



# Starch-Leather Waste Gelatin Films Cross-Linked with Glutaraldehyde

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## Abstract

Gelatin extracted from chromium-tanned leather wastes (CTLW) has a lower molar mass because of the more aggressive extraction process needed to disrupt chromium-collagen bonds when compared to gelatin extracted from the bones, skin, and connective tissues of animals. As a consequence, CTLW gelatin is more hydrophilic, thus it is harder to apply in the production of polymers. To overcome this issue, in this study, films produced with starch, CTLW gelatin, commercial gelatin (as a comparative), and their blends were cross-linked with glutaraldehyde. The cross-linking reduced the crystallinity of the films, impairing the reorganization of gelatin chains into a triple helix structure, which balanced the effect of the higher molecular chain, while not altering the films' tensile strength. It increased the elongation at break and reduced the solubility and the swelling degree by up to 53% and 69%, respectively. These results stand as a great advance for the practical use of starch-CTLW gelatin films.

**Keywords** Leather waste · Gelatin · Starch · Glutaraldehyde · Cross-linking

## Introduction

The production of starch–gelatin polymer films started in the late nineties aiming at allying the properties of materials made of proteins and polysaccharides in a single polymer [1, 2]. After more than three decades, this blend is still the subject of research [3–16]. Films produced from gelatin (or collagen hydrolysate) obtained from leather wastes are of special interest [9, 12, 17–20].

The use of starch in the production of polymeric materials is justified by its low cost and wide commercial availability. In turn, proteins such as gelatin usually result in polymeric materials of better mechanical properties [21].

Polymeric starch and gelatin films are commonly studied for applications in the food industry, especially as packaging [2, 4, 22–24]. The addition of essential oils and ethyl lauryl alginate, for example, may confer them antifungal properties [5, 25]. This blend is also applied in biomedicine, especially in drug delivery systems [3, 10, 26] and might be used in agriculture as well [27–30].

The latter application may be more suitable when the gelatin used is recovered from wastes such as chromium-tanned leather wastes, since the films would be in direct contact with soil instead of human skin or food items. Because starch–gelatin polymers are hydro-biodegradable, the hydrolysis of the long-chain polymer molecule results in smaller molecules used as nutrients by microorganisms [27–30]. The feasibility of the starch–gelatin film's practical applications, however, depends directly on the production of less hydrophilic films that could withstand, at least for a certain period of time, the conditions of application. This is especially true for CTLW gelatin films, which are more hydrophilic than the ones produced from commercial gelatin [9].

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One of the ways of decreasing the interaction of starch and gelatin films with water is through cross-linking. Marucci and Ruseckaite [31] and [32] indicate the reduction in the biodegradation of crosslinked starch–gelatin films and Al-Hassan and Norziah [4] obtained higher mechanical resistance and lower solubility values on films crosslinked with transglutaminase.

Gelatin crosslinking can be accomplished by the addition of chemical agents such as transglutaminase, terephthalaldehyde [33] and glutaraldehyde—the latter is the most widely studied in the crosslinking of collagen for biomedical applications [34, 35]. The glutaraldehyde-promoted crosslinking occurs due to the bonds created between the amine groups of the gelatin and the hydroxyl groups of the glutaraldehyde, which results in the formation of a Schiff base [17].

In this work, the effects of glutaraldehyde crosslinking on the properties of starch–gelatin films extracted from chromium-tanned leather wastes were studied. A reduction in the hydrophilicity of starch–gelatin films results in a more sustainable material with better properties, which might be used as an alternative to synthetic polymers derived from petroleum.

## Materials and Methods

### Materials

Gelatin extracted from the chromium-tanned leather wastes (CTLW) and colorless and unflavored commercial grade gelatin (Refeisucos, Brazil) were used in the production of polymer films with native corn starch (Refeisucos, Brazil). The films were plasticized with glycerol (Kinetics, Brazil) and crosslinked with glutaraldehyde (50% v/v) (Neon, Brazil). The complete extraction and purification process of gelatin extracted from CTLW was reported by Scopel, Restelatto, Baldasso, Dettmer and Campomanes Santana [36].

The gelatin ash content was determined by subjecting the samples to a temperature of 600 °C in a muffle furnace (Model 1963, Fornitec, Brazil) for 4 h and measuring their masses before and after calcination.

The gelatin molar mass was determined by gel permeation chromatography on a Viscotek GPC Max VE-2001 equipment, using 2SB-807 HQ and 2SB-806M HQ columns.  $\text{NaNO}_3$  in aqueous solution was used as the mobile phase at 0.5 mL/min and 35 °C.

The starch gelatinization temperature was determined by differential scanning calorimetry (DSC) performed on a simultaneous thermal analyzer (STA, Jupiter 449, Netzsch, Germany), according to the methodology presented by Karlsson & Eliasson (2003). The starch amylose content was determined by the spectrophotometric method recommended by Martínéz & Cuevas (1989).

### Polymeric Films Production

In order to produce the polymeric film samples by casting, starch and CTLW or commercial gelatin were mixed with deionized water, which was added to a final filmogenic solution volume of 213 mL. The pH of both solutions was equal to  $7 \pm 0.5$ . According to Farris et al. (2010), neutral to slightly alkaline pH values are more favorable to gelatin crosslinking, which indicates the chosen pH of  $7.0 \pm 0.5$  is appropriate for the intended reaction [37]. The solution was then heated at 85–90 °C for 15 min under constant stirring and mixed with glycerol. Glutaraldehyde (when used) was added to the solution (at a concentration of 3%) after it had been cooled to 25 °C. Finally, the solution was casted onto a 21.0 × 27.5 cm Teflon-coated plate. The samples were dried at  $21 \pm 3$  °C and relative humidity of  $60 \pm 7\%$  for 4 days. Each film composition was produced in triplicates and the experiments were properly randomized. Two experimental designs were used to evaluate the crosslinking effect promoted by glutaraldehyde.

The first experimental designs was used to evaluate how glutaraldehyde altered the properties of the films produced with a single natural polymer (starch-S, CTLW gelatin-L, or commercial gelatin-C). For this, samples were produced following a  $2 \times 3$  complete factorial experimental designs. Each film was produced with 6 g of the natural polymer and 1.6 g of glycerol (both on a dry basis).

The second experimental designs was used to evaluate how glutaraldehyde, in different concentrations (0.0, 1.5 and 3.0%), altered the properties of the films produced with the blend of starch and CTLW or commercial gelatin. For this, samples were produced following a  $2^k$  factorial design with a center point. Each film was produced with 2 g of starch, 4 g of gelatin and 1.6 g of glycerol (all of them on a dry basis).

The films were stored at a constant relative humidity of 60% (maintained with a saturated solution of magnesium nitrate in a closed environment) until further characterization.

### Films Characterization

The thickness of the films was determined using a layer thickness gauge (Model 345, Elcometer) capable of measuring variable thicknesses from 0 to 12,500  $\mu\text{m}$  with an accuracy of 2.5  $\mu\text{m}$ . Twenty measurements were taken for each film sample.

The solubility test was performed using  $2 \times 2$  cm samples. They were dried in a desiccator for 48 h to determine the initial dry mass and were then placed in a plastic container with 70 mL of deionized water and shaken in an

orbital shaker at 90 rpm and 27 °C for 24 h. Afterwards, the samples were again dried in a desiccator for 72 h to determine their final mass. Mass measurements were performed on an analytical balance (Model AUY220, Shimadzu, Japan). The mass loss percentage corresponded to the solubility of the film.

Water vapor permeability (WVP) was determined according to ASTM E96-00. 7 g of 4–8 mm silica gel were placed in plastic containers with 2.5 cm opening diameters. The openings of the containers were then sealed with film samples so that water vapor transfer could occur only through the film. This system was placed in a test chamber with relative humidity controlled at 75% (using a saturated sodium chloride solution) at 20 °C and atmospheric pressure. The mass of water absorbed by silica, and thus transferred through the film, was determined by weighing the system every hour for 8 h.

The degree of swelling was determined using 2 × 2 cm samples of previously dried films. They were weighed and then submerged in deionized water at 23 °C. After 5 h, the films were removed from the water and weighed again. The degree of swelling was calculated as the percentage of water (m/m) absorbed by the dry film.

Mechanical properties were determined according to the ASTM method D882-2012. For each film, five 2 × 10 cm specimens were stored at 23 ± 2 °C and 50 ± 10% of humidity for at least 40 h. Then, they were tested in a universal machine (Emic, Model DL2000, Brazil) at a separation rate of 25 mm.min<sup>-1</sup> and using a 20 kN load cell.

UV-visible absorption spectra of the films were obtained in an Evolution 60 spectrophotometer (Thermo Scientific) within the range of 200 to 550 nm using a film carrier. Color was determined using a Hunter Lab

spectrophotometer. Six layers of each film were overlaid to allow proper color measurement.

The X-ray diffraction (XRD) test was performed on an X-ray diffractometer (Shimadzu XRD-6000), at room temperature with a 2-theta range of 2°–40° and with scan step of 0.03°.

Statistical analysis was performed with the Software Statistica 10 (Statsoft). The statistical difference between means was determined by the Tukey test using a 95% confidence level.

## Results and Discussion

### Raw Material Characterization

The amylose content in starch was found to be 27% (± 0.6). Consequently, amylopectin content is equal to 73%, which is within the values typically found for native corn starch [38, 39]. Starch gelatinization temperature was found to be equal to 84.5 °C, which is a similar value to the ones found by other authors [39, 40]. This is, therefore, the minimum temperature for the filmogenic solution to be heated so that the starch can effectively undergo the gelatinization process.

Commercial and CTLW gelatin presented ash contents of 0.24% ± 0.04 and 1.2 ± 0.3% respectively. The higher ash content of CTLW gelatin will play a part in changing some of the films properties as later discussed.

Another gelatin property that alters films properties is its molar mass [41, 42]. Figure 1 presents the number average molar mass ( $\bar{M}_n$ ), mass average molar mass ( $\bar{M}_w$ ), z average molar mass ( $\bar{M}_z$ ), polydispersity ( $\bar{M}_w/\bar{M}_n$ ), and the molar mass distribution curve for both commercial and CTLW gelatin.

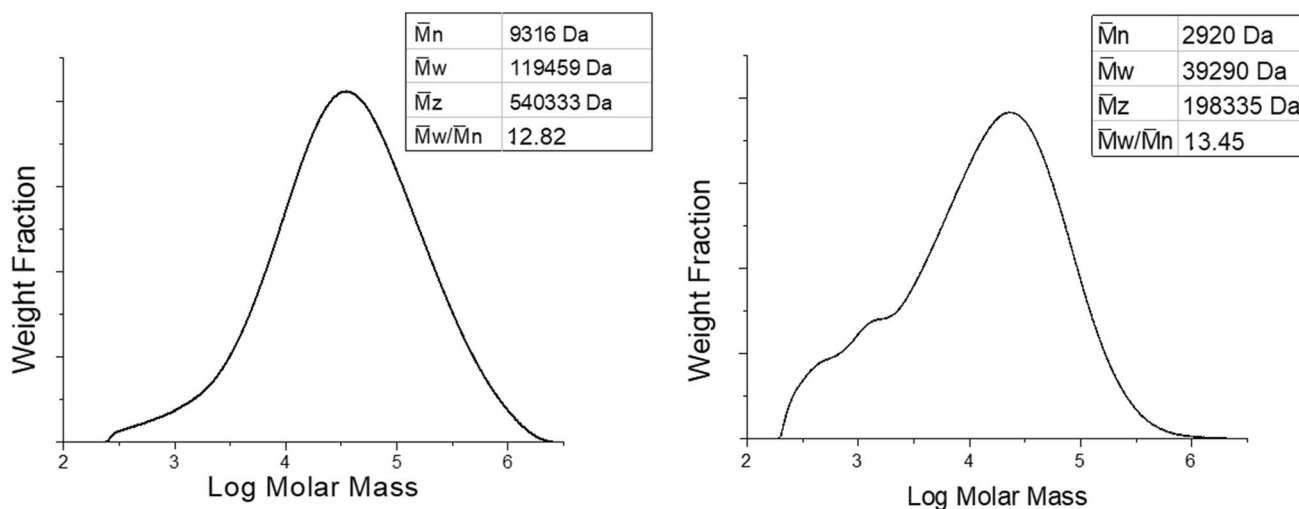


Fig. 1 Molar mass distribution curve and molar mass averages for CTLW gelatin (left) and commercial gelatin (right)

The broad molar mass distribution seen in Fig. 1, especially for CTLW gelatin, indicates that the size of the molecular chains are not homogeneous, which is confirmed by polydispersity values of 13.45 and 12.82 for CTLW and commercial gelatin respectively. According to Akcelrud [43], higher polydispersity values indicate the existence of a tail of low molar mass in the curve. This is observed in both samples, but mainly for CTLW gelatin, which has a tail of lower molar mass that corresponds to the gelatin molecules that had their chain more hydrolyzed during extraction.

$\bar{M}_w$  values (39 and 119 kDa, for CTLW and commercial gelatin respectively) are within the typical range of molar mass for gelatin extracted through the alkaline process, which ranges from 20 to 120 kDa [44]. The lower value found for CTLW gelatin was expected due to the need to break chromium-collagen bonds for the extraction of this gelatin. The hydrolysis process, thus, becomes more aggressive than the one used for commercial gelatin extraction, which is made from non-tanned animal skin, bones, and cartilage. Nevertheless, molecules with a molar mass higher than 120 kDa can be observed in both samples. It is also possible to observe that the CTLW sample presents a higher proportion of lower molecular mass chains than the commercial gelatin sample.

### Films Color and UV–Visible

The main visual characteristic resulting from the cross-linking of the gelatin chains by the action of glutaraldehyde is a yellowish color, as Fig. 2 shows. The color stems from the reaction of  $\text{NH}_2$  groups of some amino acids that form the protein with the  $\text{C}=\text{O}$  group of the aldehyde, resulting in a Schiff base, characterized by the presence of a  $\text{C}=\text{N}$  group where the carbon is bonded to two groups other than hydrogen atoms [45].

Two methods were used to prove the occurrence of crosslinking: color measurement and UV–visible analysis. Color measurement (Fig. 3) shows how samples with the addition of glutaraldehyde alter their color, tending towards yellow. Therefore, higher glutaraldehyde concentrations also result in yellower colors.

Figure 4 presents the UV–Visible spectra of the films produced with the individual natural polymers and the starch–gelatin blend. A peak at approximately 440 nm is observed for samples produced with both gelatins (commercial and CTLW) when cross-linked with glutaraldehyde. This is the wavelength at which the absorption of Schiff bases typically occurs, proving the occurrence of cross-linking and formation of the Schiff base [46]. The same peak is observed for all the films produced with the starch–gelatin blend when

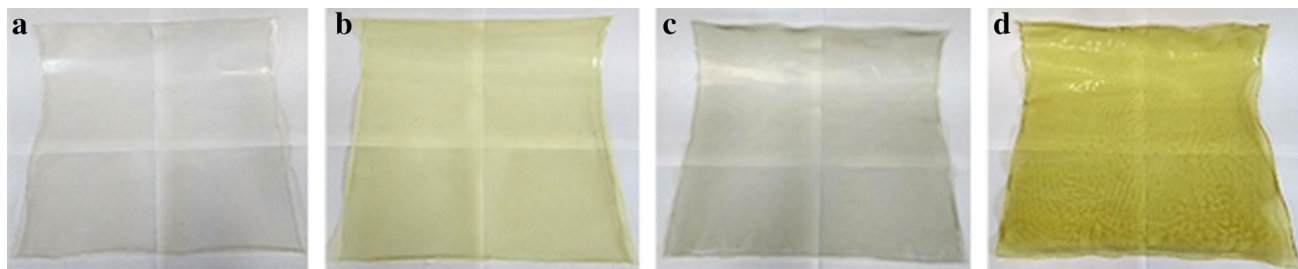
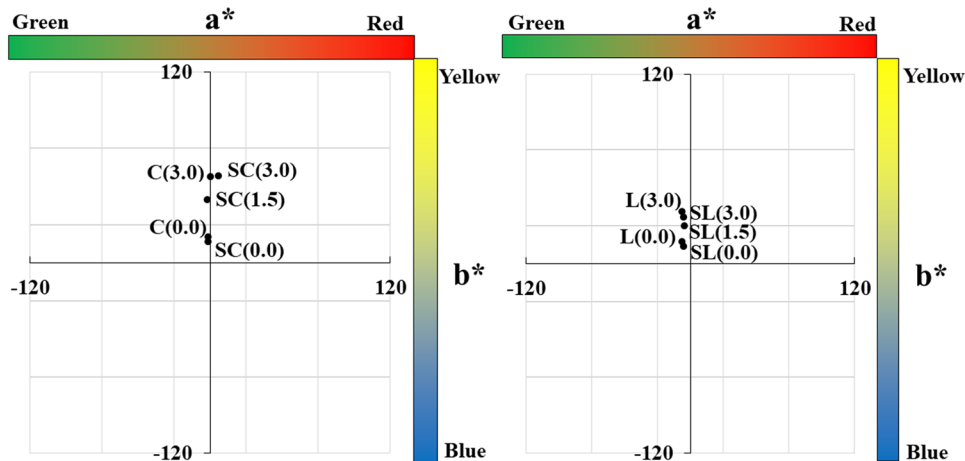
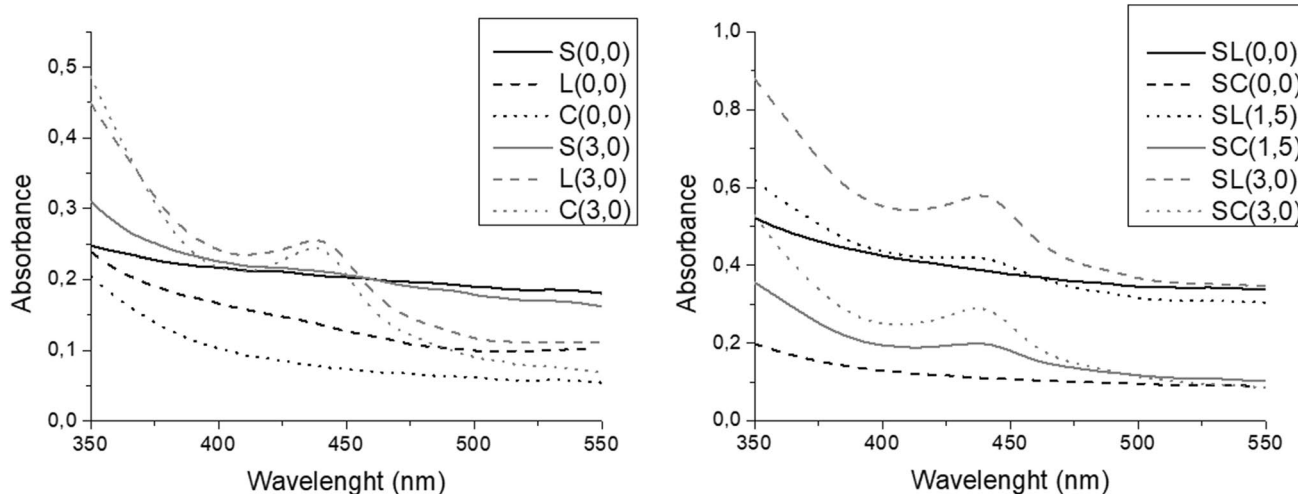


Fig. 2 Starch-commercial gelatin film without a and with b glutaraldehyde; starch-CTLW film without c and with d glutaraldehyde

Fig. 3 Color measurement of films. In the samples codes, S stands for starch, L for gelatin obtained from chromium tanned leather wastes, C for commercial gelatin, SL for starch–gelatin obtained from chromium tanned leather wastes blends, SC for starch–commercial gelatin blend. The number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)





**Fig. 4** Color measurements of films. In the samples codes, S stands for starch, L for gelatin obtained from chromium tanned leather wastes, C for commercial gelatin, SL for starch–gelatin obtained from

chromium tanned leather wastes blends, SC for starch–commercial gelatin blend. The number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)

cross-linked with glutaraldehyde, which indicates that the presence of starch did not impair the cross-linking.

Both analysis, color and UV–visible, indicate the occurrence of cross-linking between the gelatin and the glutaraldehyde, with higher cross-linking degree (yellower films) for the compositions with more glutaraldehyde. The effects of the cross-linking, as well as the effects of the different properties presented by CTLW and commercial gelatin in film composition, will be discussed below.

### X-Ray Diffraction

X-ray diffraction results (Fig. 5) show a peak at approximately  $2\theta = 7.5^\circ$  and a halo at  $2\theta = 20^\circ$ . The first peak corresponds to the gelatin protein chains which were reconstituted as triple helices (structures in which the collagen molecules are found before being partially hydrolyzed and extracted as gelatin). The halo, in turn, is related to the randomly distributed gelatin or starch chains with no spatial organization, which is the amorphous phase [47–49]. The intensity of the peak at  $2\theta = 7.5^\circ$  indicates the amount of chains reconstituted as triple-helix. An intensity reduction in this peak indicates that triple-helix reconstruction was prevented [8].

The addition of glutaraldehyde creates bonds between the gelatin chains, which prevent their conformation into helical structures. This causes a reduction in peak intensity upon addition of the crosslinking agent [50]. It is also noted that the addition of more glutaraldehyde further reduces the peak intensity, indicating that a higher cross-linking degree results in a reduction in the films crystallinity.

The films produced with CTLW gelatin have two additional fine peaks in the XRD spectrum: one at  $2\theta = 14.5^\circ$  and the other at  $2\theta = 29.5^\circ$ . These peaks occur due to the

presence of salts, especially those of calcium and sodium. Calcium comes from leather tanning and also from the gelatin extraction process, which employs calcium oxide as the alkalinizing agent [36]; and the sodium comes from skin preservation before tanning, which is performed using sodium chloride, as well as from the tanning process itself [51].

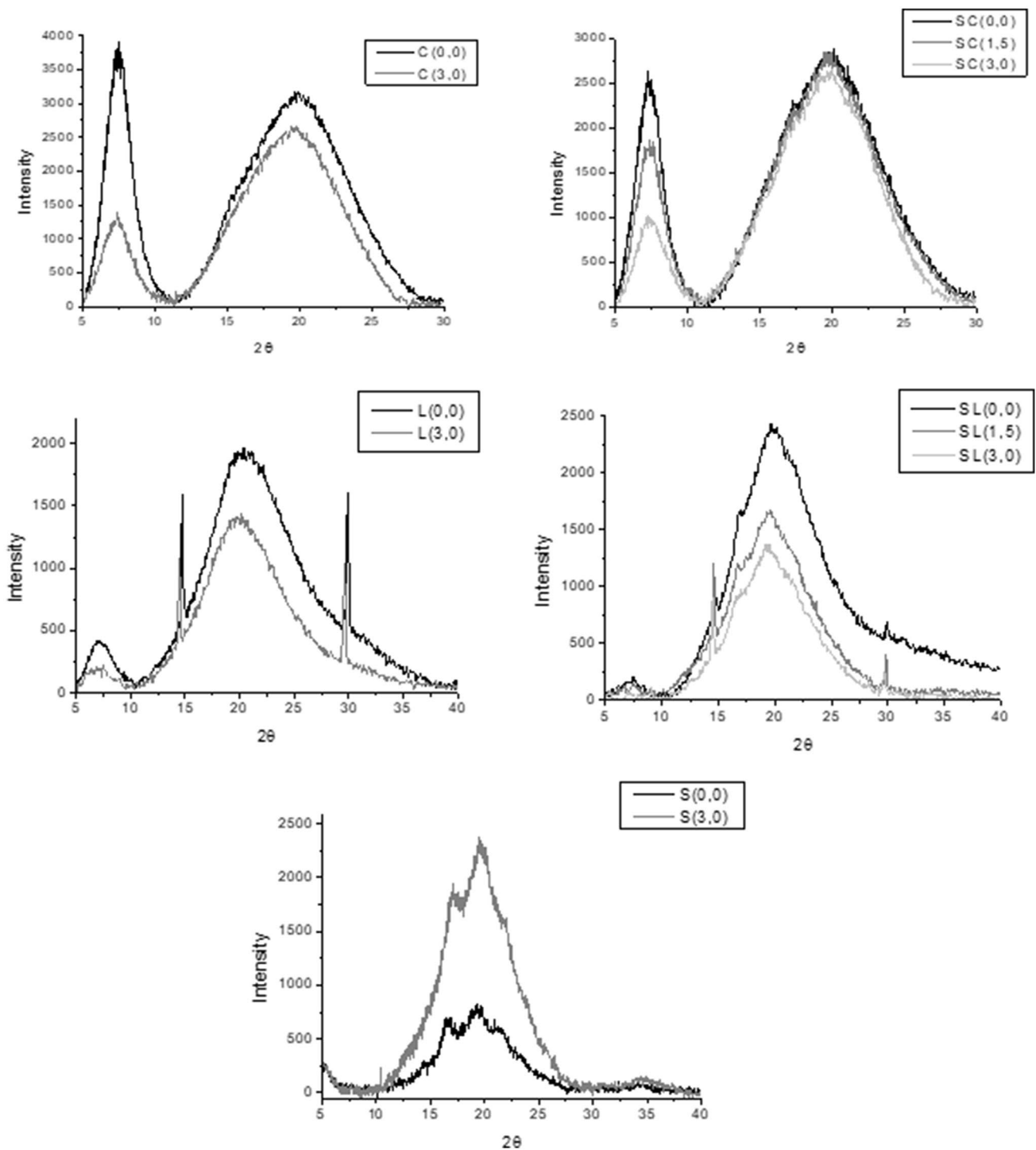
The XRD spectrum of the starch films without gelatin addition showed an amorphous halo and crystalline peaks at  $2\theta = 17^\circ$ ,  $19.5^\circ$  and  $21.5^\circ$ . Both amylose and amylopectin (the polymeric chains that form starch) can form organized, crystalline structures after the films have dried. Amylose rapidly rearranges into a crystalline structure in the presence of high moisture content (first stages of drying), while amylopectin takes days to undergo the retrogradation process [52]. The amylose chains rearrange into a double helix conformation during the drying of the film [53]. According to Souza de Miranda et al. [54], the peak found at  $2\theta = 17^\circ$  represents the crystallization of the amylose chains, whereas the one at  $2\theta = 19.5^\circ$  represents the crystallization of the amylopectin chains.

Crystalline peaks from starch retrogradation are not visible in the XRD spectra for starch–gelatin blends. This indicates that the presence of gelatin (both commercial and the one extracted from CTLW) inhibited the formation of crystalline structures of the starch due to the disruption of the starch–starch interactions.

### Mechanical Properties

Tables 1 and 2 present a summary of the results for the mechanical properties of the films. The same total dry mass was used in the production of all films. The reduction in the





**Fig. 5** XRD spectra of the films. In the samples codes, S stands for starch, L for gelatin obtained from chromium tanned leather wastes, C for commercial gelatin, SL for starch–gelatin obtained from chro-

mium tanned leather wastes blends, SC for starch–commercial gelatin blend. The number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)

thickness of the starch films compared to the gelatin ones is explained by a higher packing degree of the starch chains [55]. Overall, the addition of glutaraldehyde did not affect the thickness of the material.

The mechanical properties' results indicate that all films presented a hard and tough plastic behavior. Except for the films produced only with starch, the samples tended to strain harden (due to the orientation of the crystalline regions of

**Table 1** Thickness, yield strength, elongation at yield, tensile strength at break and elongation at break of the natural polymer films

Sample <sup>1</sup>	Thickness (μm)	Yield strength (MPa)	Elongation at yield (%)	Tensile strength at break (MPa)	Elongation at break (%)
S(0.0)	93 ± 1 <sup>b</sup>	8.3 ± 1.7 <sup>b</sup>	14 ± 1 <sup>abc</sup>	7.5 ± 0.8 <sup>ab</sup>	74 ± 18 <sup>b</sup>
S(3.0)	92 ± 1 <sup>b</sup>	5.1 ± 0.6 <sup>a</sup>	19 ± 2 <sup>ac</sup>	5.4 ± 0.4 <sup>b</sup>	94 ± 6 <sup>d</sup>
L(0.0)	111 ± 4 <sup>c</sup>	3.7 ± 0.1 <sup>a</sup>	18 ± 2 <sup>a</sup>	8.5 ± 0.5 <sup>a</sup>	144 ± 13 <sup>c</sup>
L(3.0)	99 ± 2 <sup>a</sup>	3.9 ± 0.7 <sup>a</sup>	19 ± 2 <sup>a</sup>	9.9 ± 0.8 <sup>a</sup>	157 ± 12 <sup>c</sup>
C(0.0)	107 ± 4 <sup>a</sup>	25.6 ± 1.7 <sup>d</sup>	13 ± 4 <sup>bc</sup>	27.7 ± 1.5 <sup>c</sup>	36 ± 4 <sup>a</sup>
C(3.0)	110 ± 3 <sup>a</sup>	20.5 ± 1.2 <sup>c</sup>	11 ± 3 <sup>b</sup>	27.8 ± 2.8 <sup>c</sup>	55 ± 10 <sup>ab</sup>

\*When evaluating the results of the same column, different letters indicate statistically different means (p value < 0.05)

<sup>1</sup>In the samples codes, S stands for starch in the composition, L for gelatin obtained from chromium tanned leather wastes, C for commercial gelatin and the number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)

**Table 2** Thickness, yield strength, elongation at yield, tensile strength at break and elongation at break of the natural polymer blend films

Sample <sup>1</sup>	Thickness (μm)	Yield strength (MPa)	Elongation at yield (%)	Tensile strength at break (MPa)	Elongation at break (%)
SL(0.0)	99 ± 2 <sup>b</sup>	5.0 ± 0.1 <sup>a</sup>	15 ± 2 <sup>ab</sup>	6.4 ± 0.6 <sup>a</sup>	85 ± 10 <sup>ac</sup>
SL(1.5)	102 ± 3 <sup>b</sup>	5.4 ± 0.3 <sup>a</sup>	17 ± 2 <sup>a</sup>	7.4 ± 0.6 <sup>a</sup>	108 ± 7 <sup>ad</sup>
SL(3.0)	99 ± 2 <sup>b</sup>	4.6 ± 0.5 <sup>a</sup>	16 ± 5 <sup>a</sup>	7.2 ± 0.8 <sup>a</sup>	132 ± 45 <sup>d</sup>
SC(0.0)	107 ± 1 <sup>a</sup>	19.2 ± 1.5 <sup>c</sup>	12 ± 1 <sup>bc</sup>	20.4 ± 1.2 <sup>bc</sup>	33 ± 3 <sup>b</sup>
SC(1.5)	105 ± 1 <sup>a</sup>	15.2 ± 0.9 <sup>b</sup>	10 ± 2 <sup>c</sup>	21.4 ± 1.3 <sup>c</sup>	54 ± 10 <sup>bc</sup>
SC(3.0)	107 ± 2 <sup>a</sup>	15.3 ± 1.4 <sup>b</sup>	16 ± 1 <sup>ab</sup>	19.2 ± 1.6 <sup>b</sup>	95 ± 14 <sup>a</sup>

\* When evaluating the results of the same column, different letters indicate statistically different means (p value < 0.05)

<sup>1</sup>In the samples codes, SL stands for the blend of starch–gelatin obtained from chromium tanned leather wastes, SC for starch–commercial gelatin blend and the number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)

the polymer) after the yield point. Therefore, for most samples, tensile strength at break was higher than yield strength [56].

Due to its lower molecular mass, to the presence of higher amounts of salts, and to the presence of low molar mass molecules (gelatin that is hydrolyzed to collagen hydrolysate), the use of CTLW gelatin, when compared to commercial gelatin, resulted in the production of films with lower strength values and higher elongation values, specially elongation at break. The same behavior was observed for both the pure natural polymer films and the blends. The  $\bar{M}_n$  values are associated with variations in the mechanical properties since they indicate different numbers of loose ends in the polymer chains. Minor chains interrupt molecular interaction, and reduce cohesion and mechanical resistance. They act as plasticizers in the system [43]. Hygroscopic salts, as the calcium and sodium ones found in CTLW gelatin, also act as plasticizers in starch–gelatin films due to their ability to absorb water, a natural plasticizer of this material [57].

Elongation at break increased with glutaraldehyde addition in films produced with the blend of starch and CTLW

or commercial gelatin. An increase is also observed in the films produced with the natural polymers alone when cross-linked with glutaraldehyde, which is not, however, statistically significant due to the high standard deviation found for the results. An increase in tensile strength caused by cross-linking with glutaraldehyde was also expected, however, this did not occur. This is justified by the reduction in the crystallinity of the material. While the crosslinking made the material more resistant, the loss in crystallinity made the material less resistant. The balance of the two effects resulted in the maintenance of the tensile strength of the material practically being unchanged by the addition of glutaraldehyde.

As for the reduction in tensile strength when comparing the values found for the single-polymer films and for the starch–gelatin blend films, Garcia et al. [10] indicated that an increase in the tensile strength of gelatin films due to the addition of starch happened because of the formation of a more cohesive internal structure of the material. Analogously, lower values of strength obtained for films produced by the starch and gelatin blend may result from the formation of a less cohesive internal film structure.

**Table 3** Solubility, water vapor permeability and swelling degree of the natural polymer films

Sample <sup>1</sup>	Solubility (%)	Water vapor permeability (g mm day <sup>-1</sup> m <sup>-2</sup> kPa <sup>-2</sup> )	Swelling (mass %)
S(0.0)	20.1 ± 1.5 <sup>a</sup>	13.2 ± 1.4 <sup>b</sup>	238 ± 19 <sup>a</sup>
S(3.0)	21.3 ± 0.3 <sup>a</sup>	13.3 ± 0.9 <sup>b</sup>	253 ± 8 <sup>a</sup>
L(0.0)	65.9 ± 3.9 <sup>e</sup>	10.2 ± 0.6 <sup>a</sup>	- <sup>2</sup>
L(3.0)	56.3 ± 0.9 <sup>d</sup>	10.8 ± 1.0 <sup>a</sup>	685 ± 62 <sup>c</sup>
C(0.0)	49.0 ± 1.5 <sup>c</sup>	16.5 ± 0.7 <sup>c</sup>	482 ± 36 <sup>b</sup>
C(3.0)	25.8 ± 0.6 <sup>b</sup>	17.1 ± 0.5 <sup>c</sup>	163 ± 26 <sup>a</sup>

\*When evaluating the results of the same column, different letters indicate statistically different means (p value < 0.05)

<sup>1</sup>In the samples codes, S stands for starch in the composition, L for gelatin obtained from chromium tanned leather wastes, C for commercial gelatin and the number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)

<sup>2</sup>No swelling results are shown for this sample because it ended up disintegrating during the test

**Table 4** Solubility, water vapor permeability and swelling degree of the natural polymer blend films

Sample <sup>1</sup>	Solubility (%)	Water vapor permeability (g mm day <sup>-1</sup> m <sup>-2</sup> kPa <sup>-2</sup> )	Swelling (mass %)
SL (0.0)	52.9 ± 1.8 <sup>e</sup>	11.0 ± 1.2 <sup>a</sup>	- <sup>2</sup>
SL (1.5)	44.7 ± 0.4 <sup>a</sup>	10.4 ± 0.5 <sup>a</sup>	503 ± 14 <sup>e</sup>
SL (3.0)	30.6 ± 0.7 <sup>d</sup>	11.0 ± 0.7 <sup>a</sup>	350 ± 24 <sup>c</sup>
SC (0.0)	44.1 ± 1.1 <sup>a</sup>	16.2 ± 1.7 <sup>b</sup>	411 ± 30 <sup>d</sup>
SC (1.5)	25.4 ± 0.8 <sup>c</sup>	15.8 ± 2.3 <sup>b</sup>	187 ± 11 <sup>b</sup>
SC (3.0)	20.5 ± 1.3 <sup>b</sup>	16.0 ± 0.5 <sup>b</sup>	126 ± 19 <sup>a</sup>

\* When evaluating the results of the same column, different letters indicate statistically different means (p value < 0,05)

<sup>1</sup>In the samples codes, S stands for starch in the composition, L for gelatin obtained from chromium tanned leather wastes, C for commercial gelatin and the number in parenthesis is the percentage of glutaraldehyde used in the film composition (0.0, 1.5, or 3.0%)

<sup>2</sup>No swelling results are shown for this sample because it ended up disintegrating during the test

## Water Interaction Properties

Tables 3 and 4 present solubility, water vapor permeability, and swelling degree results for the starch–gelatin films.

Reduction in solubility is one of the main changes in properties caused by cross-linking [33, 34, 58]. While starch–gelatin films had their solubility reduced by the addition of glutaraldehyde, the same was not observed for pure starch films. Also, the films produced with pure corn starch presented a strong smell of glutaraldehyde right after drying, indicating that the cross linking agent did not react and was still volatilizing. Films produced with gelatin, on the other hand, had their solubility reduced and did not present the

same characteristic of glutaraldehyde still volatilizing after drying, which indicates that the cross linking agent in fact reacted with the gelatin chains.

The films with gelatin in their composition had their solubility and swelling degree reduced both by the addition of 1.5% and 3.0% glutaraldehyde, in the presence or absence of starch, for both CTLW and commercial gelatin. The use of commercial gelatin resulted in the production of less hygroscopic films (lower solubility and swelling degree). According to data from previous works, the presence of hydrolyzed collagen (gelatin molecules that were hydrolyzed and had their molecular mass further reduced in the extraction process) makes CTLW gelatin films more hydrophilic than films produced with commercial gelatin [9, 59]. The presence of hygroscopic salts, which have high affinity with water molecules, also increases the water interaction properties of films produced with CTLW gelatin [9, 57]. The higher molecular mass of the commercial gelatin reduces the solubility of the films produced with it [60]. Finally, the amount of glutaraldehyde in the films' composition directly affected the solubility and swelling degree, both showing greater reductions with the highest amount of glutaraldehyde added. This indicates that a higher cross-linking degree was achieved with the addition of more glutaraldehyde.

The addition of 3% glutaraldehyde reduced the film solubility by 14.5% and 42.0% for the films produced with the CTLW gelatin and with the CTLW-gelatin-starch blend respectively. This reduction was equal to 47% and 53% for the samples produced with the commercial gelatin and with the commercial gelatin-starch blend respectively. The crosslinking, however, was more effective in reducing the swelling degree of the films, which was reduced by 66% and 69% for samples produced with commercial gelatin and with the commercial gelatin-starch blend, respectively, with the addition of 3% glutaraldehyde. The swelling degree reduction data for films produced with CTLW gelatin were not calculated due to the impossibility of determining the degree of swelling for uncrosslinked samples, which solubilized during the test.

The solubilization of a polymer comprises two stages: formation of a swollen gel (by the diffusion of molecules in the polymeric structure) and formation of the actual solution. The swelling process does not occur, or is impaired, in the presence of crosslinks. As for the second stage of solubilization, it is impaired by crystallinity, hydrogen bonds and crosslinks [61].

Thus, in the swelling stage, crosslinking has a direct effect on reducing the diffusion of molecules in the polymer structure. However, for the solubilization of the material, one must also take into account its crystallinity. As verified by XRD, while the addition of glutaraldehyde promoted the crosslinking of the gelatin chains, it reduced their ability to rearrange into a helical structure, reducing



their crystallinity. That is why the reduction in the degree of swelling was greater than the reduction in the solubility for the crosslinked films.

The reduction in the films degree of swelling with the addition of glutaraldehyde (and with increasing concentration of the cross linking agent) provides an indication of the increase in the number of bonds formed by the glutaraldehyde-promoted cross-linking. A higher density of crosslinks results in a smaller space for penetration of a solvent and a consequently a lower degree of swelling [43].

Water vapor permeability was not altered by the addition of glutaraldehyde. Although more soluble and hygroscopic, samples produced with CTLW gelatin have lower permeability than films produced with commercial gelatin. This may be explained by the effect of fillers on the gas permeability of polymer composites. The gas permeability coefficient decreases considerably when powder fillers are introduced into the polymers in amounts of up to 5–10%. It continues to decrease, but less rapidly, when the load content is further increased to 20–30% by volume. The permeability increases markedly when the charge content is high (40–50%). This permeability characteristic is related to the complexity of the gas transfer mechanism in a heterogeneous system such as the polymer-charge system. In heterogeneous systems, the phase that determines the transfer of gas through a material is the continuous phase, in this case the polymer phase. Usually, the polymer molecules are adsorbed on the surface of the filler, and therefore more densely packed structures are formed, with a lower gas permeability. When the filler content in the polymer phase is high, ruptures appear, that is, the phase continuity is interrupted. In a polymer with high amounts of filler, open capillaries are formed, which guarantee phase gas transfer: the permeability through diffusion is replaced by the flow of molecular or viscous gas [62]. The salts remaining in the CTLW gelatin act as fillers when it comes to water vapor permeability. When in high amounts, as verified in previous works of this research group [9], the salts would increase the films water vapor permeability. However, when in low amounts, as in the films discussed in this paper, the salts act as a barrier to water vapor permeability, reducing it.

## Conclusion

Glutaraldehyde was not effective as a cross-linking agent for starch molecules. However, it highly improved the water interaction properties of films produced with gelatin (with or without starch addition), proving its effectiveness in cross-linking this protein. The color of all films produced with gelatin and glutaraldehyde changed to yellow due to the cross-linking reaction, producing a Schiff-base. XRD results indicated that cross-linking impaired

the reconstitution of gelatin chains into a triple helix, reducing the crystallinity degree of samples produced with glutaraldehyde. The lower molar mass and the presence of hydrolyzed collagen and hydrophilic salts in the composition of CTLW gelatin made the films produced with it less strong, with higher elongation results and more soluble than the ones produced with commercial gelatin. The effect of the cross-linking in the molecular structure of the films was compensated by the reduction in crystallinity with regard to mechanical properties, which were not highly affected by cross-linking. The water interaction properties of solubility and swelling degree, on the other hand, were reduced by cross-linking, which was the main objective of this work. Therefore, the cross-linked films presented better water-interaction properties than the non-crosslinked films, which will allow them, especially the ones produced with CTLW gelatin, to better withstand the application conditions when used in high-humidity situations, such as agricultural environments.

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## References

1. Arvanitoyannis I et al (1997) Edible films made from gelatin, soluble starch and polyols, Part 3. *Food Chem* 60:593–604
2. Arvanitoyannis I, Nakayama A, Aiba S (1998) Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydr Polym* 36:105–119
3. Garcia VAS et al (2017) Gelatin and pregelatinized starch orally disintegrating films: properties and stability of vitamin C. *J Appl Polym Sci* 134:1–19
4. Al-Hassan AA, Norziah MH (2017) Effect of transglutaminase induced crosslinking on the properties of starch/gelatin films. *Food Packag Shelf Life* 13:15–19
5. Moreno O et al (2017) Active starch-gelatin films for shelf-life extension of marinated salmon. *LWT—Food Sci Technol* 84:189–195
6. Podshivalov A et al (2017) Gelatin/potato starch edible biocomposite films: correlation between morphology and physical properties. *Carbohydr Polym* 157:1162–1172
7. Wang W et al (2017) Performance of high amylose starch-composited gelatin films influenced by gelatinization and concentration. *Int J Biol Macromol* 94:258–265
8. Wang K et al (2017) Mechanical and barrier properties of maize starch-gelatin composite films: effects of amylose content. *J Sci Food Agric* 97:3613–3622
9. Scopel BS et al (2017) Cornstarch-gelatin films: commercial gelatin versus chromed leather waste gelatin and evaluation of drying conditions. *J Polym Environ* 26:1998–2006
10. Garcia VAS et al (2018) Gelatin/starch orally disintegrating films as a promising system for vitamin C delivery. *Food Hydrocoll* 79:127–135

11. Moreno O et al (2018) Starch-gelatin antimicrobial packaging materials to extend the shelf life of chicken breast fillets. *LWT* 97:483–490
12. Scopel BS et al (2019) Mechanical and water interaction properties of polymeric films produced with starch and gelatin extracted from leather waste as affected by different composition. *JALCA* 114:1–9
13. Malherbi NM et al (2019) Corn starch and gelatin-based films added with guabiroba pulp for application in food packaging. *Food Packag Shelf Life* 19:140–146
14. Shi C, Tao F, Cui Y (2018) New starch ester/gelatin based films: developed and physicochemical characterization. *Int J Biol Macromol* 109:863–871
15. Marvzadeh MM et al (2017) Preparation and characterization of bionanocomposite film based on tapioca starch/bovine gelatin/nanorod zinc oxide. *Int J Biol Macromol* 99:1–7
16. Afshar S, Baniyasi H (2018) Investigation the effect of graphene oxide and gelatin/starch weight ratio on the properties of starch/gelatin/GO nanocomposite films: the RSM study. *Int J Biol Macromol* 109:1019–1028
17. Catalina M et al (2011) Influence of crosslinkers and crosslinking method on the properties of gelatin films extracted from leather solid waste. *J Appl Polym Sci* 119:2105–2111
18. Ocak B (2018) Film-forming ability of collagen hydrolysate extracted from leather solid wastes with chitosan. *Environ Sci Pollut Res Int* 25:4643–4655
19. Dang X, Shan Z, Chen H (2018) Biodegradable films based on gelatin extracted from chrome leather scrap. *Int J Biol Macromol* 107:1023–1029
20. Langmaier F et al (2008) Biodegradable packing materials from hydrolysates of collagen waste proteins. *Waste Manag* 28:549–556
21. Aung SPS et al (2018) Environment-friendly biopolymers for food packaging: starch, protein, and poly-lactic acid (PLA). In: *Bio-based materials for food packaging: green and sustainable advanced packaging materials*. Springer, Singapore
22. Zhong QP, Xia WS (2008) Physicochemical properties of edible and preservative films from chitosan/cassava starch/gelatin blend plasticized with glycerol. *Food Technol Biotechnol* 46:262–269
23. Fakhouri FM et al (2009) Effect of fatty acid addition on the properties of biopolymer films based on lipophilic maize starch and gelatin. *Starch - Stärke* 61:528–536
24. Acosta S et al (2015) Physical properties and stability of starch-gelatin based films as affected by the addition of esters of fatty acids. *Food Hydrocolloid* 49:135–143
25. Acosta S et al (2016) Antifungal films based on starch-gelatin blend, containing essential oils. *Food Hydrocolloid* 61:233–240
26. Wang Y et al (2013) Effect of the carbonyl content on the properties of composite films based on oxidized starch and gelatin. *J Appl Polym Sci* 130:3809–3815
27. Kolomazník K et al (1999) Experience in industrial practice of enzymatic dechromation of chrome shavings. *JALCA* 94:55–63
28. Gaidau C et al (2009) Additives and advanced biomaterials obtained from leather industry by-products. *Rev Chim* 60:501–507
29. Mokrejs P et al (2007) Properties of collagen hydrolyzates obtained from leather shavings. *Asian J Chem* 19:1207–1216
30. Dang X et al (2019) Recovery and utilization of collagen protein powder extracted from chromium leather scrap waste. *Environ Sci Pollut Res Int* 26:7277–7283
31. Martucci JF, Ruseckaite RA (2009) Biodegradation of three-layer laminate films based on gelatin under indoor soil conditions. *Polym Degrad Stab* 94:1307–1313
32. Martucci JF, Ruseckaite RA (2009) Tensile properties, barrier properties, and biodegradation in soil of compression-molded gelatin-dialdehyde starch films. *J Appl Polym Sci* 112:2166–2178
33. Biscarat J et al (2015) Effect of chemical cross-linking on gelatin membrane solubility with a non-toxic and non-volatile agent: terephthalaldehyde. *Int J Biol Macromol* 74:5–11
34. Ramshaw JA (2016) Biomedical applications of collagens. *J Biomed Mater Res B* 104:665–675
35. Silvipriya KS et al (2015) Collagen: animal sources and biomedical application. *J Appl Pharm Sci* 5:123–127
36. Scopel BS et al (2018) Steam explosion in alkaline medium for gelatine extraction from chromium-tanned leather wastes: time reduction and process optimization. *Environ Technol* 38:367–373
37. Farris S et al (2010) Alternative reaction mechanism for the cross-linking of gelatin with glutaraldehyde. *J Agric Food Chem* 58:998–1003
38. Naguleswaran S et al (2013) The susceptibility of large and small granules of waxy, normal and high-amylose genotypes of barley and corn starches toward amylolysis at sub-gelatinization temperatures. *Food Res Int* 51:771–782
39. Weber FH, Collares-Queiroz FP, Chang YK (2009) Caracterização físico-química, reológica, morfológica e térmica dos amidos de milho normal, ceroso e com alto teor de amilose. *Ciênc Tecnol Aliment* 29:748–753
40. Fakhouri FM et al (2015) Edible films and coatings based on starch/gelatin: film properties and effect of coatings on quality of refrigerated Red Crimson grapes. *Postharvest Biol Technol* 109:57–64
41. Gómez-Estaca J et al (2009) Physico-chemical and film-forming properties of bovine-hide and tuna-skin gelatin: a comparative study. *J Food Eng* 90:480–486
42. Muyonga JH, Cole CGB, Duodu KG (2004) Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. *Food Hydrocoll* 18:581–592
43. Akcelrud L (2007) *Fundamentos da Ciência dos Polímeros*. Manole, Barueri
44. Gennadios A (2002) *Protein-based films and coatings*. CRC Press, Boca Raton
45. Englert C et al (2007) Bonding of articular cartilage using a combination of biochemical degradation and surface cross-linking. *Arthritis Res Ther* 9:R47
46. Kuk M, Bland J (2002) Effect of pH on a gossypol complex with phospholipids: Gossypol-PE. *JAACS* 79:1045–1048
47. Qiao C et al (2017) Molecular interactions in gelatin/chitosan composite films. *Food Chem* 235:45–50
48. Dammak I, Lourenço RV, Sobral PJA (2019) Active gelatin films incorporated with Pickering emulsions encapsulating hesperidin: Preparation and physicochemical characterization. *J Food Eng* 240:9–20
49. Soliman EA, Furuta M (2014) Influence of phase behavior and miscibility on mechanical, thermal and micro-structure of soluble starch-gelatin thermoplastic biodegradable blend films. *Food Nutr Sci* 05:1040–1055
50. Chaibi S et al (2015) The role of crosslinking on the physical properties of gelatin based films. *Colloid Polym Sci* 293:2741–2752
51. Covington AD (2015) *Tanning chemistry: the science of leather*. Royal Society of Chemistry, Cambridge
52. Rindlav-Westling A et al (1998) Structure, mechanical and barrier properties of amylose and amylopectin films. *Carbohydr Polym* 36:217–224
53. Romero-Bastida CA et al (2015) Effect of the addition order and amylose content on mechanical, barrier and structural properties of films made with starch and montmorillonite. *Carbohydr Polym* 127:195–201
54. Souza de Miranda C et al (2015) Effect of the glycerol and lignin extracted from Piassava fiber in cassava and corn starch films. *Mater Res* 18:260–264

55. Al-Hassan AA, Norziah MH (2012) Starch–gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocoll* 26:108–117
56. CROW (2015) Polymer properties database. In Polymer database. <https://polymerdatabase.com/polymer%20physics/Stress-Strain%20Behavior.html> Accessed 24 Feb
57. Jiang X et al (2016) Studies of the plasticizing effect of different hydrophilic inorganic salts on starch/poly (vinyl alcohol) films. *Int J Biol Macromol* 82:223–230
58. Etxabide A et al (2017) Effect of pH and lactose on cross-linking extension and structure of fish gelatin film. *React Funct Polym* 117:140–146
59. Selmin F et al (2015) Aminoacids as non-traditional plasticizers of maltodextrins fast-dissolving film. *Carbohydr Polym* 115:613–616
60. Wolf BA (1985) Solubility of polymers. *Pure Appl Chem* 57:323–336
61. Canevarolo SV Jr (2006) *Ciência dos polímeros: um texto básico para tecnólogos e engenheiros*. Artliber, São Paulo
62. Tager A (1978) *Physical chemistry of polymers*. Mir, Moscow

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