

## THE EFFECT OF PLASTICIZER CONTENT AND DISACCHARIDE TYPE ON THE MECHANICAL, BARRIER AND PHYSICAL PROPERTIES OF BOVINE GELATIN-BASED FILMS

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### Abstract

Gelatins are regarded as alternative raw materials to prepare films for food packaging. However, the improvement of their mechanical and water barrier properties is necessary in order to obtain useful materials in service conditions. To improve these functional properties, two strategies have been carried out in this work. First, glycerol was added as plasticizer to increase the flexibility of the films. Second, lactose or sucrose was added to react with gelatin and increase water resistance of gelatin-based films. Commercial gelatin, glycerol and lactose or sucrose were employed in this work and processing of the films was carried out by solution casting. All gelatin films obtained were transparent and flexible. Moreover, the hydrophobic character of the films was increased and the film solubility was decreased by the addition of glycerol and disaccharides. As was observed via FTIR, the changes were due to the interactions between gelatin and glycerol and Maillard reaction between gelatin and disaccharides.

Keywords: Gelatin films, Maillard reaction, Solubility, FTIR, Hydrophobicity, Mechanical properties.

### 1. Introduction

The intensive application of plastics, neither biodegradable nor renewable, in packaging is generating serious problems in the management of wastes, specially municipal wastes, due to the fact that the materials used for those purposes are generally disposed after one unique use. The employment of biodegradable and/or

compostable materials obtained from renewable sources could become a suitable approach to solve those problems. Moreover, the increasing use of this kind of materials would contribute to promote a sustainable development of materials. In this context, the use of renewable resources to produce biodegradable materials with good properties is an area of work that has reached a great interest in the last years [1, 2].

Proteins, lipids and polysaccharides have been used as biodegradable polymers, and these novel materials are widely used in food industry [3, 4]. Among biopolymers, the materials based on proteins are provided with the additional value of being abundant, having relatively low costs, and interesting functional properties such as very good film forming ability. In the case of the films based on proteins used in food industry, proteins might be even edible, adding nutritional value to food. This is the case of gelatin, which is a protein obtained by collagen denaturation [5, 6]. Two types of gelatin are commercially produced, type A gelatin from acid-treated collagen and type B gelatin from alkali-treated collagen [7, 8]. In both cases, amino acid composition mainly consists of glycine, proline and hydroxyproline [9]. This amino acid composition greatly affects the processing conditions employed to obtain the film. In the case of gelatins, solution casting is the method usually carried out and the film formation from the solution implies a good control of pH in order to allow conformational changes [10].

Although gelatin-based films show important advantages referred to their renewable origin and their biodegradability after disposal, these proteins have some disadvantages inherent to their chemical structure, such as brittleness and hydrophilic character and thus, addition of other components, preferably also natural substances, is necessary in order to obtain green composites with improved functional properties [11, 12]. Two are the approaches that can be carried out to address the drawbacks related to the low flexibility and the low water resistance shown by gelatin films: the addition of plasticizers, and the addition of chemicals able to react with the biopolymer.

Plasticizers are added to protein-based films in order to reduce interactions among protein chains. The reduction of those interactions that stabilize film network results in a greater mobility of protein molecules. Plasticizers must be added at a certain amount to obtain the film with improved flexibility without significant decrease of functional properties. Plasticizers such as glycerol, polyethylene glycol, and sorbitol are generally used for protein films to improve mechanical properties. However, the differences in composition, size, structure and shape of plasticizers directly influence their ability to function in the film network [13, 14]. Previous works have shown that glycerol improves mechanical behaviour of protein-based films [15, 16] and thus, this polyol was added to the formulations prepared in this work .

In addition to physical interactions between gelatin and glycerol, the improvement of functional properties by using chemical means has also been studied in this work. In a previous work [17], some attempts were made to improve the functional properties of proteins through protein-sugar graft reactions based on Maillard reaction. This reaction produces coloured reaction products, depending on the stage of the reaction as well as other factors, such as pH, type of reactants, temperature, water activity and concentration of reactants. Therefore, the use of

different disaccharides such as lactose and sucrose is supposed to have different effects on the properties of gelatin-based films due to their different susceptibility to react with the amino groups of proteins. In this context, the objective of this work was to study the effect of disaccharide type, as well as plasticizer content, on mechanical, barrier, and physical properties of bovine gelatin-based films prepared by solution casting.

## **2. Experimental**

### **2.1. Materials**

The commercial bovine gelatin (GE) used in this study was type A gelatin with bloom 200/220 and was purchased from Sancho de Borja S.L. (Zaragoza, Spain). This gelatin meets quality standard for edible gelatin (1999/724/CE). NaOH, glycerol (GLY), lactose (LAC), and sucrose (SUC) were supplied by Panreac (Barcelona, Spain). NaOH was used to adjust solution pH. Glycerol, which was food grade, was used as plasticizer. Lactose and sucrose were used to promote Maillard reaction with gelatin and to analyze the effect of the type of disaccharide employed.

### **2.2. Film preparation**

Films were prepared by solution casting. Film forming solutions were prepared by mixing 100 mL distilled water, 5.0 g gelatin, and 1.5 g sucrose or lactose. Solutions were heated at 80°C at 150 rpm for 30 min. After that, glycerol was added to solutions to obtain 30 or 40 wt% concentration, and solutions were maintained at 80°C for other 30 min under stirring at 150 rpm. Solution pH was appropriately adjusted to 10.0 with NaOH (0.1 M). Subsequently, solution was poured into Petri dishes (140 mm diameter), which were kept at a conditioned room for 48 h to evaporate water.

Films were designated as GE30GLY and GE40GLY to indicate 30 and 40 wt% glycerol, respectively, and GE30GLY30LAC, GE40GLY30LAC, GE30GLY30SUC and GE40GLY30SUC to indicate the incorporation of 30 wt % lactose or sucrose. All films were conditioned in a controlled bio-chamber (ACS Sunrise 700V) at 25°C and 50% relative humidity for 48 h before testing.

### **2.3. Film thickness**

Film thickness was measured to the nearest 0.001 mm with a hand-held digimatic micrometer (QuantuMike Mitutoyo). The values obtained for each sample at five different locations were averaged.

### **2.4. Moisture content (MC) and total soluble matter (TSM)**

TSM was expressed as the percentage of film dry matter solubilised after 24 h immersion in distilled water. Two methods of determination were used and compared [18]. In the first method (dry), three specimens of each sample were weighed ( $m_w$ ) and subsequently dried in an air-circulating oven at 105°C for 24 h.

After this time, samples were reweighed ( $m_0$ ) to determine MC values. Afterwards, samples were immersed in 30 mL of distilled water in the presence of sodium azide (0.02%) in order to prevent the microbial growth. The beakers were stored in an environmental chamber at 25°C for 24 h with occasional gentle stirring. After this time, specimens were dried in an air-circulating oven at 105°C for 24 h and weighed ( $m_f$ ). In the second method (wet), dry matter and soluble matter were not determined on the same sample in an effort to avoid heating sample prior to immersion in water. Instead, samples were directly immersed in water and beakers were stored in an environmental chamber at 25°C for 24 h with occasional gentle stirring to determine TSM (wet) values. Initial dry matter mass was the value obtained by MC measurements for the same sample.

MC and TSM values were calculated by Eqs. (1) and (2), respectively:

$$MC (\%) = \frac{m_w - m_0}{m_w} \times 100 \quad (1)$$

$$TSM (\%) = \frac{m_0 - m_f}{m_0} \times 100 \quad (2)$$

## 2.5. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were carried out on a Nicolet Nexus FTIR spectrometer using ATR Golden Gate device (Specac). A total of 32 scans were performed at 4  $\text{cm}^{-1}$  resolution and measurements were recorded between 4000 and 800  $\text{cm}^{-1}$ .

## 2.6. Contact angle determination

A contact angle meter (model Oca20, dataphysics instruments) was used to perform contact angle measurements on the surface of the films prepared in this study. A film sample (20 mm  $\times$  80 mm) was put on a movable sample stage and levelled horizontally; then, a drop of about 3  $\mu\text{L}$  of distilled water was placed on the surface of the film using a microsyringe. The contact angle was measured in a conditioned room by recording contact angle values. Image analyses were carried out using SCA20 software. Ten replicates were made per formulation.

## 2.7. Water vapour permeability (WVP)

WVP of the films was determined according to ASTM E96-00 [19]. Films were cut into circles of 7.40 cm diameter and the test area was 33  $\text{cm}^2$ . The setup was subjected to a temperature and relative humidity of 38°C and 90%, respectively. Water vapour transmission rate (WVTR) was calculated by Eq. (3):

$$WVTR \left( \frac{\text{g}}{\text{s} \cdot \text{cm}^2} \right) = \frac{G}{t \times A} \quad (3)$$

where  $G$  is the change in weight (g),  $t$  is time (s), and  $A$  is the test area ( $\text{cm}^2$ ). WVP was calculated by Eq. (4):

$$WVP \left( \frac{\text{g}}{\text{cm} \cdot \text{s} \cdot \text{Pa}} \right) = \frac{WVTR \times L}{\Delta P} \quad (4)$$

where  $L$  is the thickness of the test specimen (cm) and  $\Delta P$  is the partial pressure difference of the water vapour across the film (Pa). WVP for three specimens of each sample was calculated and reported.

## 2.8. Mechanical properties

An electromechanical testing system (MTS Insight 10) was used to determine mechanical properties. Tensile strength and elongation at break were determined according to ASTM D1708-93 [20]. Bone-shaped specimens (4.75 mm wide and 22.25 mm long) were cut and five replicates were tested for each composition.

## 2.9. Statistical analysis

Data were subjected to one-way analysis of variant (ANOVA) by means of an SPSS computer program (SPSS Statistic 20.0). Post hoc multiple comparisons were determined by the Tukey test with the level of significance set at  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Moisture content (MC) and total soluble matter (TSM)

In order to analyze the effect of additives on moisture and water resistance, MC and TSM values were measured and are shown in Table 1.

**Table 1. MC and TSM Values of Gelatin-Based Films.**

	MC	TSM (wet)	TSM (dry)
GE30GLY	11.51±0.37 <sup>ab</sup>	100 <sup>a</sup>	93.46±1.44 <sup>a</sup>
GE40GLY	14.65±0.27 <sup>c</sup>	100 <sup>a</sup>	90.88±0.45 <sup>a</sup>
GE30GLY30LAC	10.64±0.25 <sup>a</sup>	100 <sup>a</sup>	41.39±0.36 <sup>b</sup>
GE30GLY30SUC	11.03±0.43 <sup>a</sup>	100 <sup>a</sup>	65.88±0.64 <sup>c</sup>
GE40GLY30LAC	12.65±0.21 <sup>b</sup>	100 <sup>a</sup>	28.77±0.45 <sup>d</sup>
GE40GLY30SUC	13.23±0.15 <sup>b</sup>	100 <sup>a</sup>	46.90±0.32 <sup>e</sup>

<sup>a-e</sup> Different superscripts in the same column indicate 95% significant differences among film formulations.

MC values were significantly ( $p < 0.05$ ) different for the films with different glycerol contents, but were not significantly ( $p > 0.05$ ) different for the films with different contents of lactose or sucrose. On the one hand, the results obtained are directly attributable to the presence of glycerol, which is a hydrophilic plasticizer and enhances water absorption by gelatin films. Similar results were observed for soy protein-based films [11]. On the other hand, the addition of lactose or sucrose decreased MC values due to the decrease of free polar groups, which react through Maillard reaction. This decrease was more noticeable for GE40GLY30LAC and GE40GLY30SUC films and higher for lactose-incorporated films due to a higher extension of Maillard reaction in those systems and thus, less amount of free polar groups to absorb humidity.

TSM values were determined by two methods used in the literature in order to analyze the effect of temperature [21-23]. In the case of TSM (wet) values, all gelatin films were soluble in water ( $p > 0.05$ ), regardless of glycerol or disaccharide

content. In the case of TSM (dry) values, a significant ( $p < 0.05$ ) decrease was observed with respect to TSM (wet) values. Since TSM (dry) method implies a drying process, as explained in experimental section, these results indicated the effect of temperature on film solubility. On the one hand, these results indicate that temperature would favour the interactions between glycerol and the polar groups of gelatin, as shown by TSM (dry) values for GE30GLY and GE40GLY. On the other hand, temperature would favour Maillard reaction between amino groups in gelatin and hydroxyl groups in sucrose and lactose, reducing solubility as a result of the products formed by Maillard reaction. It is well known that temperature influences Maillard reaction; therefore, drying specimens prior to immersion in water could cause cross-linking, leading to lower TSM (dry) values [24]. The decrease in TSM values is higher for the films with lactose due to the reducing character of this disaccharide [17].

### 3.2. FTIR spectroscopy

Proteins are constituted by amino acid residues joined by peptide bonds. Amide bands in FTIR spectra represent the different vibrational modes of those peptide bonds. Figure 1 shows the FTIR spectra of films with and without lactose or sucrose. Absorption peaks related to the peptide bond in gelatin appeared at  $1625\text{ cm}^{-1}$ ,  $1537\text{ cm}^{-1}$ , and  $1236\text{ cm}^{-1}$ , and are known as amide I, amide II, and amide III bands, respectively. Amide I band is associated with the carbonyl group, amide II with N-H bending, and amide III with C-N stretching [15]. The band at  $3280\text{ cm}^{-1}$  is assigned to hydroxyl and amino groups and the peak observed at  $2933\text{ cm}^{-1}$  corresponds to  $\text{CH}_2$  groups. Finally, the bands observed at  $1032$  and  $1106\text{ cm}^{-1}$  correspond to C-O bonds in glycerol [11]. The main changes in FTIR spectra appeared around  $1000\text{ cm}^{-1}$  region, which is related to C-O bonds. In the case of the films prepared with lactose, a broad band appeared in this region, which would indicate the formation of the Maillard reaction products (MRPs). MRPs are due to the reaction between amino groups in gelatin and free hydroxyl groups in the anomeric carbon of lactose [17].

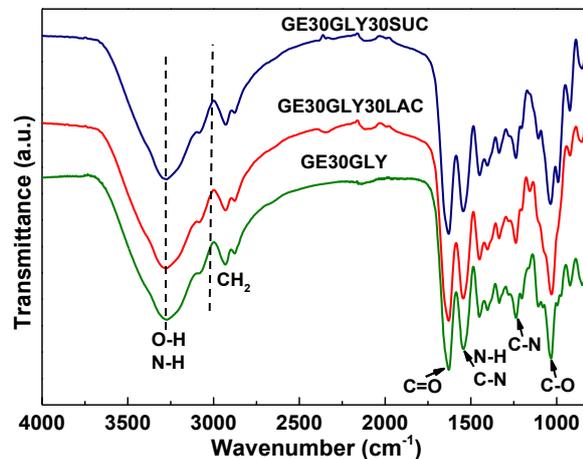


Fig. 1. FTIR Spectra of Gelatin-Based Films.

### 3.3. Water contact angle (WCA) and water vapour permeability (WVP)

To understand the effect of glycerol, lactose and sucrose on the hydrophilic or hydrophobic character of the films prepared in this study, WCA was determined and shown in Table 2.

**Table 2. WCA and WVP of Gelatin-Based Films.**

	WCA (°)	WVP 10 <sup>12</sup> (g.cm/cm <sup>2</sup> .s.Pa)
<b>GE30GLY</b>	98.66±2.29 <sup>a</sup>	2.424±0.044 <sup>a</sup>
<b>GE40GLY</b>	101.10±2.53 <sup>a</sup>	3.475±0.056 <sup>b</sup>
<b>GE30GLY30LAC</b>	103.49±2.21 <sup>a</sup>	2.270±0.013 <sup>a</sup>
<b>GE30GLY30SUC</b>	106.66±1.32 <sup>b</sup>	2.426±0.024 <sup>a</sup>
<b>GE40GLY30LAC</b>	106.10±3.03 <sup>b</sup>	2.595±0.032 <sup>a</sup>
<b>GE40GLY30SUC</b>	110.33±1.56 <sup>b</sup>	2.691±0.015 <sup>a</sup>

<sup>a-b</sup> Different superscripts in the same column indicate 95% significant differences among film formulations.

In this study, contact angles increased ( $p < 0.05$ ) with glycerol addition and thus, the hydrophobic character of the films also increased. This behaviour indicates that gelatin-glycerol interactions could result in a higher exposure of the hydrophobic groups of gelatin, as shown by the surface analysis of the films performed by X-ray photoelectron spectroscopy (XPS) carried out in a previous work [25]. This exposure of the hydrophobic groups of gelatin increased with the addition of sucrose or lactose ( $p > 0.05$ ), showing values higher than 100°, so that films with sucrose or lactose can be considered as hydrophobic films.

The addition of glycerol promotes gelatin-glycerol interactions, decreasing interactions among protein chains, which could explain the increase ( $p < 0.05$ ) in WVP values, as can be seen in Table 2. The increase in chain mobility promoted by glycerol could cause an easier diffusion of water through the film [26, 27]. Furthermore, the addition of sucrose or lactose caused a decrease ( $p > 0.05$ ) in WVP values due to the crosslinking reaction, which lead to a more compact structure, as shown by scanning electron microscopy (SEM) in a previous work [17].

### 3.4. Mechanical properties

The effect of glycerol, sucrose and lactose on mechanical properties of gelatin films is shown in Table 3.

**Table 3. Tensile properties of gelatin-based films.**

	TS (MPa)	EB (%)
<b>GE30GLY</b>	16.988±1.828 <sup>a</sup>	30.761±4.063 <sup>a</sup>
<b>GE40GLY</b>	7.756±0.677 <sup>d</sup>	47.233±5.056 <sup>c</sup>
<b>GE30GLY30LAC</b>	14.170±1.113 <sup>b</sup>	37.221±3.932 <sup>ab</sup>
<b>GE30GLY30SUC</b>	11.333±0.417 <sup>c</sup>	45.661±2.843 <sup>bc</sup>
<b>GE40GLY30LAC</b>	6.994±0.571 <sup>d</sup>	43.122±2.214 <sup>bc</sup>
<b>GE40GLY30SUC</b>	6.128±5.056 <sup>d</sup>	46.236±3.313 <sup>bc</sup>

<sup>a-d</sup> Different superscripts in the same column indicate 95% significant differences among film formulations.

On the one hand, tensile strength (TS) values significantly decreased ( $p < 0.05$ ) with glycerol content and with sucrose or lactose addition when 30 wt% glycerol was used, but there was no significant difference ( $p > 0.05$ ) for the films with sucrose or lactose plasticized with 40 wt% glycerol. On the other hand, elongation at break (EB) values significantly increased ( $p < 0.05$ ) with glycerol content, but no significant difference ( $p > 0.05$ ) was found for the films with lactose or sucrose. The increase of flexibility in the films plasticized with glycerol is in agreement with the increase of WVP values. As explained above, the looser structure of the network caused by the reduction of protein-protein interactions due to the presence of glycerol, led to a greater chain mobility [28]. It is also worth noting that EB values achieved in this work are higher than the values obtained in a previous work [29].

#### 4. Conclusions

Transparent and flexible gelatin-based films were prepared in this work and the characterization of functional properties showed the potential used of the films as food packaging. Some concluding observations from the investigation are given below.

- Elongation at break increased by the addition of glycerol; this fact can be related to the promotion of gelatin-glycerol interactions.
- Addition of disaccharides caused an increase in contact angle values, which were higher than  $100^\circ$ , indicating that these gelatin-based films can be considered hydrophobic.
- Addition of disaccharides caused chemical interaction by Maillard reaction, which was favoured by the effect of temperature, as shown by the decrease in the film solubility from 91% to 29% in the case of the films prepared with lactose.
- Water resistance of the films was significantly improved for lactose-modified films.

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