

Research Article

Enhancing Water Resistance, Tensile Properties and UV-Shielding Capacity of Bovine Gelatin Films Modified with Epoxidized Soybean Oil and Glyoxal during Processing

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Abstract

The present work is focused on the use of epoxidized soybean oil (ESO, 10 wt%) as hydrophobizing additive, glycerol as plasticizer (Gly, 30 wt%), and glyoxal (GLX, 2 wt%) as low-toxicity cross-linking agent to endow bovine gelatin (BGe) films with improved moisture-related and UV-light barrier properties. Therefore, we aimed to investigate the effect of such modifications on the physicochemical and structural properties of the obtained gelatin films. Modification was produced during intensive mixing and hot-press molding as a more environmentally sound and less time-consuming processing method. ESO improved moderately the moisture content (MC) and water vapor permeability (WVP) of BGe film by 19% and 29%, respectively, but the total solubility of BGe-10ESO film evidenced the inability of ESO to induce covalent cross-linking. When GLX was added to the BGe-10ESO formulation, several target film properties were upgraded; the film kept about 78% of its weight after 24 h of soaking in water, WVP improved by 56.8%, the tensile strength (TS) increased 48% while the stretching ability dropped in about 41%. The significant reduction in available free amino groups, from 100% (control) up to 21.9%, together with the increased color from light yellow to light orange confirmed that amino groups in BGe were involved in imine-based cross-linked structure when treated with glyoxal. Further, the high blocking ability of BGe-10ESO-2GLX film against UV-radiation may be useful in delaying UV-induced lipid oxidation when it is placed in contact with foods.

Keywords: Bovine gelatin, Compression molding, Chemical cross-linking, Physicochemical properties

Highlights

- Bovine gelatin was blended with 30 wt.% GLY, 10 wt.% ESO and 2 wt.% GLX during intensive mixing
- Films were manufactured by compression molding as a lab-scale approach to industrial processing
- The hydrophobic nature of ESO modestly reduced WVP of BGe film
- The combination of ESO and GLX significantly enhanced water resistance, MC, WVP, TS, and UV-protection

Introduction

Packaging has been conceived to protect foods from water vapor, oxygen, and microbial spoilage, enabling foods to be safe at the time of consumption [1]. Synthetic polymers have long been the principal packaging materials fulfilling the requirements mentioned above, but because of environmental and economic issues, the focus on bio-based packaging items has steadily grown over the last decades. The advantages of using naturally occurring polymers over fossil-sourced ones are their renewability and, most of the times, the biodegradability or compost ability of the finished product that offers an alternative to disposal in a landfill [2].

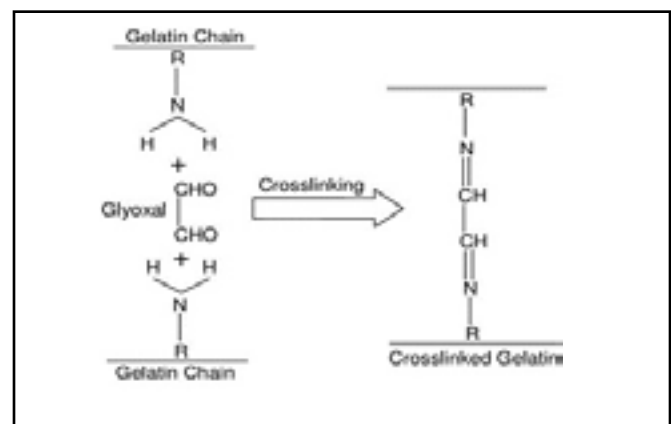
Amongst the potential candidates, proteins have gained increasing importance as having great prospective as packaging materials due to their worldwide abundance and excellent film-forming ability throughout casting and thermoplastic processing [3]. Gelatin is a water-soluble protein obtained from the denaturation of collagen from connective tissues and bones of mammals, poultry, and fish [4-15]. It is renewable, classified as “generally recognized as safe” substance by the U.S. Food and Drug Administration [16] and provides films with low gas permeability and satisfactory mechanical properties at low and intermediate relative humidity produced by casting [3,5-9,11,12]. Unfortunately, gelatin films might undergo post-processing changes when stored at high relative humidity compromising their large-scale applications [5,6].

The intended industrial applications of gelatin films can be expanded by taking advantage of the synergistic effect of hydrophobic additives [15], and chemical cross-linking agents [5-14]. Epoxidized soybean oil (ESO) is a renewable and inexpensive candidate for such applications [17] because it shares many of the characteristics and properties of the conventional epoxy cross-linking agents [12,18], with the additional benefit of its inherent hydrophobic character. ESO has improved the hydrophilic/hydrophobic balance and mechanical properties of compression-molded wheat gluten (WG) [19] and soybean protein isolate (SPI) films [20].

Regarding the cross linking agents, glyoxal (GLX) is preferred over commonly used aldehydes because of its more controllable reaction rate at ambient temperature [10], and lower toxicity, as compared with formaldehyde and glutaraldehyde (oral LD50GLX > 2000 mg/kg body weight, [21]). The reaction between gelatin and glyoxal at high pH values (i.e. close to pKa of lysine) is mainly governed by the well-known Schiff base formation (imine moieties) [7,8,10,13,14] (Scheme 1).

Currently, there are many data in the literature on gelatin films cross-linked with glyoxal using solvent-casting technique [7,8,13,14], however, no comparative study was found using thermo mechanical processing. Thermo mechanical methods usually have lower energy demand and pro-

cessing time compared to the casting method, thus being preferred for industrial application [3]. Several thermoplastic techniques have been employed for production of gelatin films. Krishna, et al. [22], compared the properties of gelatin films manufactured by extrusion and solution-casting. Films extruded displayed the highest elongation and comparable WVP that casted films, making the extruded films useful as food wrap. Andreucetti, et al. [23], studied the performance of gelatin films modified by yucca and obtained by casting, blown extrusion, and extrusion. Extrusion gave rise to films with better mechanical properties whereas blown extrusion demonstrated lower WVP. Interestingly, all films showed compact inner structure regardless the processing method. Contrarily, Chuaynukul, et al. [24], found that gelatin films produced by solvent-casting had better overall properties compared to those obtained by thermo-compression, due to partial degradation during the thermal step. Therefore, processing conditions need to be further improved to minimize such detrimental effects. Martucci and Ruseckaite [5], produced gelatin



Scheme 1

films cross-linking with dialdehyde starch (DAS) and plasticized with glycerol by compression molding. In general, the addition of 10 wt.% DAS produced a more rigid polymer network that resulted in films with increased moisture resistance, reduced solubility and enhanced WVP and oxygen barrier, and still biodegradable in soil. Despite of differences between reported results and the need of optimized conditions, all the studies analyzed agree well with the fact that thermoplastic processing is a feasible manufacturing method to produce gelatin films as a step towards commercial applications [22].

In this context, the aim of the present work is to explore the effect of ESO (10 wt%) and GLX (2 wt%) on several target properties of bovine gelatin films plasticized with glycerol (30 wt%) manufactured by compression molding assisted by intensive mixing, as a laboratory-scale precursor to continuous industrial processing.

Materials and Methods Materials

Bovine gelatin (BGe) (mesh 8, type B, 250 Bloom) was kindly provided by Rousselot Hurlingham, Argentina), with a proximate percentile prevalence of arginine (6.6%), lysine (5.9%), glycine (23%), proline (10%), hydroxyproline (9.9%), alanine (9.3%), glutamic acid (10.1%) and aspartic acid (8.7%). Epoxidized soybean oil (ESO), purchased from Unipox (Lavallol, Argentina), was used as received as a reactive modifier at various weight proportions (on dry gelatin basis). Glycerol (99%) was obtained from Anedra (Tigre, Argentina) and buffer solution pH 9.5 was prepared at the laboratory. Glyoxal (GLX, 40 wt% aq. solution) was obtained from Sigma-Aldrich (USA)

Source of bacteria

Gram-negative *Escherichia coli* O157:H7 ATCC 32158 (ATCC, American Type Culture Collection) and Gram-positive *Staphylococcus aureus* ATCC 25923 bacteria were used as model human pathogens commonly found in food. These strains were obtained in Eosin-methylene blue (EMB) and Baird Parker agar respectively. Vegetative cells of each microorganism were streaked on Mueller Hinton agar and incubated at $37 \pm 0.5^\circ\text{C}$ for 24 h. Microbial broth was then suspended in double distilled sterile water. The density of bacteria suspension was adjusted until the visible turbidity was equal to 0.5 Mc Farland standard before testing.

Preparation of gelatin films

Gelatin films were produced according to our previous work [5] and exploratory studies [25]. BGe (10 - 15 g) was manually mixed with 30 wt% Gly (on dry gelatin basis) and the required amount of buffer pH 9.5 at room temperature and left to stand for 24 h to obtain a dough-like material. Then, ESO (10 wt. % dry BGe mass) and the pre-mixture were fed into a Haake type mixer and processed at 60°C , at a speed of 0.83 Hz for 15 min. After cooling up to room temperature, blends were pelletized and converted into films using a hydraulic press (EMS, San Justo, Argentina). Pellets were placed in a preheated stainless steel mold coated with anti-adherent Teflon foil and compressed at 120°C , at a pressure of 50 kg/cm^2 applied for 10 min. The cooling step was performed under pressure until room temperature was reached. In the case of GLX-modified films, the appropriate amount of GLX (2wt% dry BGe mass) was added 1 min before ending the mixing step. The material was then processed by compression as above described. Samples were labeled as follows: BGe (control with glycerol and no other additive), BGe-10ESO and BGe-10ESO-2GLX, all of the films plasticized with 30 wt% glycerol. Film samples were conditioned at $65 \pm 2\%$ relative humidity (RH) and $25 \pm 2^\circ\text{C}$ in an environmental chamber before testing.

Methods and techniques Fourier transform infrared spectroscopy (FTIR):

Spectra were recorded on a Mattson Genesis II spectrometer (Madison, USA) between 400 and 4000 cm^{-1} using an attenuated total reflection (ATR) accessory in transmission mode (depending on the sample), applying 32 scans with a resolution of 4 cm^{-1} .

Thickness: The film thickness was measured using a manual micrometer Mitutoyo (Takatsu-ku, Japan) with an accuracy of $\pm 0.01\text{ mm}$. The reported values are the average of three readings taken randomly on each film sample.

Tensile properties: Film samples were cut into dumbbell-shaped specimens. Tensile strength (TS) and elongation at break (ϵ) were calculated from stress-strain curves obtained by using a Universal test machine Instron4467 (Buckinghamshire, UK) equipped with a load cell of 0.5 kN. All measurements were performed at a speed of 3 mm/min and room temperature, following the procedure described in ASTM D638-94b. Ten replicates of each formulation were measured and averaged.

Thermogravimetry analysis (TGA):

Non-isothermal degradation measurements were performed in a TGA 50 Shimadzu thermal gravimetric analyzer (Tokyo, Japan). Tests were running from room temperature up to 800°C at 10°C/min and under nitrogen atmosphere to avoid thermo-oxidative reactions (15 mL/min).

Color parameters and UV-Visible light barrier: Color parameters were determined using a LovibondRT 500, (Amesbury, United Kingdom), and recorded in the Hunter Lab scale. UV and Visible light barrier properties of films were determined by assessing their light absorption at the wavelength ranging from 200 to 800 nm, using an UV-Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan). The film specimens were cut in rectangular strips and placed in the spectrophotometer test cell directly. Film opacity was expressed as the area under the integrated recorded curve, and it was expressed as absorbance units per thickness unit [26]. The UV-shielding capacity was determined as the absorbance at 280 nm. The values reported are the average of six reading for each type of film.

Moisture content (MC) and Total soluble matter (TSM):

Film samples were weighed to $\pm 0.0001\text{ g}$ accuracy and oven-dried for 24 h at 105°C . Dried samples were then conditioned at $25 \pm 2^\circ\text{C}$ and $65 \pm 2\%$ RH, following the procedure de-

scribed in ASTM E104-96. The equilibrium moisture content (MC) was expressed as the weight of absorbed water when equilibrium was reached. The reported values are the average of three measurements.

TSM was determined followed by the dry method described elsewhere [27].

Free amino groups: The percentage of free amino groups (FAG) was determined by UV-visible spectrophotometry using ninhydrin (2,2-dihydroxyl-1,3-indanedione, NHN) [14]. A 50±1 mg of sample was heated in a solution of ninhydrin (0.4 g/100 mL) for 20 min. Subsequently, the absorbance of the resulting solution was measured with a Shimadzu 1601 PC spectrophotometer (Tokyo, Japan) at 570 nm. The following ratio expressed the percentage of free amino groups:

$$\text{FAG(\%)} = \frac{(\text{NHN}_{\text{reactiveamine}})_{\text{blank}} - (\text{NHN}_{\text{reactiveamine}})_{\text{sample}}}{(\text{NHN}_{\text{reactiveamine}})_{\text{blank}}} \quad (1)$$

Water vapor permeability (WVP): WVP was assessed using the modified desiccant method described by ASTM E96-95. The film was sealed on a glass permeation cup containing CaCl₂ (0% RH) using silicone vacuum grease and a rubber band to hold the film in place. The cups were placed in an environmental chamber maintained at room temperature (23±2°C) and 65±2 % RH. The weight gain of the cups was recorded every hour during an 8 h period with an analytical balance (±0.0001 g). WVP of the films was calculated as follows:

$$\text{WVP ((g/Pa}\cdot\text{s}\cdot\text{cm))} = \frac{W}{(A\cdot t\cdot\Delta P)} \cdot e \quad (2)$$

In vitro antibacterial activity of films:

Antibacterial activity on films was assessed using the agar diffusion method described elsewhere [6]. Films were aseptically cut into a 10 mm diameter disc and placed on agar plates, previously seeded with 100 µL of inoculums containing approximately 105–106CFU/mL of each tested bacteria. The plates were incubated at 37°C for 24 h. The diameter of the growth inhibition zone surrounding the film discs was measured with a manual caliper from the center of the film (Mitutoyo, Japan). Each assay was performed by triplicate on two separate experimental runs.

Statistical Analysis

Values obtained in all these experiments were statistically analyzed by one-way analysis of variance (ANOVA). Differences

between pairs of means were assessed by confidence intervals using the Tukey test. The level of significance was p>0.05.

Results and Discussion processing conditions

The feasibility of transforming gelatin into films through intensive mixing followed by compression molding has been already established [3,5,22,24]. However and the best of our knowledge, there is no reports on gelatin cross-linked with glyoxal in the melt state. Therefore, the knowledge of the optimal processing parameters is critical for BGe film formation by intensive mixing and compression molding.

The optimum combination of ingredients was fixed from exploratory studies [25]. Intensive mixing was used prior to compression molding. For blending BGe powder with GLY as plasticizer (30 wt%), ESO (10 wt%) as potentially reactive hydrophobizing agent, buffer solution pH 9 as processing aid and GLX (2 wt%) as cross-linking agent.

Mixing was performed at a fixed speed of 0.83 Hz while temperature and time were varied to analyze their effect on the blending process. Mixing temperatures were selected from glass transition temperature (T_g) values of the different blends (c.a. varying from 45 to 60°C, [25]), to ensure chain mobility. For all torque values, temperature showed a decline at the beginning of the process due to differences in the temperature of the sample (c.a. ambient temperature) and that of the chamber (Figure 1). After a few minutes, the temperature increased slightly until reaching a stable level up to the end of the process (Figure 1a-d). As expected, the torque showed a dependence on the temperature; at 40°C, the torque was unstable because the mixing temperature was lower than the lowest T_g, and therefore, unsuitable for processing (Figure 1a). At 50 and 60°C, and as the mixing process progressed, the blend flowed leading to a decrease in torque. The best torque-temperature combination was found for 60°C (Figure 1c). Mixing time (10 or 15 min, Figure 1c and d), had a marginal effect at least at the temperatures studied, therefore 15 min was fixed. GLX was incorporated in the 14th min to avoid gelation within the mixer chamber [19].

Temperature has been reported to be an essential factor in the formation of stable films during compression molding and extrusion [3,5]. Hence, molding temperature was established from TGA studies performed on BGe, BGe-10ESO and BGe-10ESO-2GLX blends, all with 30 wt% glycerol (Figure 2). All blends degraded in a gradual process with four stages in the TG curve. The first step, observed up to 170°C was attributed to the volatilization of absorbed and bounded water in gelatin [5,28]; the second stage peaking at 259°C, was attributed to

glycerol volatilization (boiling point 270°C) [5,28]; the third and main step, starting at 292°C, was assigned to protein chain scission and showed maxima at 326°C and 334°C for BGe-10ESO and BGe-10ESO-2GLX, respectively, showing the stabilizing effect of GLX due to cross-linking during heating. The last step ($T > 600^\circ\text{C}$), was associated with the decomposition of more thermally stable structures due to cross linking reactions produced during heating [28]. The most important mass loss for all materials was observed well above 200°C (Figure 2). Therefore,

120°C and 140°C were tested for the molding step. Processing pressure was established at 50 kg/cm², according to our previous works [5,25].

Pressing at 140°C produced uneven films with increased yellowish coloration. Such effect was more severe in GLX-containing films that resulted extremely fragile and dark brown in color. The initially increased flow-ability of the blends at 140°C was counterbalanced by excessive evaporation of water (used as a processing aid), becoming

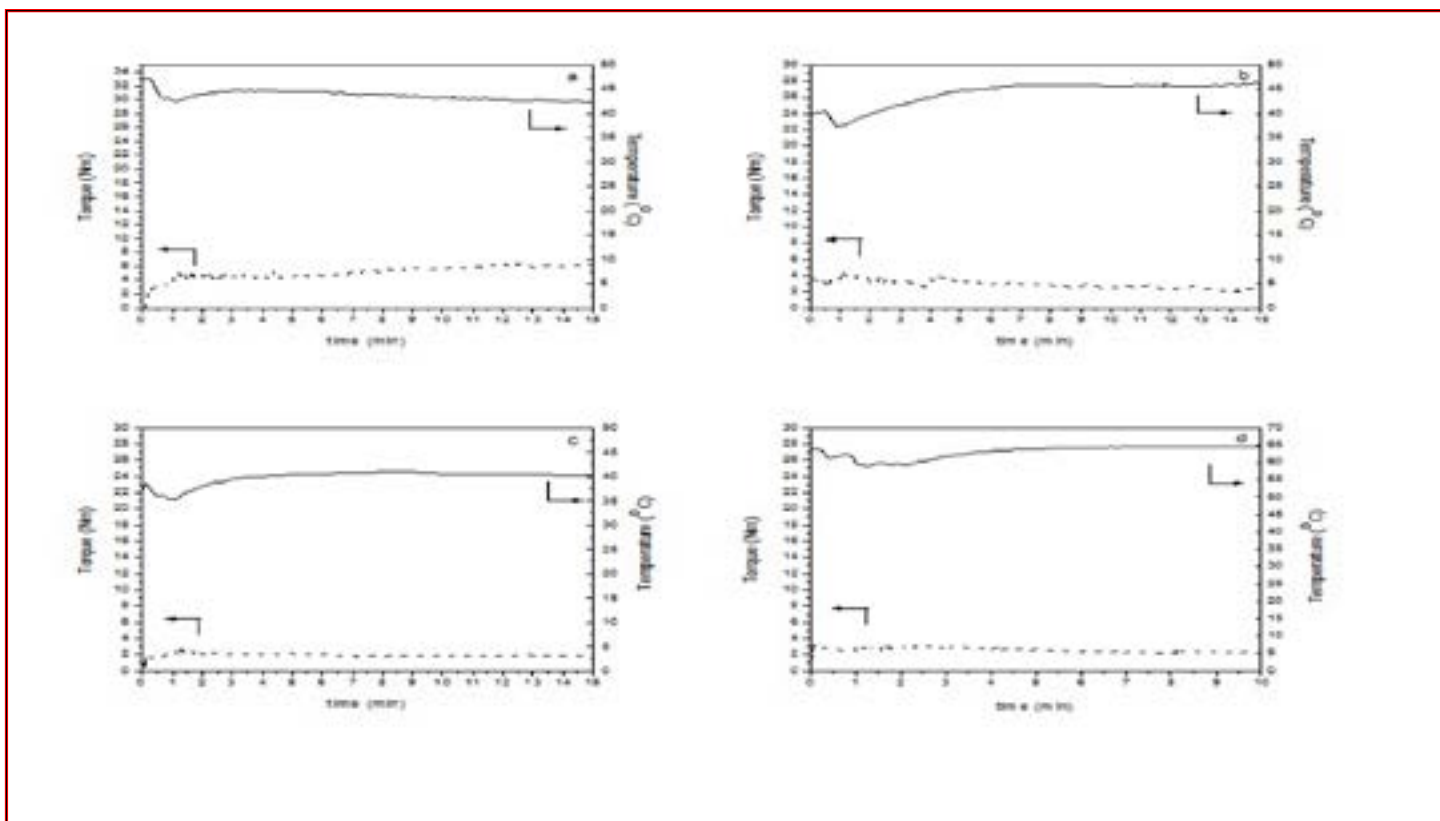


Figure 1

more viscous and more easily degraded. Molding at 120°C produced more acceptable, uniform easy to handle films. BGe-10ESO film had light yellow tint characteristic of both, BGe and ESO, whereas the film with GLX, was light orange, giving indirect evidence of the formation of imine moieties (Schiff's bases) due to cross-linking induced under compression molding conditions, as already observed for other protein-aldehyde systems obtained by thermoplastic processing [5,29]. Therefore, 120°C was selected as the compression-molding temperature [5,25]. Optimum molding time was determined as being 10 min because pressing time <10 min was unsatisfactory for films formation while longer times induced partial thermal degradation. According to the results, the processing parameters were fixed as follows: (a) intensive mixing at 60°C for 15 min at 0.83 Hz, adding GLX at the 14th min, (b) preheat-

ing of the mold at 120°C for 30 s, (c) molding at 120°C, 50 kg/cm² for 10 min and, (4) cooling under pressure up to room temperature.

Visual aspect and optical properties

All the produced films were flexible with smooth surfaces, visually homogenous, and light yellow to light orange in color (Table 1). Color parameters and light transmission varied significantly with the incorporation of ESO, as compared with the control (Table 1, $p < 0.05$), because of the scattering effect of a dispersed oily phase with mean size higher than the wavelength of the visible light (400-800 nm) [15]. Cross-linking with GLX induced a visually perceptible coloration toward the yellow-light orange tint, revealed by the

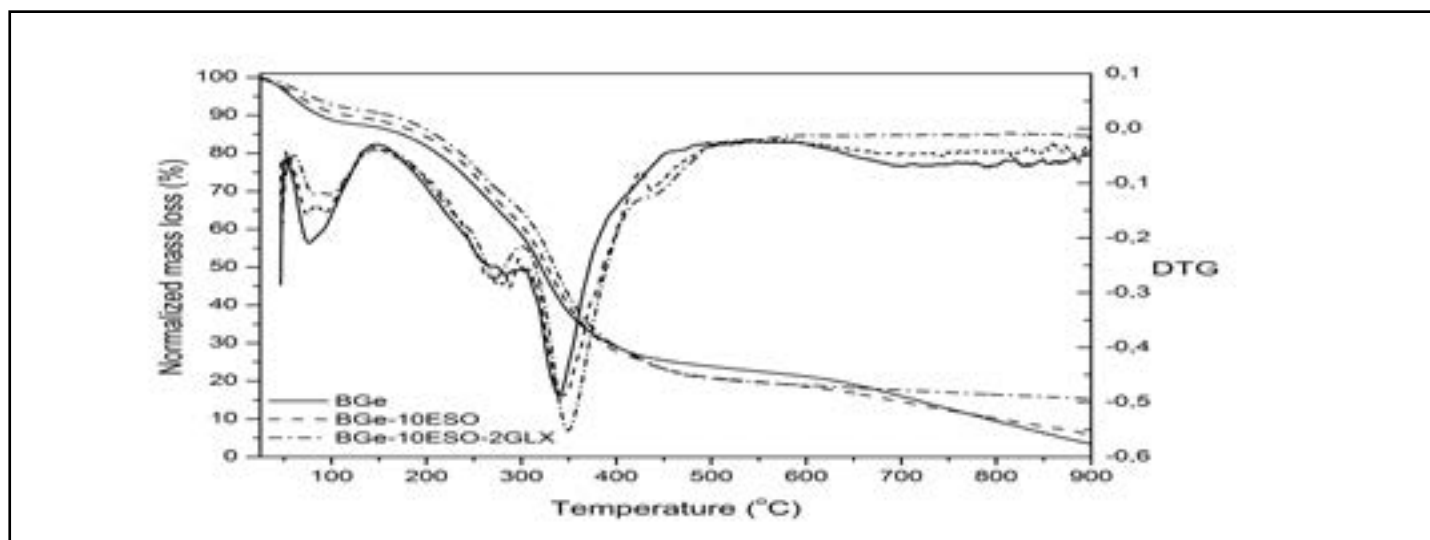


Figure 2

significant increment in a^* and b^* parameters (Table 1, $p < 0.05$), due to the colored Schiff's bases generated upon cross-linking with GLX, as already observed for other protein – aldehyde systems [5,7,8,27,29].

Interestingly, cross-linking reduced opaqueness as compared with BGe-10ESO film (Table 1, $p < 0.05$) suggesting increased miscibility between components. According to Figure 3, the combination of ESO and GLX increased the shielding of the film against UV-light by blocking the transmission at 280 nm by up to 99.9%. The UV barrier property of the gelatin film would result mainly from augmented imine moieties associated with the cross-linking reaction [6,28] (Scheme 1). Since bovine gelatin films are usually weak barrier to UV light due to the low content of aromatic amino acids (i.e., tyrosine 0.3 wt% and phenylalanine 0.2 wt%, [25]), BGe-10ESO-2GLX film might have the potential as wrapping antioxidant food packaging material.

Structural analysis by FTIR

ATR-FTIR was used to investigate the changes at the molecular level produced by the addition of ESO and GLX to BGe films during processing (Figure 4). All BGe-based films showed relevant bands at ~ 3285 - 3290 , 1633 , ~ 1548 , and ~ 1230 cm^{-1} typical of Amide A (N-H stretching and free O-H coupling by hydrogen bond), amide I (C=O stretching), amide II (N-H bending) and amide III (C-N and N-H stretching), respectively, in accordance to the literature [5,11,12]. A band at 1038 cm^{-1} characteristic of glycerol (C1 and C3-OH stretching) was also detected in all films [12]. On the other hand, spectrum of pure ESO was characterized by a strong absorption at 1743 cm^{-1} assigned to C=O stretching of triglyceride ester bond and peaks at 2925 and 2844 cm^{-1} attributable to CH_2 and CH_3




of alkyl chains of fatty acids esterified with glycerol and the internal oxirane rings were visualized as low intensity peaks at 842 and 823 cm^{-1} (C-O-C stretching) [17]. In the spectrum of BGe-10ESO, oxirane bands were no longer visible (Figure 4) suggesting the reaction, i.e. masking or cross-linking, between ESO, BGe and/or GLY [12,18].

The inclusion of GLX into BGe-10ESO formulation induced additional changes as compared with the spectrum of BGe-10ESO. Amide I and amide II bands experienced slight shifts (from 1631 to 1629 cm^{-1} and from 1545 to 1547 cm^{-1} , respectively, Figure 4), associated with the formation of new C=N (imine) covalent bonds [12], involved in the reaction between the aldehyde group in GLX and amino-side chain groups in BGe, mainly from arginine and lysine [12,13,14,18]. Such small change in the C=O region suggests that secondary structure of BGe was preserved, because of the amino groups able to react with GLX are located in the periphery of the proteins, as already observed for zein-GLX films processed by reactive extrusion [29]. Unfortunately, the characteristic peaks of GLX (C=O stretching, 1636 cm^{-1}) and imine groups (C=N stretching, 1670 - 1640 cm^{-1}) are located in the same region than Amide I, thus the disappearance of GLX nor the appearance of new C=N moieties cannot be evidenced by FTIR. Consequently, cross-linking reaction was followed by free amino groups consumption and indirect experimental evidences.

Free amino groups and total soluble matter

To further confirm the presence of covalent links between film components the concentrations of free amino groups (FAG) and total soluble matter (TSM) in control and modified BGe films were determined.

Table 1: Visual aspect, free amino groups (FAG), total soluble matter (TSM) and thickness of control, BGe-10ESO and BGe-10ESO-2GLX films.

Samplename	Visual aspect	Thickness (μm)	FAG (%)	TSM (%)
BGe		96.3 \pm 5.2 a	100 \pm 0 a	100 \pm 0 a
BGe10ESO		92.5 \pm 8.7 a	69.3 \pm 2.3 b	99.2 \pm 0.5 b
BGe-10ESO-2GLX		85.0 \pm 6.6 a	20.9 \pm 1.6 c	21.4 \pm 0.5 c

Equal letters in the same column indicate that there is no significant difference between the results. Tukey test, 95% confidence

Table 2: Color parameters, opacity and tensile properties of control, BGe-10ESO and BGe-10ESO-2GLX films.

Samplename	Color parameters			Opacity (UA*nm)	Tensileproperties	
	b*	a*	ΔE		TS (MPa)	ϵ (%)
BGe	4.72 \pm 0.34 a	-1.70 \pm 0.033 a	0 \pm 0 a	228 \pm 24 a	3.65 \pm 1.2a	95.7 \pm 17.2a
BGe-10ESO	-1.65 \pm 0.03 b	5.65 \pm 0.56 b	1.41 \pm 0.24 b	570 \pm 46 b	3.31 \pm 0.8a	88.6 \pm 18.9a
BGe-10ESO-2GLX	53.77 \pm 0.28 c	8.76 \pm 0.93 c	55.49 \pm 0.76 c	457 \pm 17 c	5.4 \pm 0.8b	56.4 \pm 15.7b

Equal letters in the same column indicate that there is no significant difference between the results. Tukey test, 95% confidence

After soaking in distilled water for 24 h, control and ESO-added BGe films dissolved completely, evidencing the lack of covalent cross-linking. The significant reduction of FAG showed by BGe-10ESO film (Table 2, $p < 0.05$), resulted from masking reaction (one-point fixation reaction) that is favored at $\text{pH} > 8$ [12,18]. Nevertheless, differences in results reported for WG [19] and SPI [20] films modified by ESO, might be ascribed to differences in protein type, amino acid composition and protein conformation. The low reactivity of ESO towards cross-linking reaction can be explained in terms of the reduced accessibility and reactivity of internal epoxy groups in ESO [17], compared with other polyepoxy reagents such

as BDDGE [12,18], where oxirane rings are terminal. Conversely, the significant drop in TSM (i.e. ~ 78%) and FAG (~ 30 %) promoted by GLX, as compared to BGe-10ESO (Table 2), gave indirect experimental evidence of the presence of covalent cross-linking induced by GLX.

Moisture content and WVP

The highest MC value was obtained for control BGe film in accordance to the hydrophilic character of both gelatin and glycerol, which causes water molecules retention inside the network (Table 2,

$p < 0.05$). The incorporation of ESO decreased MC significantly, more likely due to the hydrophobic character of the fatty acid alkyl chains esterifying glycerol in ESO [15,17], that diminished the tendency of BGe film toward water molecules [15]. The incorporation of GLX further improved both parameters (Table 2), because of the presence of covalent cross-links involving polar amino groups, reducing the water

The combination of 10 wt% ESO and GLX reduced significantly WVP (Table 2 $P < 0.05$). Since differences in thickness were marginal were (Table 2) the improvement in WVP can be ascribed to the synergetic effect of the hydrophobic character of ESO and the decreased concentration of FAG due cross-linking reaction. Although WVP of BGe-10ESO-2GLX film was lower than those reported for casted gelatin films cross linked with glyoxal (430×10^{-15} g/Pa·s·cm) [7], compression-molded gelatin films cross linked with 10 wt.% of starch dialdehyde (1700×10^{-15} g/Pa·s·cm) [5], thermos-compressed bovine gelatin ($2.84-3.4 \times 10^{-12}$ g/

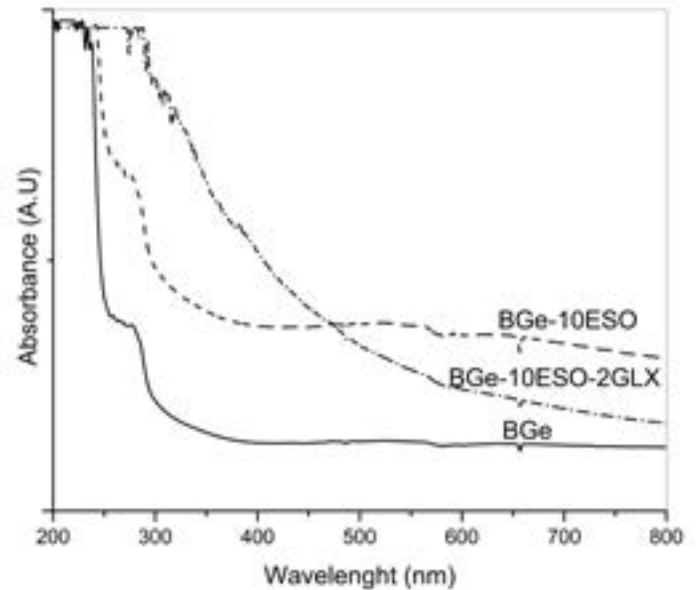


Figure 3

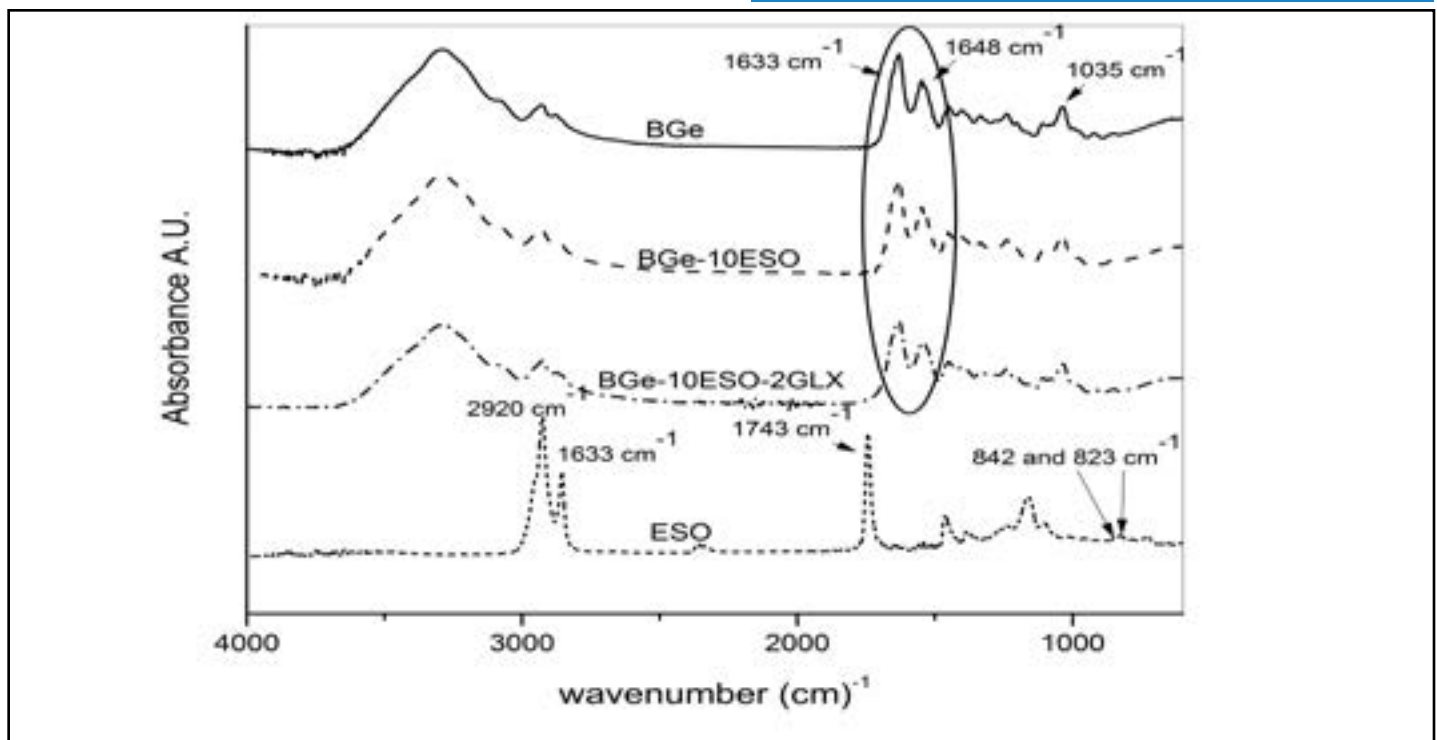


Figure 4

Pa·s·cm) [24], and gelatin films cross linked with 1 wt% glutaraldehyde (94×10^{-15} g/Pa·s·cm) [11], the WVP is still higher than values of synthetic films such as high-density polyethylene (HDPE, 2.4×10^{-15} g/Pa·s·cm), polyvinyl chloride (PVC, $0.7-2.4 \times 10^{-15}$ g/Pa·s·cm) and low-density polyethylene (LDPE, $3.6-9.7 \times 10^{-15}$ g/Pa·s·cm) [30].

Tensile properties

The tensile strength (TS) and elongation at break ($\epsilon\%$) values of control

and modified BGe films are listed in Table 2. Mechanical parameters of control BGe film were in the same range than those reported in the literature for BGe plasticized with glycerol and processed by compression molding [5]. TS and $\epsilon\%$ remained statistically unaltered after blending with 10 wt.% ESO (Table 2, $p > 0.05$), despite the significant reduction in MC, suggesting that ESO has no plasticizing effect, contrarily to results observed for gelatin modified with olive oil under micro fluidization pressure [15] and gelatin modified with olive oil under micro

fluidization pressure. As expected, glyoxal gave rise to more resistant but less stretchable films (Table 2), in accordance to a more reticulated material. The increment in TS with the drop in the elongation at break of gelatin films (plasticized and unplasticized) upon cross-linking was already published for glyoxal [7,8] and other aldehyde-based cross-linking agents such as glutaraldehyde [11], DAS [5], and formaldehyde [8].

In vitro antibacterial activity

BGe and BGe-10ESO-2GLX films were tested against *E. coli* and *S. aureus* using the agar diffusion method and the results are illustrated in Table 3. No inhibition was detected for BGe film. Differences with data reported by other on the intrinsic antibacterial activity of BGe could be related to several factors including amino acid composition, molar mass, and the type of tested bacteria [6]. The lack of inhibition halos with the inclusion of ESO and GLX (at least at the levels used herein) allows us to ascertain that BGe-10ESO-2GLX film did not exert any antibacterial activity, at least, against the microorganisms tested.

Conclusions

In the present study, we propose a simple approach to get bovine gelatin films with enhanced functional properties by the appropriate combination of glyoxal and ESO. Glyoxal was easily incorporated to BGe-10ESO mixture under mild mixing conditions, and the produced blend had suitable flow ability to be processed by compression molding. The whole transforming process is solvent-free and takes less than 1h as a whole, being less-time consuming and more environmentally friendly than other lab-scale processing methods such as casting. Regarding performance, BGe-10ESO-2GLX film kept ~80% of its integrity in ambient temperature water, enhanced the moisture content by ~30%, lowered the WVP by 57% and increased TS by ~47% while still maintaining adequate stretch ability (~56 %). No documents were found presenting data demonstrating the manufacture of gelatin films cross-linking with glyoxal using thermoplastic technology. Thus, our results are original. Additionally, the cross linked film showed UV-light barrier making it suitable for the design of antioxidant films for packaging. Based on the overall results, BGe-10ESO-2GLX film can be tailored as an external protective layer in the design of eco-friendly active all-gelatin based multilayer films, that is the subject of an oncoming paper.

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