active oxygen species scavenging and hydroperoxide destroying capacity [23]. In vivo, they have not modified the antioxidant enzymes functionality of glutathione peroxidase, catalase and superoxide dismutase. They are considered in diet a value added component [24]. Additionally, melanoidins have demonstrated a protective effect on rat hepatocytes from lipid oxidation and protein oxidation induced by Adriamycin [25] and exerted protective effects against oxidative stress on human hepatoma HepG2 cells [24]. However, the mechanism of antioxidant effect of MRPs still hindered by difficulties in pigments purification and due to the fact that color may have resulted from multiple chromophores [21].

The present paper concerns the elucidation of optimum conditions to crosslink gelatin with alginate by MR. So, different pH at different temperatures were used. The degree of crosslinking, release of gelatin, free amino groups and browning intensity were performed. The physical and chemical characterization was done by Fourier Transform Infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The antioxidant activity of MRPs was also determined using two different methods, the reducing power and radical scavenging. For validation of these methods, two known antioxidants, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, were examined, respectively. Furthermore, anti-inflammatory effect of MRPs was evaluated by protein renaturation.

Materials and methods

Materials

Pharmaceutical and food grade pig skin gelatin in powder form (Type A, 300 Bloom), sodium alginate (NaAlg) (Mw = 12–40 kDa), 2,4,6-trinitrobenzenesulfonic acid (TNBS), Bicinchoninic Acid (BCA), bovine serum albumin (BSA), butylated hydroxytoluene (BHT) and ascorbic acid were purchased from Sigma-Aldrich company. All other chemicals and reagents used were at an analytical grade.

Preparation of crosslinked alginate/gelatin films via MR

MR was studied first from prepared solutions of gelatin and NaAlg by following three steps at different pH and temperatures. (1) Gelatin aqueous solution (solution A) was obtained at 5 wt% in bi-distilled water. The pH was adjusted to about 7, 8, 9, 10 or 11 with 2.0 mol L^{-1} aqueous NaOH under a gentle stirring to avoid a bubble formation at $T = 70$ °C for 30 min. (2) NaAlg aqueous solution (solution B) was prepared separately at 5 wt% under stirring for 30 mn at $T = 70$ °C. (3) Mixtures of solution A and B with ratios (1/1) (v/v) were adjusted again to pH = 7, 8, 9, 10 or 11 with 2.0 mol L^{-1} NaOH at 70 °C. The heating was maintained in a water bath under agitation at 70 °C during 6 h.

Then, uniform film was achieved by casting method. The same amount of film-forming solution was poured into 9.5 cm diameter polystyrene Petri dish then it was exposed to air dried at $T = 25$ °C for 3–4 days. The same procedure described above was applied at $T = 80$ and 90 °C. The resulting samples are coded as it is illustrated in Table 1.

Determination of degree of crosslinking

The degree of crosslinking of samples was determined by a UV assay of uncrosslinked ε-amino groups before and after crosslinking according to the method of Ofner et al. [26]. Briefly, 11 mg of each sample was mixed with 1 mL of 4% $NaHCO_3$ and 1 mL of 0.5% TNBS, and heated at 40 °C for 4 h. 3 mL of 6 N HCl were added and the mixture was maintained at 60 °C for 2 h. The reaction mixture was then extracted with ethyl ether. A 5 mL aliquot of the aqueous phase was removed from each sample and heated for 15 min in a hot water bath, cooled to room temperature, and diluted again with 15 mL of water. The absorbance of the

Table 1 Labels used for different samples as a function of the preparation conditions

T (°C)	PH				
	7	8	9	10	11
70	AG70-7	AG70-8	AG70-9	AG70-10	AG70-11
80	AG80-7	AG80-8	AG80-9	AG80-10	AG80-11
90	AG90-7	AG90-8	AG90-9	AG90-10	AG90-11

diluted solution was measured at 346 nm in a UNICAM-UV–VIS 300 spectrophotometer (USA), against a blank. The degree of crosslinking could be obtained from the differences between the absorbance values before and after crosslinking. The equation was the following:

$$\text{Degree of crosslinking } (\%) = \left(1 - \frac{\text{Absorbance of crosslinked sample}}{\text{Absorbance of non-crosslinked sample}}\right) \times 100. \quad (1)$$

Release of gelatin

Weighted amounts (50 mg) of each sample were immersed in 25 mL of distilled water, at selected times. 100 µL of each solution were taken. Fractions were collected and analyzed for gelatin content as described below. Gelatin concentration in the release solution was determined by colorimetric assay using a bicinchoninic acid protein assay kit [9]. A 4% copper (II) sulfate pentahydrate solution was mixed with an excess of bicinchoninic acid at a final ratio of 1:50 v/v. 100 µL of the release solution was added to 2 mL of the assay solution in a test tube and stored at $T = 25$ °C for 90 min. The absorbance of each solution at 562 nm was measured using UNICAM-UV–VIS 300 spectrophotometer (USA). The gelatin concentration in the release solution was determined through comparison with a calibration curve.

Determination of free amino groups

The number of amino groups was measured by pH titration [27]. In this method, 25 mL of 0.1 N HCl solution were added in excess to approximately 0.2 g of the corresponding sample, allowing enough time (24 h) to charge all proton binding groups. Subsequently the solution was titrated with 0.1 N NaOH. Pure gelatin (0.2 g) was also dissolved in the HCl solution and titrated with 0.1 N NaOH solution. Two different equivalence points could be recognized. The percentage of amino groups (% NH_2) was calculated using the equation:

$$NH_2 = \left[M_{NaOH}(V_2 - V_1) \times \frac{146.19}{W}\right] \times 100, \quad (2)$$

where, M_{NaOH} is the molarity of the NaOH solution, V_1 and V_2 are the volumes required to neutralize the excess HCl and protonated amino groups, respectively, 146.19 g/mol is the molecular weight of lysine, W is the mass of sample in the dry state before titration.

Browning intensity

Maillard reaction products (MRPs) were studied from prepared solutions at pH (7–11) (see above in Section "Preparation of crosslinked alginate/gelatin films via

MR"). The method for determining the browning intensity of MRPs was done by measuring the absorbance of each sample using UNICAM-UV–VIS 300 spectrophotometer (USA) [28]. Samples were taken and analyzed in an interval time of 30 min of heating. All model systems were prepared in triplicate.

Fourier transforms infrared spectroscopy (FT-IR)

FTIR spectroscopy analysis was acquired in transmission mode from dried films in the range of 4000–500 cm^{-1}. The spectra were recorded with a JASCO FT/IR-4200 spectrophotometer (USA) at a resolution of 2 cm^{-1}.

X-ray diffraction study (XRD)

XRD measurements were carried out on samples by means of a D8 Advance diffractometer from Bruker (Germany) equipped with a Cu–K_α radiation ($\lambda = 1.5418$ Å) operating at 40 kV and 40 mA. The 2θ diffraction diagrams were determined between 5° and 90° at a scanning rate of 0.02°/min.

Determination of the anti-oxidative activity

DPPH radical scavenging activity

DPPH radical-scavenging activity of the MRPs was determined according to the method of Vhangani and Wyk [18] with a slight modification. 0.6 mL of each sample was quickly added to 2 mL of a 0.1 mM methanol solution of DPPH. After incubating the solution at RT for 30 min, the absorbance was read against a blank at 517 nm. BHA (400 µM) was used as a positive control under the same assay condition. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition of DPPH } (\%) = \left[\left(A_{\text{blank}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100, \qquad (3)$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the sample. The IC_{50} value was derived from the % inhibition of DPPH at different concentration.

Reducing power determination

The reducing power of the MRPs was determined according to the method of Norajit et al. [29] with some modifications. 1 mL of MRPs was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min. Then the solution was centrifuged, and the supernatant (2.5 mL) was added to 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The blank was prepared in the same manner as the samples

except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm with UNICAN-UV–VIS 300 spectrophotometer (USA). Ascorbic acid was used as a positive control under the same assay conditions. Results were the average of three measurements. The reducing power was expressed as an increase in absorbance at 700 nm.

Evaluation of in vitro anti-inflammatory activity

Anti-inflammatory activity of samples was evaluated by protein denaturation method as described by Alhakmani et al. [30] with slight modifications. Diclofenac sodium and ibuprofen, powerful non steroidal anti-inflammatory drugs were used as standard drugs. The reaction mixture consisting of 2 mL of different concentrations of samples (100–500 μg/mL) or standard ibuprofen/diclofenac sodium (100 μg/mL and 200 μg/mL) and 2.8 mL of phosphate buffered saline (pH 6.4) were mixed with 2 mL of egg albumin (from fresh hen's egg) and incubated at 27 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm using double distilled water as blank. For control test, 2 mL of egg albumin and 2.8 mL phosphate buffer saline (pH 6.4) were used. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Inhibition } (\%) = \left(\frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}} \right) \times 100, \tag{4}$$

where A_{sample} absorbance of tested sample; A_{control} absorbance of control.

Results and discussion

Determination of degree of crosslinking

TNBS has been used as a UV chromophore in various procedures to determine primary amino groups in peptides, proteins, and foodstuffs [31]. Production of amino groups is facilitated by the alkaline medium, and therefore, under basic conditions (at pH >9.0, isoelectric point of gelatin), deprotonated gelatin becomes the dominant species [32].

The degree of crosslinking is determined by measuring the amount of unreacted gelatin present by TNBS assay. The results reported in Fig. 1 indicate that pH = 8 was sufficient to crosslink about 08% of the ε-amino groups at $T = 70$ °C. Furthermore, the degree of crosslinking increases with pH and significantly up to about 26% when a high temperature is used. It was also observed that the degree of crosslinking increased with the temperature to about 56.5% for AG90-11, 47.08% for AG80-11 and 45% for AG70-11.

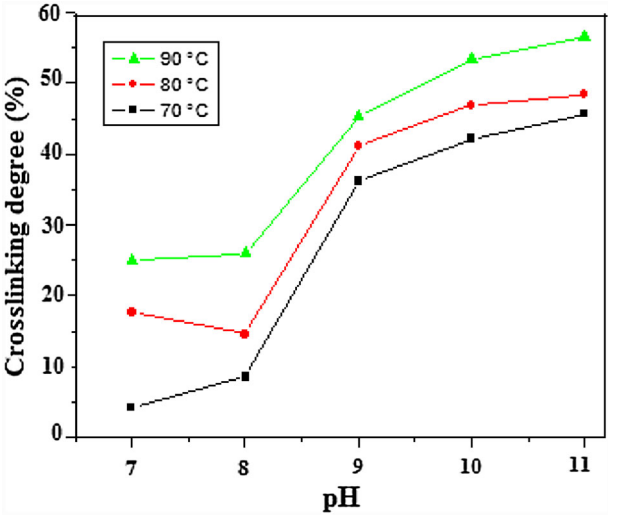

Fig. 1 Crosslinking degree as a function of pH and temperature

Release of gelatin

As it is shown in Figs. 2 and 3, the percentage of released gelatin decreases when the pH and temperature increase, and grows with time, except for AG80-10 which shows high values than those of AG70-10 after $t = 2$ h (Fig. 3d). Furthermore, for AG80-7, the percentage of released gelatin from t = 25 min to 3 h was lower than those obtained for AG90-7 (Fig. 3a). The values measured increase from 21.5 to 42% for AG80-7and from 23.5 to 47% for AG90-7, but after $t = 3$ h, the percentage of released gelatin become identical (45.5%).

We also note that the cumulative release of gelatin from the film at high pH (pH = 11) is remarkably low and it accounts for about 9% for AG90-11 after $t = 4$ h of storage in distilled water against the highest amount of gelatin to about 55% from studied sample at 70 °C (AG70-7). The diminution in release of gelatin is due not only to the formation of random coil conformation of α chains [33], but also from the reduction of the intra- and inter-molecular interactions of gelatin [34], which promotes proximity of reactive functions, like lysine, with other constituents of its environnement. So, the reaction between the amino groups from lysine is facilitated and lead to the formation of intermolecular links between gelatin chains and alginate through the Maillard reaction. Additionally, as shown in Fig. 2, this reaction greatly reduces the release of gelatin when the pH increases and confirms the stabilization induced by alginate on gelatin. These results are similar to those previously obtained on oxidized alginate-gelatin films [9].

Determination of free amino groups

The amount of free amino groups can be calculated by pH-titration [35]. Starting with an excess of HCl the addition of NaOH increases the pH-value. The first sharp

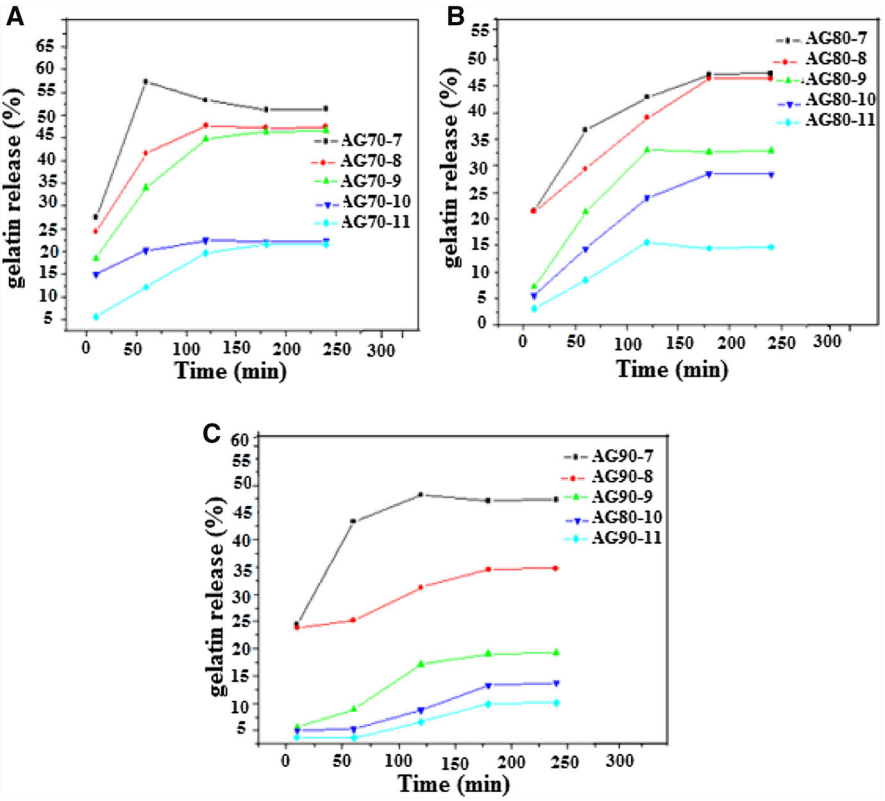

Fig. 2 Gelatin release (%) as a function of the storage time in distilled water, pHs and temperature (**a** 70 °C, **b** 80 °C and **c** 90 °C)

increase corresponds to the excess of HCl. The amount of NaOH between the first and the second jumping point corresponds to the amount of free amino groups. Figure 4 shows the pH-titration of the gelatin and gelatin-alginate crosslinked by Maillard reaction. The two steps at 10 and 13 mL of 0.1 N NaOH in the diagram indicate 37.35% of free amino groups of gelatin, used as basis material.

Therefore and apart from prepared films at 70 °C and pH = 7 (AG70-7) which exhibit two steps at 20 mL and 22.5 mL of NaOH with 18.41% of free amino groups, all other materials does not present significant amounts of amino groups.

Browning intensity

In the first stage of the MR, the carbonyl group reacts with the amino group giving rise to colorless compounds which do not absorb in the visible spectrum. Further progress of the MR involves the production of high molecular weight compounds MRPs, termed melanoidins [18] with different absorbance values.

The brown color obtained during the present study was used as an indicator of the Maillard reaction and its possible link to anti-oxidative capacity. Figure 5 shows

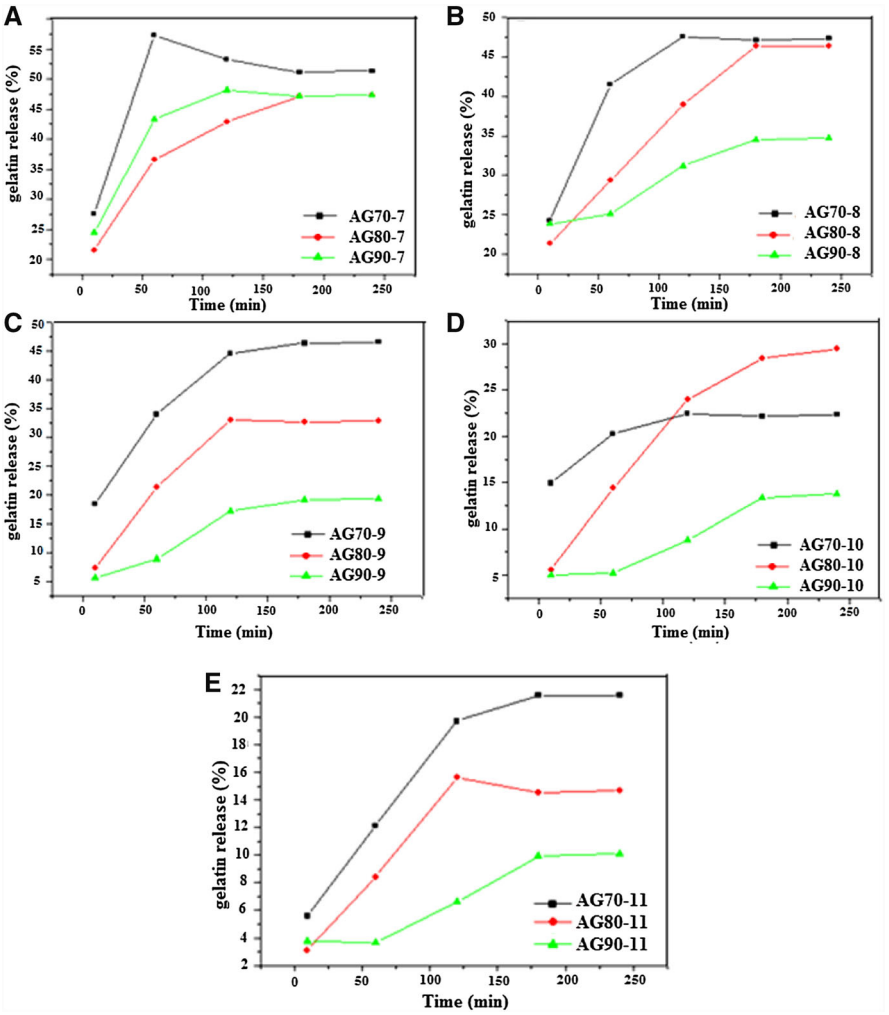

Fig. 3 Gelatin release (%) as a function of the storage time in distilled water, temperature and pHs (**a**–**e** pH = 7–11, respectively)

browning intensity as a function of reaction temperature (70, 80 or 90 °C) and time. After 2 h of heating at pH = 11, there is an increase in the browning intensity as the heating time increase characteristic by the increasing of the absorbance for all samples.

As shown in Fig. 5a (T = 70 °C), the absorbance at 280 nm increases with the heating time. The absorbance at this wavelength was used to follow Maillard reaction in its early stages [36]. However, changes in absorbance at 290 nm are detected as a measurement of intermediate products of the Maillard reaction which are colored compounds, as reported in literature [28, 37, 38]. At the same time, a characteristic absorbance peak at 325 nm appears (Fig. 5b) (T = 80 °C), and

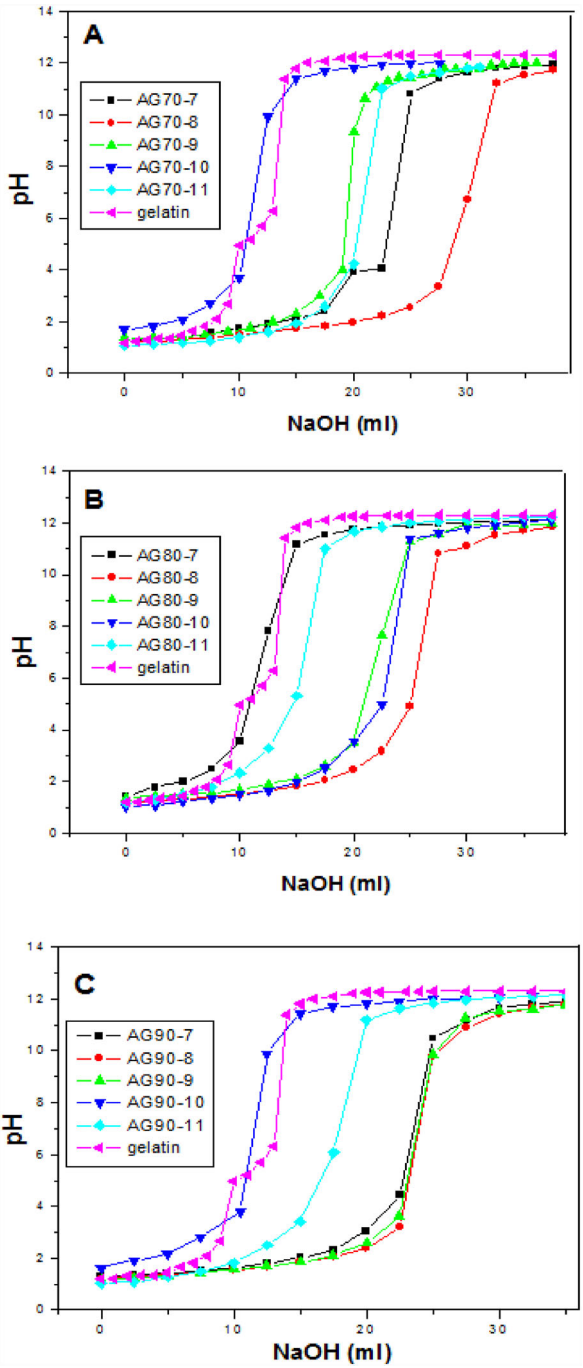

Fig. 4 pH-titration of gelatin and alginate/gelatin crosslinked via Maillard reaction at different temperature and pHs

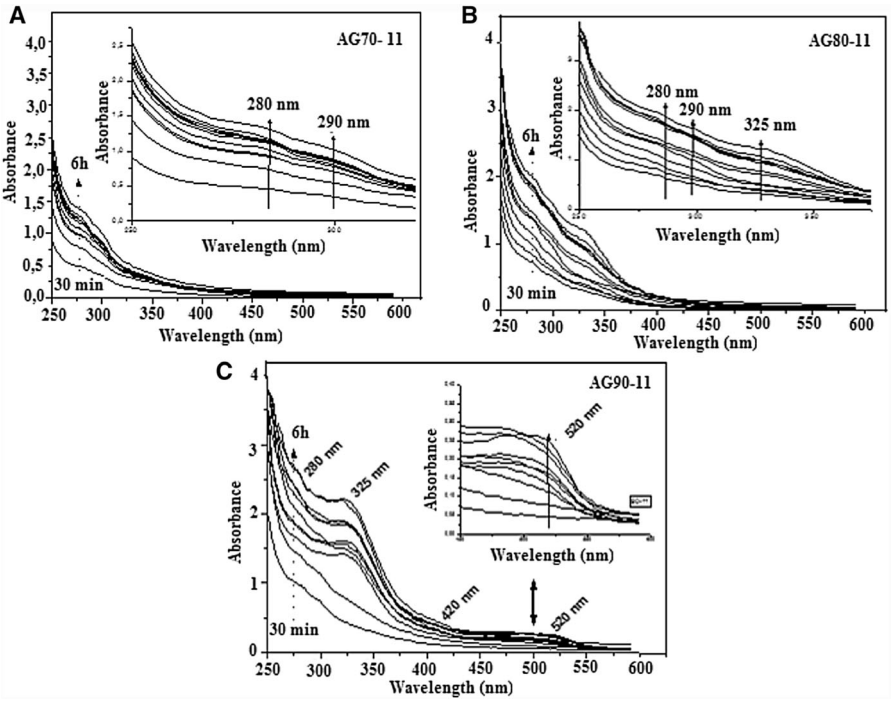

Fig. 5 Browning intensity as a function of reaction temperature (**a** 70 °C, **b** 80 °C and **c** 90 °C) and time

increases with heating time indicating the formation of a new Maillard reaction product and this trend was also found by Kim and Lee [39] and Li et al. [40]. The intensity of this peak was higher at $T = 90$ °C, which may be mean that the concentration of the Maillard reaction product rises in high temperature. Additionally, two new bands appear at 420 nm and 520 nm (Fig. 5c) ($T = 90$ °C) with increasing intensities. It is an indication of the formation of Melanoidins according to Ramonaitytė et al. [41], Li et al. [42], Nursten [14].

Moreover, the results of the effect of the pH were schematized in Fig. 6. At $T = 70$ °C, the peak at 280 nm appeared on all the curves and increased in intensity with pH. Also, a new peak (290 nm) was detected at pH equal to 11. The same findings were obtained at $T = 80$ °C, the two peaks previously obtained were observed on all graphs. Likewise as given above, at pH = 11, the peak at 325 nm was detected for AG80-11 and had become higher for AG90-11. Only the latter had shown absorption band at 520 and 420 nm.

Fourier transforms infrared (FT-IR) spectroscopy

FT-IR spectroscopy is a particularly useful technique for the study of protein–carbohydrate systems [43]. The spectroscopic analysis of polymeric molecules, including proteins, is complex due to the molecular vibrations arising from numerous atoms [21].

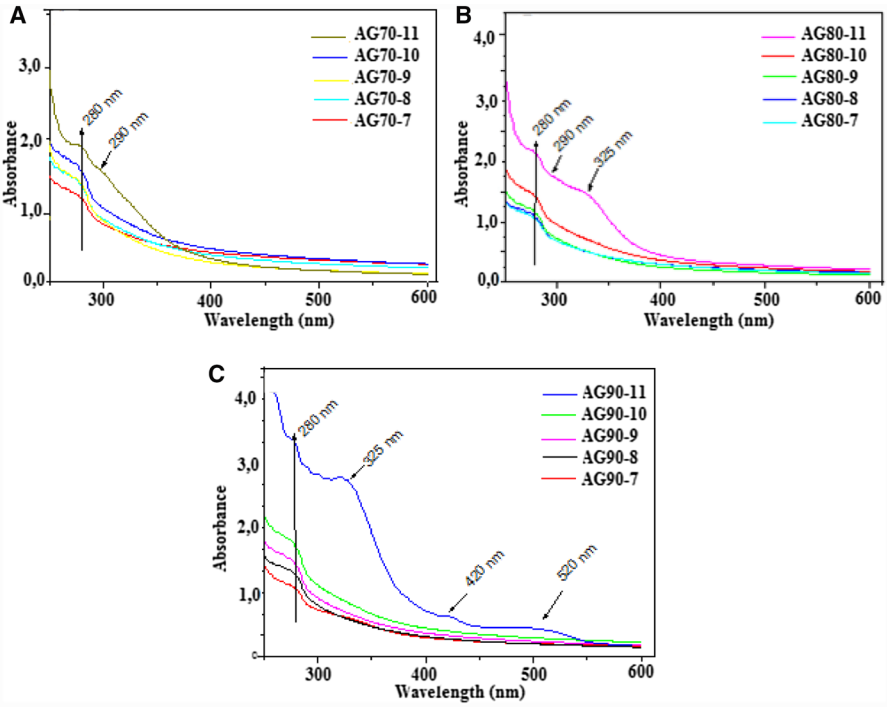

Fig. 6 Browning intensity as a function of reaction temperature (**a** 70 °C, **b** 80 °C and **c** 90 °C) and pHs (7–11)

Figure 7 shows the IR spectra of gelatin, alginate, AG under temperature variations (70–90 °C) and pHs (7–11).

About pure alginate, two characteristic absorption bands at 1595 and 1410 cm^{-1} were observed and attributed to the asymmetric stretching vibrations and symmetric stretching vibrations of carboxyl groups, respectively [2]. The characteristic bands of gelatin at 1646, 1550 and 1237 cm^{-1} were assigned to amide I (C=O and C–N stretching vibration), amide II and amide III (mainly N–H bending vibration and C–N stretching vibration), respectively [44].

Functional groups including NH_2, especially from lysine, may be lost, while the amount of those associated to Maillard products such as the Amadori compounds (C=O), Schiff base (C=N), and pyrazines (C–N) increase through Maillard reaction [45].

The region from 1800 to 800 cm^{-1} is often useful for the analysis of proteinaceous material, because the active bonds which form the amide group (C–O, N–H, and C–N) and linked to MRPs absorb the energy in this wavelength region [43].

Also, the new band at 1517 cm^{-1} was observed, suggesting that Schiff base (C=N double bond, intermediate products) formed between the carbonyl groups of NaAlg and the amino groups of gelatin [46]. The intensity of this band increases

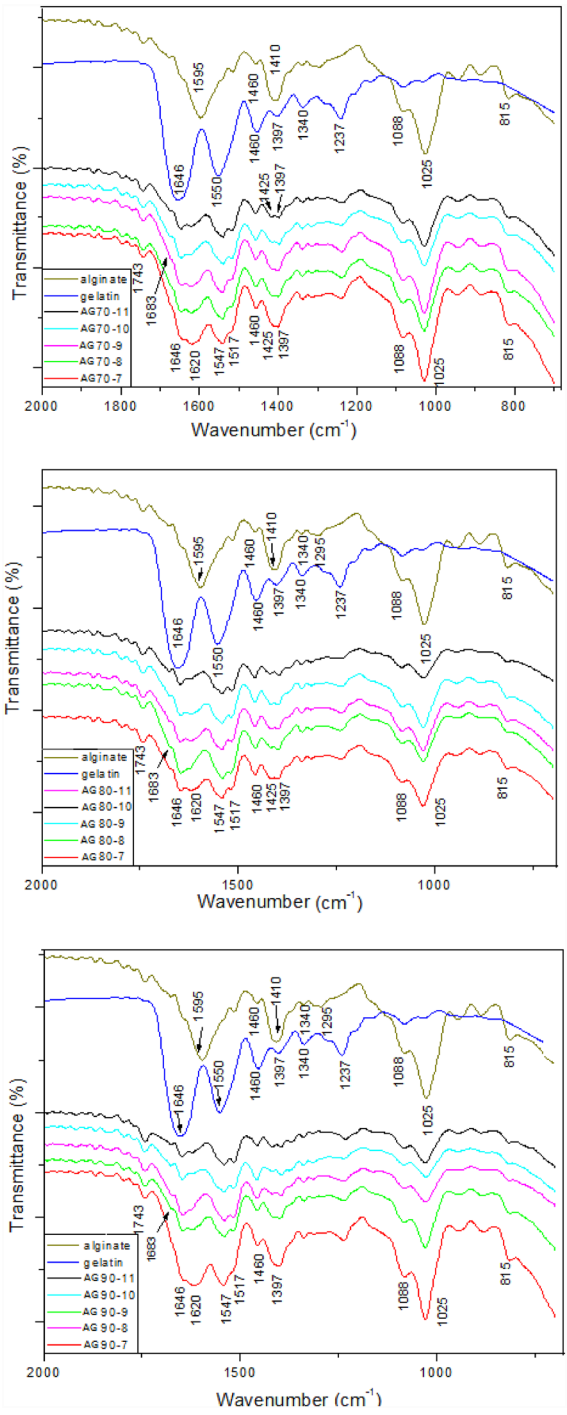

Fig. 7 FTIR spectra of: pure alginate, pure gelatin, AG as a function of temperature between 70 and 90 °C and pH (7–11)

with the pH at $T = 80$ and 90 °C. After the formation of the Schiff bases, a large variety of subsequent reactions are involved in the cross-linking of the material.

It has been reported by Kareb et al. [46], that Amadori products (C=O) give rise to peaks at 1660 and 1700 cm^{-1}. In our case, new peaks at 1743 and 1683 cm^{-1} corresponding to carbonyl (C=O) group of Amadori product was observed in the spectra of AG-(70, 80 and 90).

It can be seen that the intensity of the bands at 1600–1400 cm^{-1} decreased when NaAlg was added. These changes of the amide I and amide II bands reflected that hydroxyl groups in sugar and amino groups in gelatin were consumed during the heating process and alkali pH conditions. These results confirm that alginate was successfully crosslinked with gelatin through Maillard reaction and are in agreement with the work reported by Liu et al. [43].

In regards to Enediol formation, Alkenes (Enol products) usually absorb in the region between 1620 and 1700 cm^{-1} [47].

The formation of a new band at 1620 cm^{-1} indicated that only intermediate products of MR were formed (enediol) which decrease until disappearance at pH = 11 for AG- (70, 80 and 90 °C) noting the progress of MR.

At $T = 90$ °C, some peaks in the 1460–1397 cm^{-1} range decreased, pointing out a certain degree of interaction between gelatin and NaAlg. A decrease in 1025 cm^{-1} was observed due to the glycation of NaAlg and its participation in forming Maillard components when the heating degree and pH of the reaction increase.

Based on the obtained results a proposition for the mechanism of the Maillard reaction was presented in Figs. 8 and 9.

X-ray diffraction study

Figure 10 illustrates XRD patterns of alginate, gelatin and crosslinked materials AG70-11, AG80-11 and AG90-11. The diffractogram of alginate consists of three crystalline peaks at 14.3°, 21.3° and 37.1° [48] while the spectrum of gelatin exhibits two peaks ascribed to helical crystalline structure of collagen renatured in gelatin, the first small and narrow peak is at $2\theta = 7.75°$ and the second broad one at $2\theta = 20.08°$ [49, 50].

Therefore, after Maillard reaction, most of the characteristic peaks of alginate and gelatin disappear and new peaks appear in XRD patterns of AG70-11, AG80-11; AG90-11 (Fig. 10c–e) with diffraction peaks at new 2θ of 15.80°, 31.06° and 41.88° for AG70-11; 13.34°, 16.46°, 31.09° and 41.88° for AG80-11 and 14.65°, 31.06° and 41.15° for AG90-11, respectively. These experimental results reveal the establishment of the strong interactions between alginate and gelatin which had destroyed the close packing of the alginate molecules for the formation of regular crystallites [2] and the small angle peak at $2\theta = 7.75°$ related to the diameter of the triple helix of the gelatin. It seems important to point out from the range of $2\theta = 10°–25°$ (Fig. 10c–e) that the three new obtained materials present some different conformations in their chemical structures which are related indubitably to the evolution of Maillard reaction with studied conditions of pH and temperature.

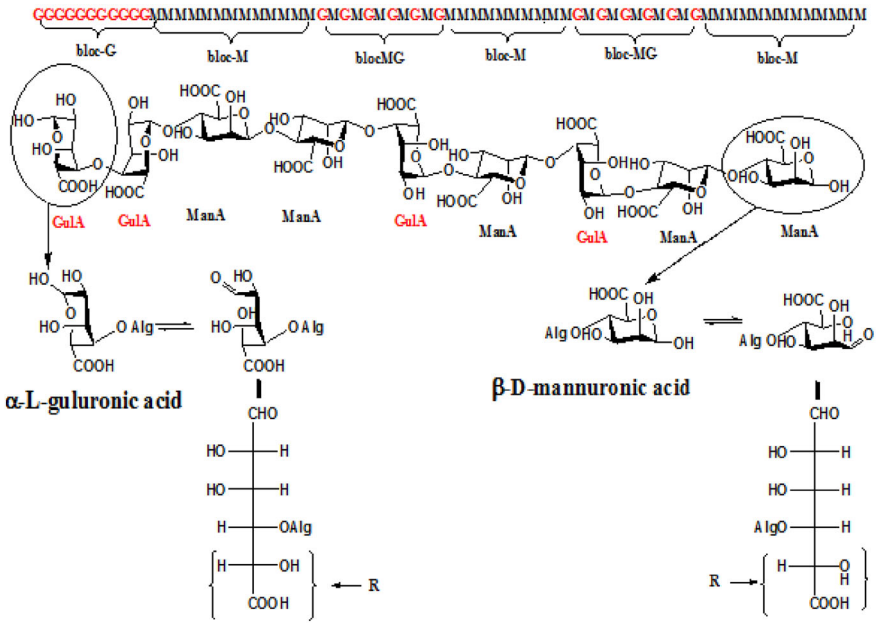

Fig. 8 Scheme of the molecular structure of alginate, and Equilibrium between cyclic and open chain forms in α-L-guluronic acid and β-D-mannuronic acid

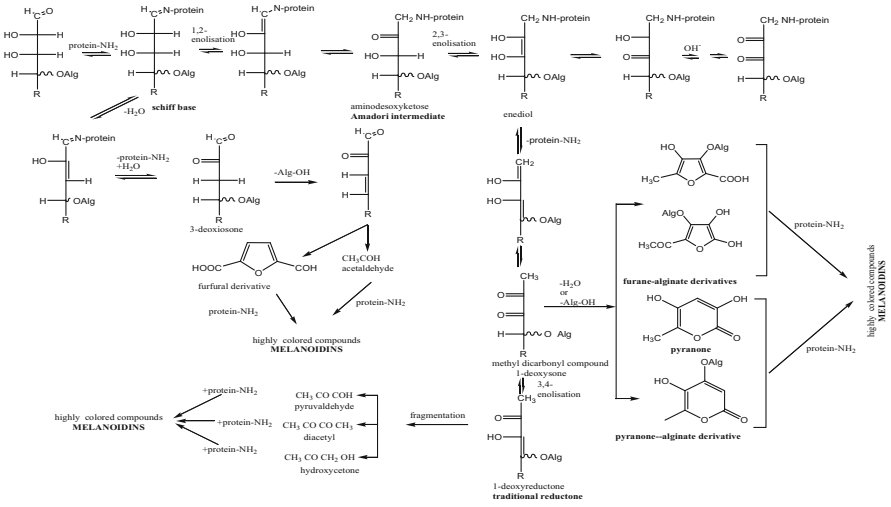

Fig. 9 Schematic representation of steps involved in the formation of principal conjugates obtained from alginate-gelatin model system during Maillard reaction

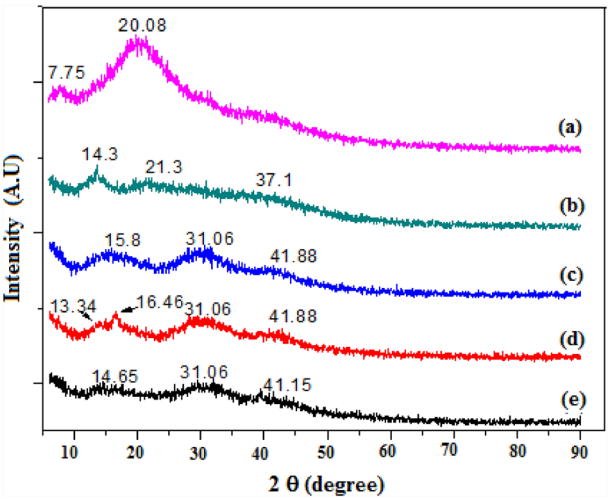

Fig. 10 XRD patterns of *a* gelatin, *b* alginate, *c* AG70-11, *d* AG80-11, *e* AG90-11

Anti-oxidative activity

DPPH radical scavenging activity

The stable DPPH-RS radical scavenging assay is based on hydrogen atom transfer and has been used widely for determining primary antioxidant activity. This assay determines free radical scavenging activity of all antioxidants, including those in pure form, as well as components of food products and plant and fruit extracts [18]. The DPPH scavenging activities of alginate without or with gelatin crosslinked at 70, 80 and 90 °C are shown in Fig. 11. The pH used for this study was fixed at 11. Lertittikul et al. [51] had found for porcine plasma protein–glucose system that the higher pH of samples resulted in a greater antioxidative activity of MRPs, compared with the lower pHs used. From Fig. 11, all our prepared materials have a significant scavenging activity against DPPH radicals, that can be explained by the formation of Maillard reaction products including intermediates or the final brown polymers; these products can act as hydrogen donors, which contribute to the antiradical activity [17]. Also, the pure alginate shows the higher IC_{50} which means lower antioxidant activity, the reaction of crosslinking result an increase of antioxidant activity, this activity is rising with degree of heating. These results suggest that there was a positive relationship between the antioxidant capacity and MRPs, particularly compounds crosslinked at higher temperature condition. Nevertheless scavenging activity against DPPH radicals for all samples remains lower than values for BHT.

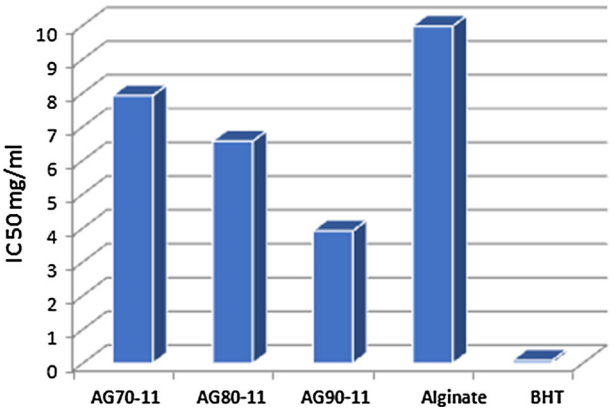

Fig. 11 IC_{50} (mg/mL) of alginate, AG70-11, AG80-11, AG90-11and BHT

Reducing power

The reducing power (RP) is a primary assay undertaken to determine the primary antioxidant activity of antioxidants obtained from components of food products and plant and fruit extracts [18]. During the reducing power assay, the presence of reductants in the samples result in reducing Fe^{3+}/ferricyanide complex, ($[Fe(CN)_6]^{3-}$), to the ferrocyanide form, ($[Fe(CN)_6]^{4-}$). The ferrous form Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [52]. It is well documented that the antioxidant capacity of MRPs increases with increasing temperature [53]. This may be caused by the formation of Amadori products in the Maillard reaction [17, 54]. According to Amarowicz [53], these reductone compounds exhibit high RP due to their ability to donate hydrogen atoms.

As can be seen in Fig. 12, the reducing power of studied samples correlated well with increasing concentration and reaction temperature. It is consistent with browning intensity this is due to the formation of prevalent antioxidants [54, 55].

The RP of ascorbic acid is found to be significantly higher than those of AG90-11, AG80-11, AG70-11. At a concentration of 0.3–2.3 mg/mL for ascorbic acid, 2.3–12.3 mg/mL for AG90-11, 2.3–21.3 mg/mL for AG80-11, 4.6–26.3 mg/mL for AG80-11, the reducing power is 0.1–1, 0.15–1.05, 0.12–0.95, 0.11–0.68, respectively.

In-vitro anti-inflammatory activity

Alginate as well as β-D-mannuronic acid show efficacy as anti-inflammatory drugs when tested in various inflammatory diseases [56].

Kitts et al. [57] had found that MRPs components derived from glucose-lysine have anti-inflammatory activities and which may potentially protect against the etiology of intestinal inflammation or other inflammatory diseases.

Results of in vitro anti-inflammatory activity of our samples at different concentrations are given in Table 2. The latter revealed that all tested compounds

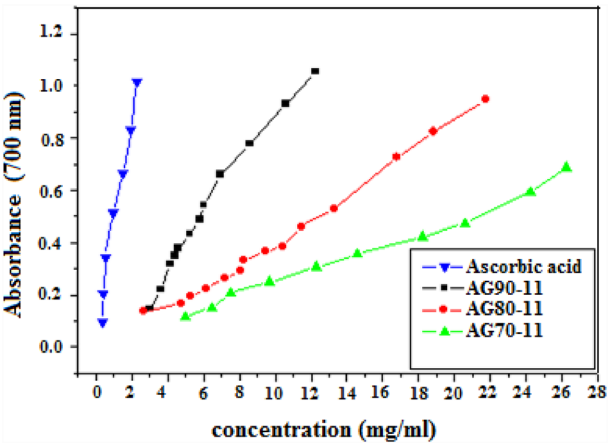

Fig. 12 The reducing power of AG90-11, AG80-11, AG70-11 and ascorbic acid

Table 2 In vitro anti-inflammatory effect of AG70-11, AG80-11 and AG90-11

AG70-11		AG80-11		AG90-11	
Concentration (mg/mL)	Inhibition (%)	Concentration (mg/mL)	Inhibition (%)	Concentration (mg/mL)	Inhibition (%)
25	19.05	20	18.67	7.5	17.8
30	19.47	25	19.59	10	55.6
35	19.31	30	20.11	15	65.7
40	20.27	35	21.49		

Table 3 In vitro anti-inflammatory effect of standard drugs

Ibuprofen		Diclofenac sodium	
Concentration (μg/mL)	Inhibition (%)	Concentration (μg/mL)	Inhibition (%)
100	98.02	100	63.31
200	125.25	200	76.51

exhibit a significant inhibition of denaturation of egg albumin as function as heating degree and AG90-11 present the highest anti-inflammatory activity compared with AG70-11 and AG80-11.

It is also noted that as mentioned in Table 3 percentage inhibition of protein observed for ibuprofen and diclofenac sodium (at 100 and 200 μg/mL) is much higher than values obtained for AG70-11, AG80-11 and AG90-11.

Conclusion

The results obtained have demonstrated that alginate can be successfully utilized to crosslink gelatin by Maillard reaction under high temperature and alkali pH conditions. The increase in the degree of crosslinking and the reduction in the release of gelatin seemed to be directly linked to the advancement of the reaction and strongly depended on the temperature and pH without any ambiguity.

The antioxidant and anti-inflammatory activities were also coincidental with the increase in browning indicating the formation of new colored compounds (MRPs) which were in permanent transformation with the reaction.

These new prepared compounds could be used in food and medicine but it is recommended that further investigations should be done to elucidate the biological behavior of the MRPs after their separation and subsequently their chemical identification.

References

1. Wang LF, Shankar S, Rhim JW (2017) Properties of alginate-based films reinforced with cellulose fibers and cellulose nanowhiskers isolated from mulberry pulp. Food Hydrocoll 63:201–208
2. Dong Z, Wang Q, Du Y (2006) Alginate/gelatin blend films and their properties for drug controlled release. J Membr Sci 280:37–44
3. Aadil KR, Prajapati D, Jha H (2016) Improvement of physcio-chemical and functional properties of alginate film by Acacia lignin. Food Packag 10:25–33
4. Mandal B, Ray SK (2013) Synthesis of interpenetrating network hydrogel from poly(acrylic acid-co-hydroxyethyl methacrylate) and sodium alginate: modeling and kinetics study for removal of synthetic dyes from water. Carbohydr Polym 98:257–269
5. Pawar SN, Edgar KJ (2012) Alginate derivatization: a review of chemistry, properties and applications. Biomaterials 33:3279–3305
6. Hattori M, Ogino A, Nakai H, Takahashi K (1996) Functional improvement of alginic acid by conjugating with β-lactoglobulin. J Agric Food Chem 61:1171–1176
7. Razzak MA, Kim M, Chung D (2016) Elucidation of aqueous interactions between fish gelatin and sodium alginate. Carbohydr Polym 148:181–188
8. Ertesvåg H, Valla S, Skjåk-bræk G (2009) Enzymatic Alginate Modification. In: Rehm BHA (ed) Alginates: biology and applications. Springer, Berlin Heidelberg, pp 95–115
9. Boanini E, Rubini K, Panzavolta S, Bigi A (2010) Chemico-physical characterization of gelatin films modified with oxidized alginate. Acta Biomater 6:383–388
10. Nguyen VT, Ko SC, Oh GW, Heo SY, Jeon YJ, Park WS, Choi IW, Choi SW, Jung WK (2016) Anti-inflammatory effects of Sodium alginate/gelatine porous scaffolds merged with fucoidan in murine microglial BV2 cells. Int J Biol, Macromol
11. Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23:563–576
12. Devi N, Kakati DK (2013) Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex. J Food Eng 117:193–204
13. Hwang IG, Kim HY, Woo KS, Lee J, Jeong HS (2011) Biological activities of Maillard reaction products (MRPs) in a sugar–amino acid model system. Food Chem 26:221–227
14. Nursten H (2005) The Maillard reaction: chemistry, biochemistry and implications. Royal Society of Chemistry, Cambridge, pp 1–208
15. Gerrard JA (2002) New aspects of an AGEing chemistry-recent developments concerning the Maillard reaction. Aust J Chem 55:299–310
16. Hiller B, Lorenzen PC (2010) Functional properties of milk proteins as affected by Maillard reaction induced oligomerisation. Food Res Int 43:1155–1166

17. Liu Q, Li J, Kong B, Jia N, Li P (2014) Antioxidant capacity of Maillard reaction products formed by a porcine plasma protein hydrolysate-sugar model system as related to chemical characteristics. J Food Sci Biotechnol 23:33–41
18. Vhangani LN, Wyk JV (2013) Antioxidant activity of Maillard reaction products (MRPs) derived from fructose–lysine and ribose–lysine model systems. Food Chem 137:92–98
19. Virág D, Kiss A, Forgó P, Csutorás C, Molnár S (2013) Study on Maillard-reaction driven transformations and increase of antioxidant activity in lysine fortified biscuits. Microchem J 107:172–177
20. Jiang Z, Brodkorb A (2012) Structure and antioxidant activity of Maillard reaction products from α-lactalbumin and β-lactoglobulin with ribose in an aqueous model system. Food Chem 133:960–968
21. Gu FL, Kim JM, Abbas S, Zhang XM, Xia SQ, Chen ZX (2010) Structure and antioxidant activity of high molecular weight Maillard reaction products from casein–glucose. Food Chem 120:505–511
22. Labuza TP, Reineccius GA, Monnier VM, O'Brien J, Baynes JW (1994) Maillard reactions in chemistry, food and health. R Soc Chem, Cambridge, pp 1–435
23. Zhuang Y, Sun L (2013) Antioxidant activity of maillard reaction products from lysine-glucose model system as related to optical property and copper (II) binding ability. Af J Biotechnol 10:6784–6793
24. Bastos DM, Monaro É, Siguemoto É, Séfora M (2012) Maillard reaction products in processed food: pros and cons, food industrial processes-methods and equipment. INTECH Open Access Publisher, Rijeka, pp 281–300
25. Liu Q, Li J, Kong B, Li P, Xia X (2014) Physicochemical and antioxidant properties of Maillard reaction products formed by heating whey protein isolate and reducing sugars. Int J Dairy Technol 67:220–228
26. Ofner CM, Bubnis WA (1996) Chemical and swelling evaluations of amino group crosslinking in gelatin and modified gelatine matrices. Pharm Res 13:1821–1827
27. Hoffmann B, Seitz D, Mencke A, Kokott A, Ziegler G (2009) Glutaraldehyde and oxidised dextran as crosslinker reagents for chitosan-based scaffolds for cartilage tissue engineering. J Mater Sci 20:1495–1503
28. Laroque D, Inisan C, Berger C, Vouland É, Dufossé L, Guérard F (2008) Kinetic study on the Maillard reaction. Consideration of sugar reactivity. Food Chem 111:1032–1042
29. Norajit K, Kim KM, Ryu GH (2010) Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract. J Food Eng 98:377–384
30. Alhakmani F, Kumar S, Khan SA (2013) Estimation of total phenolic content, in vitro antioxidant and anti-inflammatory activity of flowers of M oringa oleifera. Asian Pac J Trop Biomed 3:623–627
31. Bubnis WA, Ofner CM (1992) The determination of ε-amino groups in soluble and poorly soluble proteinaceous materials by a spectrophotometric method using trinitrobenzenesulfonic acid. Anal Biochem 133:129–133
32. Ahmad M, Benjakul S (2011) Characteristics of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as influenced by acid pretreatment and extraction time. Food Hydrocoll. 25:381–388
33. Rbii K, Violleau F, Brambati N, Buchert AM, Surel O (2011) Decrease of available lysine in thermally treated gelatin followed by LC-UV: influence on molar mass and ability to helixes formation. Food Hydrocoll 25:1409–1412
34. Zandi M, Mirzadeh H, Mayer C (2017) Effects of concentration, temperature, and pH on chain mobility of gelatin during the early stages of gelation. Iran Polym J 12:861–870
35. Gómez-Mascaraque LG, Méndezc JA, Fernández-Gutiérrez M, Vázquez B, Román JS (2014) Oxidized dextrins as alternative crosslinking agents for polysaccharides: application to hydrogels of agarose-chitosan. Acta Biomater 10:798–811
36. Carabasa-Giribet M, Ibarz-Ribas A (2000) Kinetics of colour development in aqueous glucose systems at high temperatures. J Food Eng 44:181–189
37. Decourcelle N, Sabourin C, Dauer G, Guérard F (2010) Effect of the Maillard reaction with xylose on the emulsifying properties of a shrimp hydrolysate (*Pandalus borealis*). Food Res Int 43:2155–2160
38. Riquelme N, Díaz-Calderón P, Enrione J, Matiacevich S (2015) Effect of physical state of gelatin-plasticizer based films on to the occurrence of Maillard reactions. Food Chem 175:478–484
39. Kim JS, Lee YS (2009) Enolization and racemization reactions of glucose and fructose on heating with amino-acid enantiomers and the formation of melanoidins as a result of the Maillard reaction. Food Chem 36:465–474

40. Li C, Liu F, Gong Y, Wang Y, Xu H, Yuan F, Gao Y (2014) Investigation into the Maillard reaction between ε-polylysine and dextran in subcritical water and evaluation of the functional properties of the conjugates. LWT Food Sci Technol 57:612–617
41. Ramonaityte DT, Keršiene M, Adamsb A, Tehrani KA, Kimpe ND (2009) The interaction of metal ions with Maillard reaction products in a lactose-glycine model system. Food Res Int 42:331–336
42. Li Y, Lua F, Luo C, Chen Z, Mao J, Shoemaker C, Zhong F (2009) Functional properties of the Maillard reaction products of rice protein with sugar. Food Chem 117:69–74
43. Liu Q, Kong B, Han J, Sun C, Li P (2014) Structure and antioxidant activity of whey protein isolate conjugated with glucose via the Maillard reaction under dry-heating conditions. Food Struct 1:145–154
44. Carbonaro M, Nucara A (2010) Secondary structure of food proteins by Fourier transform spectroscopy in the mid-infrared region. Amino Acids 38:679–690
45. Farhat IA, Orset S, Moreau P, Blanshard JMV (1998) FTIR study of hydration phenomena in protein-sugar systems. J Colloid Interface Sci 207:200–208
46. Kareb O, Gomaa A, Champagne CP, Jean J, Aïder M (2017) Electro-activation of sweet defatted whey: impact on the induced Maillard reaction products and bioactive peptides. Food Chem 221:590–598
47. Yaylayan V, Ismail A, Huyghues-Despointe A (1994) Investigation of the acyclic forms of reducing sugars and Amadori products by FTIR spectroscopy. Royal Society of Chemistry, Cambridge, UK, pp 69–74
48. Kuila SB, Ray SK (2014) Dehydration of dioxane by pervaporation using filled blend membranes of polyvinyl alcohol and sodium alginate. Carbohydr Polym 101:1154–1165
49. Sarker B, Papageorgiou DG, Silva R, Zehnder T, Gul-E-Noor F, Bertmer M et al (2014) Fabrication of alginate–gelatin crosslinked hydrogel microcapsules and evaluation of the microstructure and physico-chemical properties. J Mater Chem B 2:1470–1482
50. Asma C, Meriem E, Mahmoud B, Djaafer B (2014) Physicochemical characterization of gelatin-cmc composite edibles films from polyion-complex hydrogels. J Chil Chem Soc 1:2279–2283
51. Lertittikul W, Benjakul S, Tanaka M (2007) Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein-glucose model system as influenced by pH. Food Chem 100:669–677
52. Phisut N, Jiraporn B (2013) Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. Int Food Res J 20:1077–1085
53. Amarowicz R (2009) Antioxidant activity of Maillard reaction products. Eur J Lipid Sci Technol 111:109–111
54. Huang X, Tu Z, Xiao H, Wang H, Zhang L, Hu Y, Zhang Q, Niu P (2012) Characteristics and antioxidant activities of ovalbumin glycated with different saccharides under heat moisture treatment. Food Res Int 48:866–872
55. Manzocco L, Calligaris S, Mastrocola D, Nicoli MC, Lerici CR (2000) Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends Food Sci Technol 11:340–346
56. Mirshafiey A, Rehm BHA (2009) Alginate and its comonomer mannuronic acid: medical relevance as drugs. In: Rehm BHA (ed) Alginates: Biology and Applications. Springer, Berlin Heidelberg, pp 229–260
57. Kitts DD, Chen XM, Jing H (2012) Demonstration of antioxidant and anti-inflammatory bioactivities from sugar-amino acid maillard reaction products. J Agric Food Chem 60:6718–6727