Formulation and Characterization of Film Based on Gum Exudates from Brea Tree (*Cercidium praecox*)

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Abstract: In the present study, films and coatings for food applications based on a gum exudate from Brea tree (*Cercidium praecox*) were formulated and the influence of plasticizer content on their functional properties was evaluated. Brea gum is a renewable resource available in semi-desert areas, extracted by native people. Films were produced by casting method and characterized through water vapour permeability, mechanical (tensile strength and elongation at break), morphological and optical properties. Glycerol was used as plasticizer. Brea gum films presented good visual aspect, transparency and amber colour. Microscopic observation showed a dense and homogeneous structure. Film wettability increased with glycerol content due to hydrophilic nature of the plasticizer. Solubility of films in water increased with temperature. Water sorption isotherms of films at 25 °C showed that at high a_w (above 0.50), the film matrix swells altering its structure and properties. Water vapour permeability remained practically constant up to 20% of glycerol content and then increases linearly with the plasticizer. The addition of glycerol decreased the opacity and tensile strength. The best film properties were obtained at 20% of glycerol concentration.

Key words: Films, brea gum, mechanical properties, barrier properties, plasticizer effects.

1. Introduction

For centuries, man has searched effective substitutes for natural coating of food to keep them fresh isolated from physical, chemical and/or microbiological contaminants. For many years, the industry of packaging has used materials produced from various petroleum-derived monomers to elaborate varieties of plastics, which have very good functional properties but also produce serious pollution problems. Thanks to advances in science and the growing interest in the environmental impact of discarded plastics, it was developed a new series of materials of biological origin (plant, animal and microbial), whose main advantage is that they are renewable, fully degradable and even edible in some cases.

Edible films and coatings generate a modified atmosphere by creating a semi-permeable barrier against O_2, CO_2, moisture and solute movement, thus reducing respiration, water loss and oxidation reaction rates [1]. Various compounds have been used as edible film and coating, like lipid, proteins and carbohydrates. The lipids have good water barrier properties but waxy taste and bad mechanical properties. Protein and carbohydrates have good mechanicals properties and barrier properties against O_2 and CO_2. However, the main functional properties (mechanical and barrier properties) of these hydrophilic materials depend on their water content. This is due to water vapor strongly interacts with polymer matrix affecting the structure [2-4].

The brea gum (BG) is a hydrocolloid obtained as phloematic exudate of *Cercidium praecox* (Ruiz &

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Pavon) Harms [*Parkinsonia preaecox* (Ruiz & Pavon) Hawkins]. The two most common species in Argentine are *Cercidium praecox* (Brea tree) and *Cercidium australe* (Brea arbustus). The genus *Cercidium* belongs to *Leguminosae* family. Thank to their extensive root system, Brea trees can be found in arid and semi-arid regions of the America (South, Central and North). Brea trees grow scattered in the wild and gum from these untended trees is collected by native people manually. The exudates gum is obtained from superficial incisions made in the branches and tree trunk. After some weeks, gum is manually collected as partially dried tears. The exudate is purified by a simple process of solution and subsequent drying and further grinding to fine powder.

The production and composition of the BG is complex and varies to some extent depending on the geographical origin, climatic conditions and age of the trees. The BG is amber colour; it has semi-liquid consistency and faintly sweet flavour. It is highly soluble in water. Solutions are homogeneous and present acid character (pH = 4). This hydrocolloid contains residues of L-arabinose, D-xylose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid, in the approximate molar ratios of 1.7:6, 3:1, 9:0.9, associated with small amounts of protein which contributes to their emulsifying properties [5, 6]. The major structural features of BG appear to be a β-(1→4)-linked D-xylan backbone [possibly containing some (1→2)-linkages] that is heavily 2-substituted by short branch-chains containing residues of D-xylose (and L-arabinose) and D-glucuronic acid, in which both types of residue may be terminal [5].

The BG has structural and chemical similarities with other plant gums widely used in the food industry as Arabic gum [6]. Hence, BG could be a suitable candidate for incorporation as stabilizing, emulsifying and thickening additive in food formulation [7].

BG has been traditionally used as a "woodland candy" by countryside people since pre-Colombian times without producing harmful consequences. Müller et al. [8] reported a toxicological evaluation of BG in mice. Their results suggest that feeding BG at levels up to 5% to mice do not exert any toxicological effects, supporting its potential use as a food additive for human consumption.

The easy access to this inexpensive, non-toxic, hydrophilic and biodegradable polysaccharide makes viable its potential use as film matrix for different applications. Many authors formulated edible film and coating using different gum with or without addition of substances like lipids, waxes or polysaccharides to improve their water barrier properties [9-14].

The purpose of the present contribution was the formulation and characterization of films based on BG, and the determination of the effect of plasticizer content on their physicochemical and functional properties.

2. Materials and Methods

2.1 Materials

BG was donated by native community group from Tartagal, Salta, Argentina. The BG exudate from the plant in the form of small drops or tears was collected. The exudate was purified by a simple process of solution; filtration and drying in ovens at temperatures below 50 °C, then grinding to fine powder (mesh 80-ASTM). In the film elaboration, Glycerol (Mallinckrodt, USA) was added as plasticizer. P_2O_5 (Mallinckrodt, USA) was used as a desiccant. All salts used to obtain different relative humidity ambient (%RH) were provided by Aldrich (USA).

2.2 Film Preparation

Film-forming solution was prepared by mixing BG and water in a concentration of 10% w/w. Glycerol was added as plasticizer in concentrations ranged between 0 and 50% w/w. The resulting dispersion was poured on polystyrene plates. Then, they were placed in an air-circulating oven at 35 °C and 53% RH for 15 h. After that, plates were removed from the oven and

films peeled off.

2.3 Film Opacity and Colour

Opacity of films was determined according Gontard et al. [15] procedure and ASTM methods D1003-00 [16] recommendations. Film samples were cut into a rectangle and placed on the internal side of the spectrophotometer cell. The absorbance spectrum (400-800 nm) was recorded for each sample using a Spectronic Unicam Genesys 10 UV (USA) spectrophotometer. Film opacity was defined as the area under the recorded curve determined by an integration procedure. The opacity was expressed as absorbance units × nanometers (AU nm). Samples were previously conditioned at 53% RH and 25 °C.

The vision sensations that are sent to the brain create the three dimensions of colour judgment response that is often referred to as three-dimensional colour space. In the CIE Lab system, these dimensions are expressed as: *L** related to lightness varied from black (zero) to white (100), and other two related to chromaticity, a* from green (-*a**) to red (+*a**) and b* from blue (-*b**) to yellow (+*b**). The Commission Internationale de l'Eclairage (CIE) *L**, *a**, *b** colour characteristics were determined using a ColorTec, PCM colorimeter (Accuracy Microsensor Inc., Pittsford, USA), equipped with light source D65 and observation angle 10 °. Measurements were taken at 25 °C. *L**, *a** and *b** values were averaged from five readings at each film.

2.4 Contact Angle Measurement

Surface hydrophobicity was evaluated in static contact angle experiments in a Rame-Hart Model 250, contact angle goniometer. A small water droplet was released on the film surface, digital pictures were gathered and image pro software was used to calculate contact angle. The average contact angle was determined from five experiments each for the evaporative and the lower surface of BG films.

Measurements of contact angles (θ) were performed by the sessile drop method at room temperature, using a goniometer (Standard Goniometer with DROPimage standard, model 200-00, Ramé-Hart Instrument Co., Succasunna, USA). A drop of deionized water (10 μL) was placed onto the film surface by a micro syringe. The drop image was recorded by a video camera, and digitalized. The drop shape was solved numerically and fitted using a mathematical soft provided by the manufacturer. Five readings were taken on different parts of each film; the average of contact angle values and the standard deviation were calculated.

The surface free energy (γ_S) was obtained from the surface equation of state:

$$\cos\theta + 1 = 2 \sqrt{\left(\frac{\gamma_S}{\gamma_L}\right) e^{-\beta(\gamma_L - \gamma_S)^2}} \qquad (1)$$

where, θ is the contact angle, γ_L the surface tension of the liquid, $\beta = 0.0001247$ m^2 mJ^{-2}, is a given parameter experimentally determined, proposed by Kwok and Neumann [17]. The program uses an iterative process to solve this equation for γ_S. The relation needed for γ_S (mJ m^{-2}) calculation is given by Young's Eq.:

$$\gamma_S = \gamma_{SL} + \gamma_L \cos\theta \qquad (2)$$

where γ_{SL} (mJ m^{-2}) is the polymer-liquid interfacial energy, γ_L (mJ m^{-2}) is the surface tension of the liquid used. The contact angle is usually measured by the tangent at the three-phase interface (solid-liquid-vapour).

2.5 Film Solubility in Water

Film solubility in water was measured as percentage of dry matter of the film solubilized in water during a period of 1 h. The initial dry matter of each film was obtained after drying film specimens in desiccators containing P_2O_5 during a week. Samples of approximately 200 mg were weighed and immersed in 20 mL distilled water, sealed and agitated in a thermostatized bath. Films not solubilized in water were separated by centrifugation (Sigma 4K10, Germany) at 2,500 g and dried at 40 °C to determine remaining dry matter. Tests were performed by triplicate and solubility was calculated as follows:

$$\text{Solubility (\%)} = \left(\frac{\text{Initial dry weight - Final dry weight}}{\text{Initial day weight}}\right) \times 100 \quad (3)$$

2.6 Moisture Sorption Isotherms

Constant relative humidity environments were established inside sorbostats, glass jars, using salt solutions. The salts used (LiCl, CH$_3$COOK, MgCl$_2$, K$_2$CO$_3$, Mg(NO$_3$)$_2$, NaBr, NaCl, KCl) were the different salts recommended by COST-90 project, to cover a water activity (a$_w$) range from 0.10 to 0.90 [18]. All salts used were analytical grade. Film samples (rectangular strips approximately 2 cm^2 area) were first freeze-dried (Thermovac Industries Corp, USA) and storage in a desiccator with P$_2$O$_5$ during 48 h. Samples were weighed and placed on a plastic lattice by holding it on a tripod inside the sorbostats that contain the saturated salt solutions and then the sorbostats were sealed. The sorbostats were kept inside an environmental chamber maintained at constant temperature. Film samples were equilibrated in the sorbostats for 24 h before their weights were recorded. The weights of the samples were checked for 3 days more. Equilibrium was judged to have been attained when the difference between two consecutive sample weightings was less than 1 mg g^{-1} dry solid. Results at equilibrium were reported for each relative humidity as gram water sorbed 100 g^{-1} dry film. Absorption tests were done in quadruplicate at each a$_w$.

The data obtained were fitted by BET model:

$$We = \frac{Wo\, C\, a_w}{(1 - a_w)(1 + (C - 10)a_w)} \quad (4)$$

where, W_e is the equilibrium moisture content (g water 100 g^{-1} dry film), W_0 is the monolayer moisture content (g water 100 g^{-1} dry film) and C is a temperature dependent adsorption constant [19].

2.7 Water Vapour Permeability (WVP)

The apparatus and methodology described in the ASTM E96-00 [20] were used to measure the WVP of the film. Film specimens were conditioned for 48 h in a chamber at 25 °C and 53% relative humidity

(Mg(NO$_3$)$_2$ saturated solution) before being analyzed. Films were sealed on cups containing distilled water. Test cups were placed in a desiccator cabinet maintained at temperature and relative humidity controlled. A fan was used to maintain uniform conditions at all test locations over the specimen. Periodical weightings monitored the weight changes. Weight loss was plotted over time and when steady state (straight line) was reached 8 h more were registered. Film thickness was measured with an electronic micrometer (0.001 mm accuracy, Fowler, Cole-Parmer Instruments Co. USA). Thickness value was the mean value of five measurements and it was used for water vapour permeability calculations. The WVP was calculated from the slope (G) of a linear regression of weight loss versus time:

$$\text{WVP} = \frac{G\, 1}{A\, \Delta p} \quad (5)$$

where, l is the film thickness; A is the area of exposed film and Δp is the differential water vapour partial pressure across the film.

2.8 Scanning Electron Microscopy (SEM)

Cross-sections and surfaces of films were examined by SEM utilizing a JEOL JSM 6480 LV scanning microscope (USA). For cross-section observations, films were cryofractured by immersion in liquid nitrogen. Samples were stored at 25 °C over silica gel. Film samples were mounted on bronze stubs and coated with gold-palladium alloy. Samples were observed using an accelerating voltage of 20 kV.

2.9 Mechanical Properties

The tensile properties were measured using a texturometer Brookfield (USA) according to ASTM D882-00 [21] with some modifications. The films were cut into strips 25.4 mm wide and 80.0 mm long using a sharp scalpel. The ends of the strips were mounted between cardboard grips using double-side adhesive tape. The final film area exposed was 25.4 mm × 50.0 mm. The texturometer was set to tensile mode. Initial grip separation was 50 mm. Force and elongation was

recorded during extension at 20 mm min^{-1} up to break. All film strips were equilibrated during a week in a cabinet conditioned at 25 °C and 52% relative humidity using saturated magnesium nitrate solution previous tension assay. Test was carried out in triplicate for each film.

2.10 Statistical Analysis

Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure in GraphPad Prism 5.01 software. Tukey's multiple range test ($P \leq 0.05$) was used to detect differences among mean values of films properties. Nonlinear regressions were performed with GraphPad Prism. Measurements were replicated three times at least for each film tested.

3. Results and Discussion

A film based on BG, a hydrocolloid obtained as phloematic exudate of *Cercidium praecox*, and plasticized with glycerol, was elaborated. The film presented good visual aspect, transparency and amber colour.

3.1 Film Opacity and Colour

Opacity is a property of main significance if the film is to be used as a food coating or as a food packaging [15]. Low relative opacity values indicated a transparent film. In Fig. 1, opacity of BG films with different glycerol content were compared with commercial cellophane and corn starch film obtained in our laboratory. BG film presents a good transparency compared with other edible films like those obtained from corn starch. Film opacity is related to film internal structure. Film opacity was influenced by the interaction of glycerol with BG. Experimental opacities of BG films ranged between 46.8 and 72.2 Au nm, showing a better performance as compared to those of yam starch films, measured by the same method, which showed opacity ranged between 85.0 and 111.2 [22].

Colour attributes are of prime importance because they directly influence consumer acceptability. The results of evaluation of colour of BG films are shown in Table 1. There was little changes in $a*$ values with glycerol concentration whereas $b*$ values decreased significantly with the increase in glycerol content. Little change in $L*$ values were observed. These findings suggested a modification in amber colour and a smooth change of brightness with glycerol content.

3.2 Contact Angle

Wettability of films can be obtained through measurements of contact angle (θ) of a water drop on the film and the calculation of the surface energy (γ_S) that can not be directly measured. Table 2 shows that
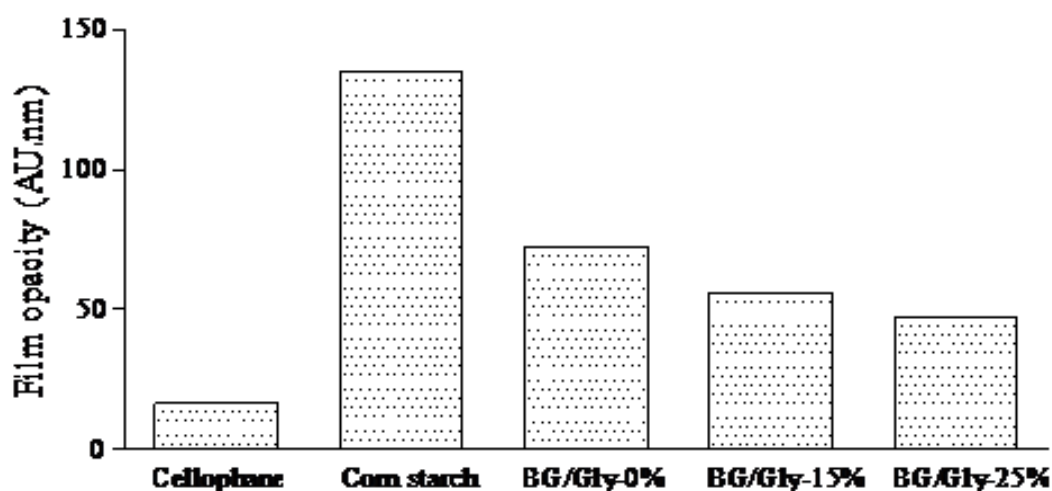

Fig. 1 Film opacity of BG films with different glycerol content (%) compared with opacity of cellophane and corn starch based film. Bars indicate standard deviation.

Table 1   Effect of glycerol content on the colour parameters of BG films.

Glycerol (%)	L^*	a^*	b^*
0	57.1 ± 1.2^a	5.9 ± 0.5^a	50.6 ± 2.3^a
15	58.6 ± 0.9^a	11.1 ± 0.9^b	34.3 ± 1.2^b
20	61.1 ± 0.5^b	13.0 ± 0.4^c	23.2 ± 0.9^c
25	64.1 ± 2.0^c	14.9 ± 1.1^d	12.8 ± 0.6^d

The mean difference is significant at $P \leq 0.05$. Similar letters refer equality between means.

Table 2   Contact angle and surface energy of BG films with different glycerol content.

Glycerol (%)	Contact angle (°)	Surface energy (mJ m^{-2})
0	71.19 ± 0.39^a	40.58 ± 0.79^a
15	67.78 ± 1.98^b	$41.87 \pm 3.05^{a, b}$
20	67.56 ± 0.97^b	42.33 ± 1.01^b
25	67.29 ± 1.36^b	43.34 ± 0.90^b

The mean difference is significant at $P \leq 0.05$. Similar letters refer equality between means.

contact angle and surface energy were smoothly altered by the increase in glycerol content. Due to hydrophilic character of glycerol, surface hydrophobicity decrease due to glycerol incorporation.
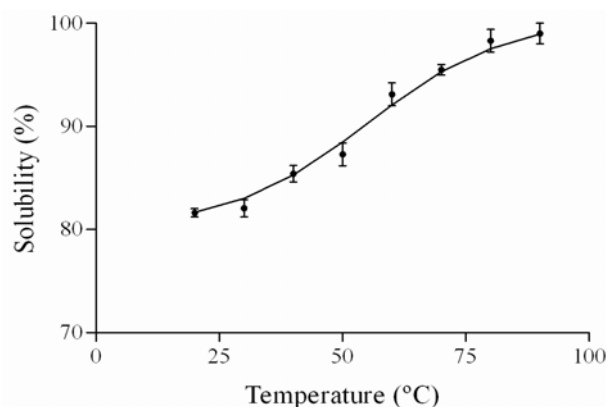
3.3 Film Solubility in Water

Solubility in water is an important property of films for food packaging applications. Some potential uses may require water insolubility to enhance product integrity and water resistance. However, in other cases, water solubility of the film before product consumption might be useful as in encapsulation of food or additives.

The effect of temperature on BG films with 20% glycerol was analyzed. BG films were highly soluble and their solubility increase with temperature (Fig. 2). Gontard et al. [15] studied edible wheat gluten films solubility in water at 20 °C and they reported solubility values ranged between 40% and 100% depending on film-forming conditions.

3.4 Moisture Sorption Isotherms

BG is a hydrophilic hydrocolloid with high solubility as showed in Fig. 2, and then the properties of BG edible film are greatly influenced by relative humidity. Moisture sorption isotherms are useful to evaluate the storage stability and properties in different

Fig. 2   BG film solubility in water as a function of temperature. Bars indicate standard deviation.
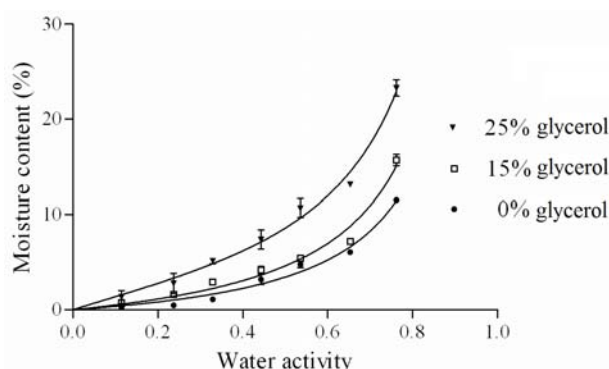
Fig. 3   Experimental data and isotherms curves predicted by BET model for moisture sorption of BG films at 25 °C. Bars indicate standard deviation.

RH conditions. Fig. 3 shows the moisture adsorption data and BET model fit curve obtained for BG film at 25 °C with glycerol concentration ranged between 0 and 25%. The moisture content of samples increases with equilibrium relative humidity. The high hygroscopicity of BG produced film disintegration at high relative humidity. Then, water content data above a_w values of 0.75, are not considered. Table 3 shows the fitting parameters obtained with BET model as a function of glycerol content using GraphPad Prism software. Similar behavior was reported in films based on starch [2], locus been gum [9] and chicken feather keratin [23]. Curves present a relative slight slope at low values of a_w, but take an exponential course at high water activities (above 0.50). The structure of the film is modified above a certain degree of freedom of water. Under these conditions, polymeric chains swell altering its structure. Moisture sorption isotherms

Table 3   Estimated BET parameters for BG films.

% glycerol	w_0	C	R^2	% E
0	3.726 ± 0.372^a	0.855 ± 0.245^a	0.977	6.12
15	4.782 ± 0.487^b	0.933 ± 0.284^b	0.971	8.64
25	6.223 ± 0.348^c	2.224 ± 0.525^c	0.965	9.63

The mean difference is significant at $P \leq 0.05$. Similar letters refer equality between means.

shown in Fig. 3 are similar in shape to Type III BET. These isotherms are observed when a liquid or vapor penetrant acts as a strong solvent or swelling agent on the polymer. The pronounced upswing at higher water activity range determines a better fit of BET than GAB equation [24]. In fact, moisture sorption data were adequately fitted with the BET model (Eq. 3) for the whole water activity range studied.

3.5 Water Vapour Permeability

The water permeability of films plays an important role in deteriorative reactions of food; therefore, it is the most extensively studied property of films. Hydrophilic materials such as BG interact with permeating water molecules through the polar groups of the film structure. Besides, plasticizers change the polymer network creating mobile regions with larger interchain distances, promoting water clustering by competing with water at active sites of the polymer matrix and the formation of micro cavities in the polymer network structure. Water sorption by biopolymers often results in swelling and conformational changes. The absorbed water plasticizes the film matrix, leading to a less dense structure where chain ends are more mobile, thus increasing water permeability [14].

Jangchud and Chinnan [25] found glycerol was the best plasticizer for water soluble polymers among several studied in their work. Based on this finding, glycerol was used as plastificant. The WVP of BG films as a function of glycerol content is presented in Fig. 4.

There were no significant differences on WVP values up to glycerol concentration of 20%. Above this value, WVP increased sharply as the concentration of glycerol increased. These results could be related to
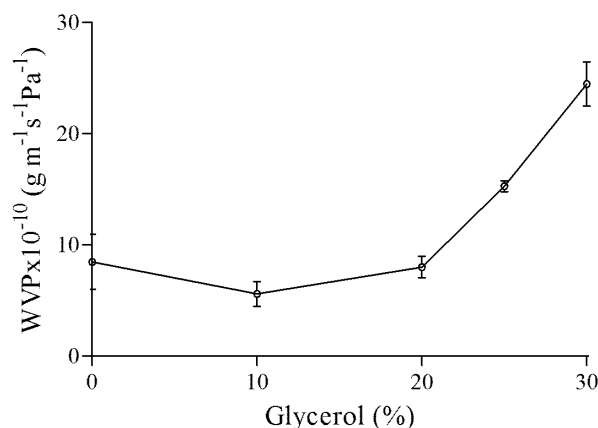
Fig. 4   Effect of glycerol content on water vapor permeability of BG films. Bars indicate standard deviation.

structural modifications of the BG network produced by the plasticizer and to the hydrophilic character of glycerol. Plasticizer causes a greater flexibility in polymeric structure, which increases water sorbed mobility within the structure. As a result, WVP increases with plasticizer film content. This behavior is commonly found in hydrophilic edible films and was reported by Wang et al. [26] for flaxseed gum based films; Yang and Paulson [27] for gellan films and Das et al. [28] for guar gum.

3.6 Scanning Electron Microscopy

Fig. 5 shows the cross section of BG film with 20% of plasticizer. SEM observation of unplasticized and low glycerol content films showed a high density structure with smooth surfaces and no pores or cracks. Cereda et al. [29] also found similar surfaces in cassava starch films. No differences were detected for BG films plasticized with different plasticizer concentration.

3.7 Mechanical Properties

Adequate mechanical strength and extensibility are generally required for a packaging film to withstand external stress and maintain its integrity as well as barrier properties during applications in packaging. BG presented excellent film forming capacity due to its polymeric nature. In the absence of plasticizers, films made from BG were brittle. The addition of a plasticizing agent to films was required to overcome
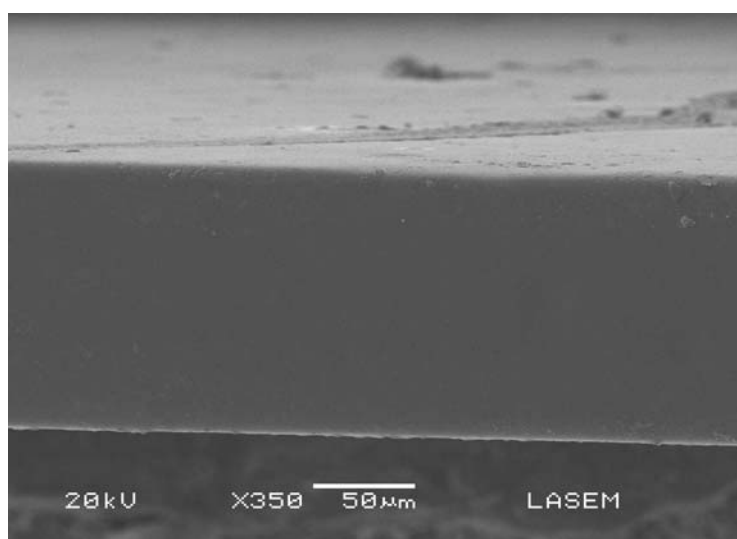
Fig. 5 Cross-section micrographs of BG films with 20% of plasticizer. SEM magnification is indicated in the micrograph.

film brittleness caused by extensive intermolecular forces. Plasticizers reduced these forces, thereby improving flexibility and extensibility of the films [30]. Tensile strength of BG films plasticized with different glycerol content was measured and is shown in Fig. 6. Tensile strength decrease linearly with glycerol concentration in the range studied. Tensile strength values obtained were similar to those reported by Carneiro-da-Cunha et al. [12] for an exudate gum from *Anacardium occidentale*. Yang and Paulson [27] reported tensile strength and elongation of gellan gum based films plasticized with glycerol in a range of 60% to 80%. They found a similar linear decrease in tensile strength between 37 and 7 MPa in the range of glycerol content studied. Film elongation varied between 30% and 37%. The relative high concentration of glycerol used was due to the numerous hydrogen bonds and cation crosslinks between the gellan molecular chain that contribute to the high mechanical strength and flexibility.

Glycerol is a small molecule as compared with BG. Glycerol as a plasticizer can penetrate into the intermolecular spaces, resulting in separation of molecular chains of polymer from each other. Furthermore, it is generally accepted that plasticization of glycerol promotes the mobility of polymer chain for edible films through increasing the free volume
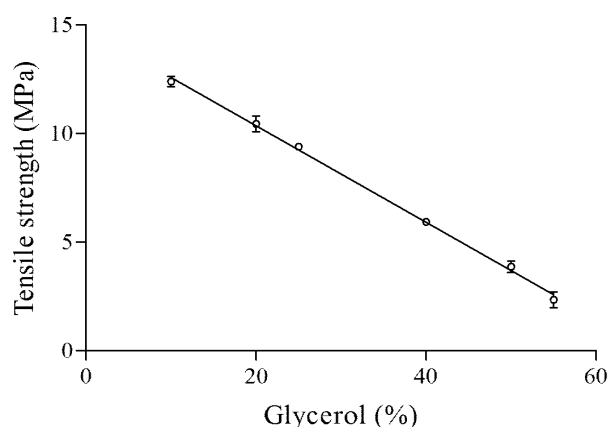
Fig. 6 Effect of glycerol concentration on tensile strength of BG films. Bars indicate standard deviation.
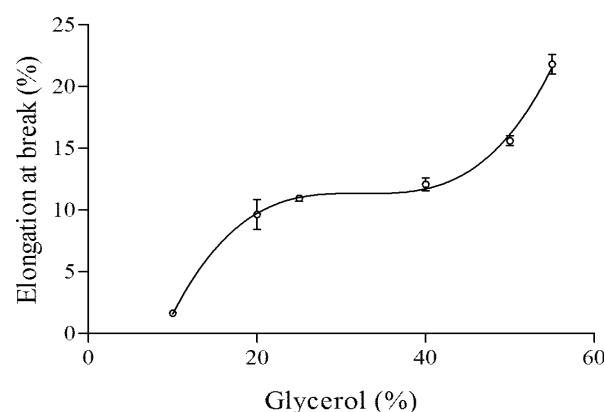
Fig. 7 Effect of glycerol concentration on elongation at break of BG films. Bars indicate standard deviation.

available to the chain [31]. Fig. 7 shows the effect of glycerol concentration on BG films. It was observed an almost constant elongation between 20% and 40% of

glycerol content. The values obtained for elongation are in agreement with those of films based on exudate gum from *Anacardium occidentale* [12].

4. Conclusions

A biodegradable and renewable biomaterial (Brea gum) was used to formulate films and coatings. BG films presented good visual aspect, transparency and amber colour. SEM observation showed a dense and homogeneous structure. Film wettability increased with glycerol content due to hydrophilic nature of the plasticizer. Solubility of films in water increased with temperature and water sorption isotherms of films plasticized with glycerol at 25 °C showed that at high a_w (above 0.50), the film matrix swell, altering its structure and properties. WVP remained practically constant up to 20% of glycerol content and then increases linearly with the plasticizer. Glycerol produced plasticization on BG films giving a more plastic like structure. By increasing chain mobility in the starch network, a decrease of tensile strength and an increase in elongation were clearly noticed. In our experiments, the best mechanical and barrier properties were found at 20% glycerol concentration. BG films may be proposed as a new biomaterial for application in food packaging and conservation.

Acknowledgments

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