Carbohydrate Polymers 101 (2014) 349–358

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Lepidium perfoliatum seed gum: A new source of carbohydrate to make a biodegradable film

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ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 31 August 2013
Accepted 19 September 2013
Available online 29 September 2013

Keywords:
Lepidium perfoliatum seed gum
Biodegradable film
Glycerol
Water vapor permeability
Mechanical properties
Thermal properties

ABSTRACT

Microstructural, physical, mechanical and thermal properties of a novel biodegradable film based on *Lepidium perfoliatum* seed gum (LPSG) were investigated. LPSG films were successfully prepared by incorporation of four levels of glycerol (40%, 50%, 60% and 70%, w/w). As expected, increasing glycerol concentration from 40 to 70% (w/w), increased water vapor permeability (WVP), elongation at break (EB%), moisture content, moisture adsorption and water solubility of LPSG films; whilst, elastic modulus (EM), contact angle, melting point (T_m), enthalpy of melting (ΔH_m) and glass transition point (T_g) decreased significantly. LPSG films became slightly greenish and yellowish in color but still transparent in appearance. The images taken from electron scanning microscopy indicated uniform surface, compact sheets with no holes or fracture. This study demonstrates that LPSG based films with desired properties can be obtained by adjusting glycerol content.

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1. Introduction

The use of synthetic polymers and plastics for packaging has grown tremendously in the last century. Plastics are cheap to manufacture and provide excellent protection for food product. Therefore, they are used as packaging material in almost every foods and drinks. However, plastics are non-degradable due to their very large polymer molecules and stable carbon–hydrogen bond, which make its decomposition by organisms rather difficult. Non-renewable nature of these plastics has created serious environmental problems due to the materials' inability to biodegrade (Muscat, Adhikari, Adhikari, & Chaudhary, 2012).

Researches on new materials from renewable resources to be used in food industries have progressed in order to decrease the problem of plastic waste disposal (Prashanth & Tharanathan, 2007; Siracusa, Rocculi, Romani, & Rosa, 2008). There has been an increasing interest in edible biodegradable films, due to their numerous advantages over non-biodegradable plastic packaging films. Edible films can be developed from different materials such as proteins, polysaccharides, lipids, and resins (Krochta, 2002). Polysaccharides and proteins posses the ability of establishing polymer interaction, thus creating a continuous network responsible for the functional properties of edible films (Giancone, Torrieri, Masi, & Michon, 2009; Perez, Carrara, Sánchez, Rodríguez Patino, & Santiago, 2009). These films show good mechanical properties, but are highly permeable to water because of their hydrophilic nature (Anker, Berntsen, Hermansson, & Stading, 2002; Kristo, Biliaderis, & Zampraka, 2007).

Several polysaccharides have good film forming capacities, including locust bean gum and guar gum (Shrestha, Arcot, & Paterson, 2003), starch (Muscat et al., 2012; Rodríguez, Osés, Ziani, & Maté, 2006), mesquite gum (Bosquez-Molina, Tomas, & Rodrıguez-Huezo, 2010), chitosan (Thakhiew, Devahastin, & Soponronnarit, 2010), cellulose and cellulose derivatives (HPMC, CMC and MC) (Rachtanapun, Luangkamin, Tanprasert, & Suriyatem, 2012; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009), *Pseudomonas oleovorans* (Alves et al., 2011), flaxseed gum (Wang, Li, Wang, Yang, & Özkan, 2011), psyllium seed (Ahmadi, Kalbasi-Ashtari, Oromiehie, Yarmand, & Jahandideh, 2012), *Salvia hispanica* mucilage (Muñoz, Aguilera, Rodriguez-Turienzo, Cobos, & Diaz, 2011) and cress seed gum (Jouki, Khazaei, Ghasemlou, & HadiNezhad, 2013).

Gum extracted from *Lepidium perfoliatum* seeds (LPSG) can be used as a potential thickening and stabilizing agent in food industries (Hesarinejad, Koocheki, & Razavi, 2013; Koocheki, Taherian, & Bostan, 2013; Soleimanpour, Koocheki, & Kadkhodaee, 2013a, 2013b). Our previous studies showed that LPSG could be an essential viscosity builder and provider of the body and mouthfeel for industrial application (Koocheki, Taherian, Razavi, & Bostan, 2009). In general, the thickening action of LPSG in aqueous system could add texture to formulations and stabilize the dispersions and emulsions. Therefore, the gum obtained from *L. perfoliatum* seeds could

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0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.072

be a new source of polysaccharides with the potentials of generating films and coatings with improved properties.

Formulation of biopolymer films requires incorporation of plasticizer to reduce their brittleness, allow easier removal from the forming support and confer plastic properties (Hernandez-Izquierdo & Krochta, 2008). Brittleness is an inherent quality attributed to the complex/branched primary structure and weak intermolecular forces of natural polymers. The plasticizer molecules decreases intermolecular forces along the polymer chains, thus improving flexibility, extensibility, toughness and tear resistance of the film; however, they also decrease its barrier properties (Karbowiak, Hervet, et al., 2006). Among plasticizers, glycerol produces the best effects in carbohydrate based films, thus leading to more stable, flexible and less brittle films. For example, glycerol was used as a plasticizer to impart film flexibility and enhanced the mechanical properties of kefiran (Ghasemlou, Khodaiyan, & Oromiehie, 2011), psyllium seed gum (Ahmadi et al., 2012) and cress seed (Jouki et al., 2013) edible films.

As mentioned above, hydrocolloids are the most widely investigated biopolymers in the field of edible films. However, to date, no research has presented the LPSG film preparation with glycerol as plasticizer. Hence, the aim of this study was to develop thin films from LPSG and to evaluate the effect of glycerol concentrations on properties of this film. For this purpose, mechanical, physical, barrier, microstructure and thermal properties of LPSG based films were investigated.

2. Materials and methods

2.1. Materials

L. perfoliatum seeds were obtained from a local medical market in Mashhad, Iran. Glycerol and potassium sulfate (used to equilibrate films at 97% RH) were purchased from Merck Co. (Germany). Anhydrous calcium chloride was procured from Scharlau (Spain). Calcium nitrate $6H_2O$ (used to equilibrate films at 50 ± 2% RH) obtained from Ghatran Chemistry (Iran).

2.2. Extraction of LPSG

L. perfoliatum seeds were manually cleaned to remove all foreign substances such as stones, dust, dirt, chaff and broken seeds. The LPSG was prepared based on the method described by Koocheki et al. (2009). In brief, *L. perfoliatum* seeds were dispersed in heated deionized water (48 ± 1.0 °C) at a water/seed ratio of 30:1. The pH was monitored continuously and adjusted at 8 while the temperature was kept constant at 48 ± 1.0 °C during the process. The seed-water slurry was stirred continuously throughout the entire extraction period (1.5 h). Finally, seeds were discarded, and ultimately, the slurry was dried in a laboratory oven (70 °C for 46 h), milled and sieved using a mesh 100 sifter. Gum extracted under this condition contains 88.23% total sugar, 4.6% protein, 6.0% moisture, 0.18% ash and no fat (Koocheki et al., 2013).

2.3. Film formation

Aqueous solutions of 0.6% (w/v) LPSG were prepared by dispersing LPSG powder in distilled water at room temperature (23 ± 2 °C). Our preliminary experiments showed that films made from higher LPSG concentrations had air bubbles which could not be removed due to the high viscosity of the solution. On the other hand, films formulated with concentration lower than 0.6% had low thickness and were difficult to peel off. In addition, film without plasticizer was brittle, and cracked during drying. Therefore, glycerol (40, 50, 60 and 70%, w/w) was added and solutions were magnetically stirred during 15 min. The solutions were kept overnight and then poured onto a series of Petri dishes (diameter 15 cm). To control film thickness, volume of each film-forming solution poured onto a plate was always 110 ml. Films were dried at 45 °C for 18 h, in a drying box (Soroush oven-so-2005, Iran) with gentle fan forced air circulation. Dried films were peeled-off and stored in a desiccator containing saturated calcium nitrate to meet the required relative humidity before analysis.

2.4. Physical properties of LPSG films

2.4.1. Film thickness and density

Thickness of the films was determined using a manual digital micrometer (QLR digit-IP54, China) to the nearest 0.001 mm. Informed values are an average of at least ten random locations of each film sheets. The film density (ρ_s) was calculated directly from the film weight and dimensions (Salgado, Ortiz, Petruccelli, & Mauri, 2010) according to the following equation:

$$\rho_s = \frac{m}{A \times \delta} \quad (1)$$

where A is the film area (4 cm × 4 cm), δ is the film thickness (cm), m is the film dry mass (g) and ρ_s is the dry matter density of the film (g/cm^3).

2.4.2. Moisture content

The moisture content was determined by film weight loss using oven drying at 103 ± 2 °C for 24 h (Soroush Oven-SO-2005). Preliminary experiments showed us that 24 h were enough to fully dry up the samples. The weight loss of the sample was determined, from which the moisture content was calculated using the following equation:

$$\mathrm{MC} = \frac{M_i - M_f}{M_i} \times 100 \quad (2)$$

where M_i and M_f are the masses of initial and dried samples, respectively.

2.4.3. Moisture absorption

The moisture absorption (MA) was determined according to the method of Angles and Dufrense (2000). Dried films (2 cm × 2 cm) were placed in a desiccator containing anhydrous Calcium Chloride (0% RH) at 25 °C for 24 h. After initial weighing, they were conditioned in a desiccator containing saturated potassium sulfate solution (97 ± 1% RH). The weight of the samples was recorded every 24 h until constant weight was reached. The moisture absorption was calculated using Eq. (3):

$$\mathrm{MA} = \frac{W_t - W_0}{W_0} \quad (3)$$

where W_t and W_0 are the weights of the sample after time t and the initial weight of the sample, respectively.

2.4.4. Water solubility

The films' solubility in water was determined according to the method reported by Gontard, Duchez, Cuq, and Guilbert (1994). Solubility is defined as the content of dry matter solubilized after 24 h immersion in water. Pieces of LPSG films (2 cm × 2 cm) were cut with a scalpel, dried at 103 ± 2 °C for 24 h in a laboratory oven, and weighed to obtain the initial film dry weight. Each piece of film was placed into a plastic tube with 50 ml of distilled water. After 24 h of immersion with gentle mechanical agitation, the remaining pieces of films were filtered and dried at 103 °C to reach a constant weight (final dry weight). The water solubility was calculated using Eq. (3):

$$\mathrm{WS}(\%) = \frac{W_0 - W_f}{W_0} \times 100 \quad (4)$$

where W_0 is the initial weight and W_f is the weight of the desiccated undissolved film.

2.5. Water vapor permeability (WVP) and water vapor transmission rate (WVTR)

The water vapor permeability through films was determined gravimetrically using the ASTM E96-00 method (ASTM, 2000). The circular test cups containing 3 g anhydrous calcium chloride (0% RH, assay cup) were sealed by films without pinholes or defects (0.00018 m^2 film area). To maintain the gradient of relative humidity of approximately 97% across the film, cups were placed inside desiccators containing saturated potassium sulfate solution at the test temperature of 25 °C. The permeability cups with the films were weighted at intervals of 2 h during the first 12 h and finally after 24, 48 and 72 h. Linear regression was used to calculate the slope of a fitted straight line in a graph of variation of mass versus time. The WVTR and the WVP were calculated from Eqs. (5) and (6) respectively:

$$\mathrm{WVTR} = \frac{\mathrm{Slope}}{A} = \frac{\Delta m}{\Delta t A} \tag{5}$$

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times X}{\Delta p} \tag{6}$$

where $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/s), A is the area of the exposed film surface (m^2), X is film thickness (m) and Δp is the water vapor pressure difference between the two sides of the film (Pa).

2.6. Contact angle

The surface hydrophobicity of the films was estimated by the sessile drop method, based on optical contact angle method. A droplet of distilled water (20 μL) was put over the surfaces of the samples and images were taken right after using a digital camera. The angle between drop and surface of the film was measured using the Image J Software. Measurements were performed at three locations for each specimen.

2.7. Opacity

Opacity of LPSG films was measured according to the method of Gontard, Guilbert, and Cuq (1992). In brief, films were cut in rectangular strips of 0.4 cm × 7.3 cm and placed in the spectrophotometer cell. A spectrum of each film was recorded using a UV–vis spectrophotometer (Shimadzu UV-160A). The area under the absorbance curve from 400 to 800 nm was determined by Adobe Photoshop CS and defined as the film opacity.

2.8. Color analysis

In order to investigate the effect of glycerol content on color of LPSG based films, the following procedure was applied:

In this research, sample illumination was achieved with four fluorescent lights (Opple, 8W, model: MX396-Y82; Taiwan, 60 cm in length) with a color index (Ra) close to 95%. The illuminating lights were placed in a wooden box, 45 cm above the sample and at the angle of 45° with sample plane to give a uniform light intensity over the film samples.

The interior walls of the wooden box were painted black to minimize background light. A color digital camera (Canon EOS 1000D, Japan) with 15 mega pixels resolution was located vertically 35 cm above the sample. The angle between the camera lens axis and lightening sources was around 45°. Images were taken and Canon Digital Camera Solution Software (version 22) was used to acquire the images in the computer in JPEG format. To achieve high uniformity and repeatability, the camera was adjusted in the manual mode and the lens was operated with aperture of 5.6 and shutter speed of 1/40 s (without zoom and flash), while the ISO was 100 and white balance was set on florescent.

To improve the background's contrast of images and segmentation (to separate the true images of the films from background) Adobe Photoshop (Adobe, v.8.0) was used. Since the *Lab* color is device independent and providing consistent color regardless of the input or output, the images taken were converted into *Lab* units. Where L is referred to as the lightness or luminance, a is defined along the axis of red–green, and b is defined along the axis of yellow–blue (Sun, 2008). In the *Lab* space, the color perception is uniform, and therefore, the euclidean distance between two colors is almost in agreement with the color difference perceived by the human eye (Pedreschi et al., 2007). The total color difference (ΔE) was calculated using Eq. (7).

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{7}$$

where ΔL, Δa and Δb represent the differentials between the color parameter of the sample and the white standard.

Yellowness (YI) and whiteness (WI) indices were also calculated using Eqs. (8) and (9)

$$\mathrm{YI} = \frac{142.86b}{L} \tag{8}$$

$$\mathrm{WI} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \tag{9}$$

In this study, the image analysis was managed using ImageJ software (National Institutes Health, Bethesda, MD, USA) version 1.40 g.

2.9. Mechanical properties

The mechanical properties of the films including tensile strength (TS), elongation at break (EB) and modulus of elasticity (EM) were determined a texture analyzer (TA-XT Plus™, Stable Micro Systems, UK) in accordance with ASTM 882-02 standard method (2002). The thickness of the films was measured at 10 random positions in the middle part of the films where the failure usually occurred, using a digital micrometer. Rectangular samples (10 mm × 140 mm) were cut and stored at 25 °C and 50 ± 2% RH, inside desiccators containing saturated solutions of calcium nitrate for 48 h. Equilibrated film specimens were mounted in the film-extension grips. Initial grip separation and cross-head speed were set at 100 mm and 12.5 mm/min, respectively. Mechanical properties were calculated using the average thickness of each film sample. The TS (MPa) was calculated by dividing the peak force with the cross section area of the film. Similarly the % elongation was measured by dividing the change in length achieved at break with the film's original length. The EM (MPa) which represents the stiffness of the films was calculated from the initial linear portion of the stress–strain curve.

2.10. Differential scanning calorimetry (DSC)

The thermal properties of films were determined by differential scanning calorimetry (Shimadzu, DSC 60). Approximately 2 mg specimen was placed in an aluminum pan. An empty aluminum pan was used as reference. Films were scanned at a heating rate of 10 °C/min between the temperature ranges of −100 and +300 °C. The glass transition temperatures (T_g) of the different films were determined as the midpoint temperature of a step-down shift in baseline, due to the discontinuity of the specific heat of the sample. The melting point (T_m) was calculated as the temperature where

Table 1
Physical properties of LPSG films with various concentrations of glycerol.[a,b]

Glycerol conc. % (w/w)	Moisture content (%)	Water solubility (%)	Moisture absorption (%)	Thickness (mm)	Density (g/cm^3)
40	23.21 ± 0.68^d	38.98 ± 0.70^c	127.39 ± 5.46^b	0.069 ± 0.004^a	0.93 ± 0.02^a
50	27.3 ± 1.03^c	45.44 ± 1.11^b	127.81 ± 5.66^b	0.070 ± 0.004^a	0.92 ± 0.02^a
60	32.69 ± 0.43^b	56.88 ± 1.40^a	152.60 ± 3.39^a	0.071 ± 0.005^a	0.91 ± 0.03^a
70	37.66 ± 0.45^a	58.04 ± 1.47^a	155.15 ± 4.54^a	0.071 ± 0.004^a	0.88 ± 0.01^a

[a] Means within each column with different letters are significantly different ($p < 0.05$).
[b] Data are means ± SD.

the peak of the endotherm occurs. The enthalpy of melting (ΔH_m) was determined as the area over the endothermic peak.

2.11. Scanning electron microscopy (SEM)

Cross-section morphology of the dried films was performed by scanning electron microscopy (LEO 1450 VP). The films containing various plasticizers were fractured in liquid nitrogen, mounted on aluminum stubs using a double-sided adhesive tape, and sputtered with a thin layer of gold–palladium using a sputter coater (SC 1620). Scanning electron microscope (SME) operated at 20 kV under vacuum condition. Films were observed at a magnification of 1000×.

2.12. Fourier-transform-infrared spectroscopy (FTIR)

Fourier transformed infrared (FTIR) spectra of the films were carried out on a AVATAR 370 FTIR Thermo Nicolet. A total of 20 scans were performed in the spectral range of 4000–400 cm^{-1}.

2.13. Statistical analyses

Significant differences among the means were evaluated by analysis of variance (one-way ANOVA), and the means were compared using the Tukey test ($p < 0.05$). Data were evaluated using Minitab software. Nine replicates for mechanical properties while two replicates for FTIR, T_g, and SEM were performed. The remaining experiments were conducted in triplicate.

3. Results and discussion

3.1. Physical properties

The density of the films (Table 1) ranged between 0.88 and 0.93 g/cm^3. However, no significant decrease was observed with increasing GLY concentration ($p > 0.05$). Increasing glycerol concentration had also no significant effect on the films thickness (Table 1). Therefore, any change in films properties was due to the presence of glycerol not the film thickness.

The moisture content of LPSG based film is shown in Table 1. The moisture increased from 23.21 to 37.66% with increasing glycerol concentration. Glycerol is a hydrophilic substance that could become immobilized between LPSG chains and interact with water molecules. Since glycerol acts as a water-holding agent (Arvanitoyannis & Biliaderis, 1999), increasing glycerol concentration made the film more hydrophilic. Therefore, the moisture content of films can be changed due to the formation of glycerol–gum and glycerol–water interactions. Other studies also reported that the moisture content of carbohydrate based films increased with increasing plasticizer concentration (Ghasemlou et al., 2011; Jouki et al., 2013; Zhang & Han, 2006).

Furthermore, the LPSG films with higher concentrations of glycerol adsorbed more moisture to some extent. As shown in Table 1, increasing glycerol content from 40 to 50% did not change film moisture adsorption, while further increase substantially increased this property. This is due to the high water absorption tendency of glycerol. Glycerol, being the small molecule, could be placed between polymer chains more easily as compared to larger plasticizer molecules, to interrupt the formation of polymer–polymer hydrogen bonds. At high plasticizer content, glycerol-rich domains could form within the polymer matrix (Chen & Zhang, 2005; Sothornvit & Krochta, 2005). Therefore, the glycerol might facilitate the moisture permeation into LPSG based film when submitted to highly moisture atmospheres. These results were similar to those achieved by Ghanbarzadeh and Almasi (2011), who also reported that the increase in plasticizer content could increase the moisture absorption of the carboxymethyl cellulose film.

The water solubility of edible films is also an important feature when choosing a film for specific applications (Arvanitoyannis, Nakayama, & Aiba, 1998). Generally, when the water solubility of a film is high, it cannot protect food from moisture or from water loss. For some applications like packaging wrap, the high solubility is an indicator of biodegradability which could be an advantage for such films (Stuchell & Krichta, 1994). Increasing glycerol concentration significantly altered the water solubility of LPSG based film from 38.98 to 58.04% (Table 1). The highest solubility was achieved when higher concentration of glycerol (70%, w/w) was incorporated. Plasticizers can reduce crosslink between biopolymer molecules, and thus increase the solubility of film (Ghasemlou et al., 2011). Because glycerol is highly hygroscopic, this may explain why glycerol-plasticized blend films have high water solubility (Tong, Xiao, & Lim, 2013). These results were similar to those achieved by Cerqueira, Souza, Teixeira, and Vicente (2012), who also reported that increasing plasticizer content in galactomannan based films increased the interaction of polymer with water. The higher mobility of the matrix was also influenced by this interaction.

3.2. WVP and WVTR of films

The permeation characteristics of small molecular weight species, such as water vapor, were investigated to determine the effect of glycerol on the barrier properties of LPSG film. The water vapor permeability is the most extensively studied property of edible films, mainly because of the importance of the water in deteriorative reactions. WVP is a phenomenon that implies water solubility and diffusion of the water molecules through the matrix of the film. Since most natural biopolymers are very prone to water absorption, WVP is considered a crucial property for such films (Prodpran, Benjakul, & Artharn, 2007). In the same way, WVTR is another property that is directly related to the hydrophilic nature of the film, i.e. more hydrophilic films will have greater WVTR (lower water vapor barrier) (Bourtoom & Chinnana, 2008; Ferreira, Nunes, Delgadillo, & Lopes-da-Silva, 2009; Tongdeesoontorn, Mauer, Wongruong, & Rachtanapun, 2009).

The WVP and WVTR of LPSG based films containing various plasticizer concentrations are shown in Table 2. The water vapor barrier of films was significantly ($p < 0.05$) affected by glycerol concentration. Increasing glycerol content from 40 to 70% increased the WVP and WVTR of the manufactured films from 17.71 to 20.86 g mm/m^2 d kPa and 679.12–771.475 g/m^2 d, respectively. Generally, WVP and WVTR of edible films mainly depend on two

Table 2
Contact angle, WVTR and WVP of LPSG film.[a,b]

Glycerol conc. % (w/w)	Contact angle (°)	WVTR (g/m² d)	WVP (g mm/m² kPa d)
40	72.9 ± 0.81[a]	679.12 ± 19.20[b]	17.71 ± 0.66[b]
50	72.05 ± 1.03[a]	735.08 ± 15.67[ab]	18.75 ± 0.23[ab]
60	70.27 ± 0.69[ab]	739.24 ± 20.84[ab]	19.51 ± 0.64[ab]
70	68.36 ± 0.05[b]	771.475 ± 21.39[a]	20.86 ± 0.64[a]

[a] Means within each column with different letters are significantly different ($p < 0.05$).
[b] Data are means ± SD.

factors: the diffusivity and solubility of water molecules in the film matrix.

The WVP values observed for LPSG films can be attributed to the developing hydrophilic nature of the films as glycerol levels increased. This is supported by the differences between moisture absorptions of LPSG films with different glycerol concentrations (Table 2). Gaudin, Lourdin, Le Botlan, Ilari, and Colonna (1999) reported that higher water content leads to increased permeability in hydrophilic biopolymer films.

Moreover, according to Guilbert and Biquet (1996), addition of a plasticizer modifies the properties of the edible film by reducing the intermolecular bonds between the polymer chains, thus increasing the WVP and WVTR of film. Therefore, we can conclude that glycerol as a low molecular weight substance can probably penetrate into LPSG network, thereby result in effective disrupting of intermolecular interaction among polysaccharide chains. Consequently, there would be greater space for water and other molecules to migrate through the film structure (Sothornvit & Krochta, 2000).

The increase in WVP and WVTR with increasing hygroscopic plasticizer concentration is a well recognized phenomenon in the manufacture of edible films (Buttler, Vergano, Testin, Bunn, & Wiles, 1996; Cuq, Gontard, Cuq, & Guilbert, 1997; Nur Hanani, McNamara, Roos, & Kerry, 2013; Sobral, Menegalli, Hubinger, & Roques, 2001). Jouki et al. (2013) also reported that the increase in glycerol concentrations significantly increased the WVTR and WVP of cress seed carbohydrate films. They inferred that WVP was a characteristic which depended on solubility coefficient and diffusion rate of water in the film. Similar observations have also been reported for flaxseed gum (Wang et al., 2011), kefiran (Ghasemlou et al., 2011) psyllium seed gum (Ahmadi et al., 2012), pullulan (Tong et al., 2013), potato starch (Talja, Helén, Roos, & Jouppila, 2007), yam starch (Mali, Grossmann, Garcia, Martino, & Zaritzky, 2002) and soybean polysaccharide (Tajik et al., 2013).

The films formed with LPSG showed similar water vapor barrier properties to carbohydrate film produced with cress seed gum (Jouki et al., 2013). However, films made of LPSG had higher WVP values compared with those of kefiran (Ghasemlou et al., 2011) and psyllium seed gum (Ahmadi et al., 2012) films.

3.3. Contact angle

Measuring the contact angle is a way to specify the surface hydrophilicity of a film. It is well known that a small contact angel below 30° shows a hydrophilic surface. Table 2 illustrates the contact angle values for LPSG films with different glycerol concentrations. Increasing glycerol concentration slightly reduced the water contact angle which is due to the hydrophilic nature of this plasticizer. Others authors have also found similar behaviors, for example Ahmadi et al. (2012) reported that the incorporation of glycerol into psyllium seed gum films, decreased the hydrophobicity of the films, which was attributed to the hygroscopic nature of glycerol.

Surface properties of the films also give information regarding the phenomenon of wetting or non-wetting of a product surface by film forming dispersions (Karbowiak, Debeaufort, & Voilley, 2006; Vargas, Albors, Chiralt, & González-Martínez, 2009). Wettability is one of the most important properties when evaluating the capacity of a solution to coat a surface where higher values of wettability (lower contact angels) are considered the most suitable to coat the surface. An increase in contact angle is known to reflect lower wetting (relatively higher hydrophobicity) property of the film surface. The contact angles of LPSG films were between 68.38° and 72.9° (Table 2) which indicates that all the formulations tested would wet, at least partially, the surface of the product.

The contact angle results were in good agreement with the results obtained in the moisture absorption test. As mentioned before, films with higher moisture contents had lower contact angles, indicating more ability to absorb water and so that higher hydrophilicity.

3.4. Color and opacity

Optical properties are needed to define the ability of films to be applied over the surface of food, since these affect the appearance of the covered product, which is an important quality factor (Mali, Grossmann, & García, 2004). Among these properties, color attributes are of prime importance because they directly influence consumer acceptability (Rao, Kanatt, Chawla, & Sharmam, 2010).

The color parameters (L, a, b) of LPSG films are shown in Table 3. Results showed that all color parameters, with the exception of L value, significantly changed when glycerol concentration increased ($p < 0.05$). Incorporation of glycerol to LPSG film matrix was able to increase b value and decrease a value. In other words, greeness and yellowness increased with increasing glycerol content. These results were consistent with visual observations. Ahmadi et al. (2012) also concluded that increasing the glycerol concentration, increased the lightness and blue–yellow color, while reduced green–red color in psyllium films.

Color changes with addition of glycerol can be more fully described using other color functions, such as ΔE which indicates the degree of total color difference from the standard color plate, YI indicates degree of yellowness, and WI indicates degree of

Table 3
Opacity and color (values including, L, a, and b, total color difference (ΔE), whiteness index (WI) and yellowness index (YI)) parameters of LPSG film.[a,b]

Glycerol conc. % (w/w)	Opacity (AU nm)	L	a	b	ΔE	WI	YI
40	17.65 ± 0.43[a]	76.21 ± 0.16[a]	−16.27 ± 0.02[a]	−6.40 ± 0.48[c]	27.81 ± 0.25[a]	70.47 ± 0.22[a]	−11.99 ± 0.93[c]
50	17.50 ± 0.78[a]	75.52 ± 0.20[a]	−17.53 ± 0.10[b]	−4.33 ± 0.19[b]	28.57 ± 0.17[a]	69.57 ± 0.19[a]	−8.19 ± 0.34[b]
60	17.08 ± 0.52[a]	76.12 ± 0. 90[a]	−18.00 ± 0.25[bc]	−1.26 ± 0.34[a]	27.91 ± 0.59[a]	69.94 ± 0.62[a]	−2.37 ± 0.67[a]
70	16.90 ± 0.79[a]	76.15 ± 0.18[a]	−18.26 ± 0.13[c]	−1.26 ± 0.17[a]	28.05 ± 0.24[a]	69.57 ± 0.23[a]	−2.37 ± 0.33[a]

[a] Means within each column with different letters are significantly different ($p < 0.05$).
[b] Data are means ± SD.

whiteness (Table 3). Increasing glycerol content resulted in a significant increase in YI, while, no significant differences in ΔE and WI values were detected among films.

Opacity is an established measurement of the transparency of a film (Abdollahi, Alboofetileh, Rezaei, & Behrooz, 2013). Generally, opacity and transparency are inversely correlated. A higher value of opacity means a lesser transparency. The opacity values of film samples are presented in Table 3, where lower relative opacity values indicate that the films are more transparent. When the glycerol content increased up to 70%, the opacity value of the resulting film slightly decreased. However, this trend was not significant, meaning that the transmission of light through the resulting films did not change with plasticizer concentration. These findings are important since film transparency or opacity are critical properties in various film applications, particularly if the film will be used as a surface food coating or for improving product appearance (Gontard et al., 1992). In many applications, an increased opacity is undesirable, although some applications need to provide protection against reactions of deterioration produced by the effect of light, offering some advantage to this type of film.

3.5. Mechanical properties

Mechanical properties of films are often presented in terms of elastic modulus (EM), tensile strength (TS) and elongation at break (EB). Due to the structural nature of TS and EB, usually films with high TS show low EB so that both properties should be analyzed simultaneously (Altenhofen da Silva, Krause Bierhalz, & Guenter Kieckbush, 2009).

The effects of glycerol content on TS, EB and EM values of edible films made from LPSG are summarized in Fig. 1. TS represents film's mechanical resistance due to cohesion forces between chains, while EB measures its plasticity, which is the capacity of a film to extend before breaking. Low elongation indicates a low deformation capacity and a brittle film structure (Banker, 1966). The increase of plasticizer concentration in LPSG based film exerts a great influence over EB values (Fig. 1a). When glycerol content increased from 40 to 70%, EB increased from 1.163 to 4.352%. On the other hand, as the glycerol concentration increased up to 50%, the TS decreased and then became constant at higher plasticizer concentrations (Fig. 1a). Moreover, EM as an index for stiffness also showed a general decrease with increasing glycerol content (Fig. 1b). Films with low TS and high EB tend to be less brittle. As a result, LPSG films plasticized with glycerol exhibited great flexibility and hence underwent fracture in a slow and sustained pace. Furthermore, the higher the glycerol content, the lower the brittleness and the higher the flexibility of the LPSG films. This most likely arises from the penetration of glycerol in the polymer matrix and reduction of inter-chain interactions which finally alters the mechanical properties of LPSG based film.

Generally, plasticizers interfere with polymer chains where, by decreasing intermolecular forces, they soften the rigidity of the film's structure and increase the polymer mobility; this decreases TS and increases EB (Leceta, Guerrero, & de la Caba, 2013). According to Jouki et al. (2013), a rising glycerol content increases film elasticity and elongation because it constrains establishment of hydrogen bonds between the polymer chains thus increasing intermolecular spacing, and therefore chain mobility. The water content of the films when glycerol is added also affects TS and EB, and accentuates the effect of the glycerol content (Ziani, Oses, Coma, & Maté, 2008).

These results are also in agreement with those reported by Ghasemlou et al. (2011), Ahmadi et al. (2012) and Jouki et al. (2013) who also found that mechanical properties of kefiran, cress seed gum and psyllium seed gum based films were affected by glycerol concentration. From the above results it can be seen that the tensile strength, modulus of elasticity and elongation properties are strongly influenced by the concentration of plasticizers used i.e. the concentration of glycerol.

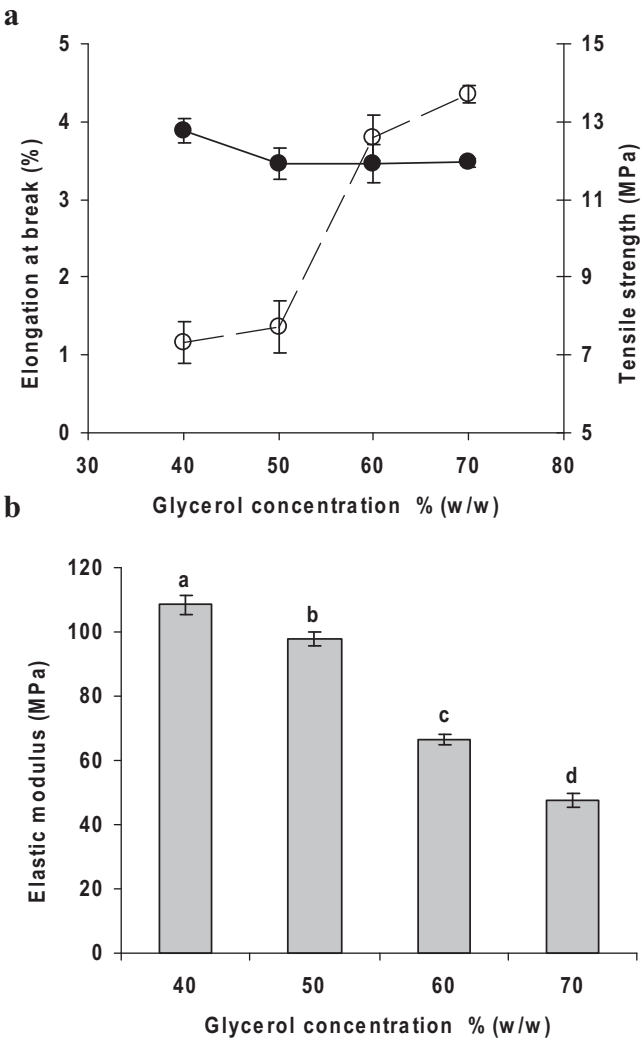

Fig. 1. Mechanical properties: (a) elongation at break (○), tensile strength (●) and (b) elastic modulus of glycerol plasticized LPSG films.

3.6. Thermal properties

DSC studies of LPSG films containing different concentrations of glycerol were performed to better understand the structure and interaction between polymers and plasticizer. The glass transition temperature (T_g) is the temperature at which the material undergoes a structural transition from the glassy state to a rubbery state (Yang & Paulson, 2000). Below T_g, films are rigid and brittle, whereas above it films become flexible and pliable. The variation of glass transition temperature (T_g) is another effective indicator of the compatibility of polymers. Results showed that glycerol was compatible with LPSG, and confirmed the effectiveness of plasticization since only one T_g was observed. If a polymer and the plasticizer were immiscible, the mixture would in fact exhibit two T_g values, corresponding to the two pure phases (Ghasemlou et al., 2011).

All T_g values were below −61.58 °C depending on the glycerol concentration of the film (Table 4). The low T_g values of LPSG films could be attributed to their inherent structural characteristics (high chain mobility) and to their relatively high hydrophilicity, which lets them absorb more water molecules than less hydrophilic

Table 4
Calorimetric values: T_m (melting point), ΔH_m (melting enthalpy) and T_g (glass transition temperature) for LPSG films.[a,b]

Glycerol conc. % (w/w)	T_g (°C)	T_m (°C)	ΔH_m (J/g)
40	−61.58 ± 1.62[a]	156.25 ± 1.35[a]	58
50	−66.69 ± 1.36[b]	143.47 ± 1.88[b]	57
60	−70.86 ± 1.97[c]	122.12 ± 1.19[c]	40
70	−73.00 ± 1.76[c]	103.00 ± 0.98[d]	31

[a] Means within each column with different letters are significantly different ($p < 0.05$).
[b] Data are means ± SD.

films. Ghasemlou et al. (2011) also concluded that plasticizers like glycerol reduce T_g values when incorporated into kefir exopolysaccharide films. According to their results, this was probably due to the ability of glycerol to penetrate between the polymer chains and therefore weaken the interaction between polysaccharides chains.

As a trend, the presence of glycerol lowered the T_g of the plasticized films (Table 4). The T_g decreased as glycerol content increased from 40 to 60% (w/w). The increase of glycerol concentration leads to an increase of the free volume and mobility of the polymer network, changing the physical structure of the LPSG film, which is in agreement with the decrease of the T_g values (Roos & Karel, 1991). T_g values were inversely associated with the moisture content of LPSG based films; in fact, also water acts as a plasticizer increasing the molecular mobility (lower T_g values) of the films. As previously mentioned glycerol changes the polymer network creating mobile regions with greater interchain distances, increasing the moisture content in the films.

Moreover, T_m and ΔH_m also decreased when the glycerol content increased (Table 4). Such a decrease in thermal stability was affected by the presence of glycerol, which reduced the interaction between polysaccharide, and thus stabilized the network structure; in other words, higher glycerol concentration required a lower enthalpy to disrupt inter-chain interactions. This result is similar to those obtained by Al-Hassan and Norziah (2012). They reported that the lower T_m and ΔH values of plasticized starch–gelatin edible films could be attributed to the reduction of intermolecular forces and increase in the mobility of polar polymer chains. Mali, Sakanaka, Yamashita, and Grossmann (2005) also reported that glycerol affected cassava starch films properties due to the hygroscopic character of glycerol that tends to provide additional water into the film matrix.

3.7. Microstructure

A microstructural study of the films gives information about how the film's components are arranged, which has an impact on film properties. SEM studies were performed for better understanding of water vapour transmission and mechanical properties. Fig. 2 shows cross sections and surface image of LPSG films prepared with different glycerol content. When glycerol was incorporated into the LPSG film formulation, the surface view suggested a uniform surface with no holes or fracture. Thus, glycerol offers good miscibility and compatibility when incorporated into LPSG. The cross section micrographs revealed that the sheets stacked in compact and dense layers, which showed glycerol incorporated uniformly in the matrix. So, we can postulate that, the use of a plasticizer like

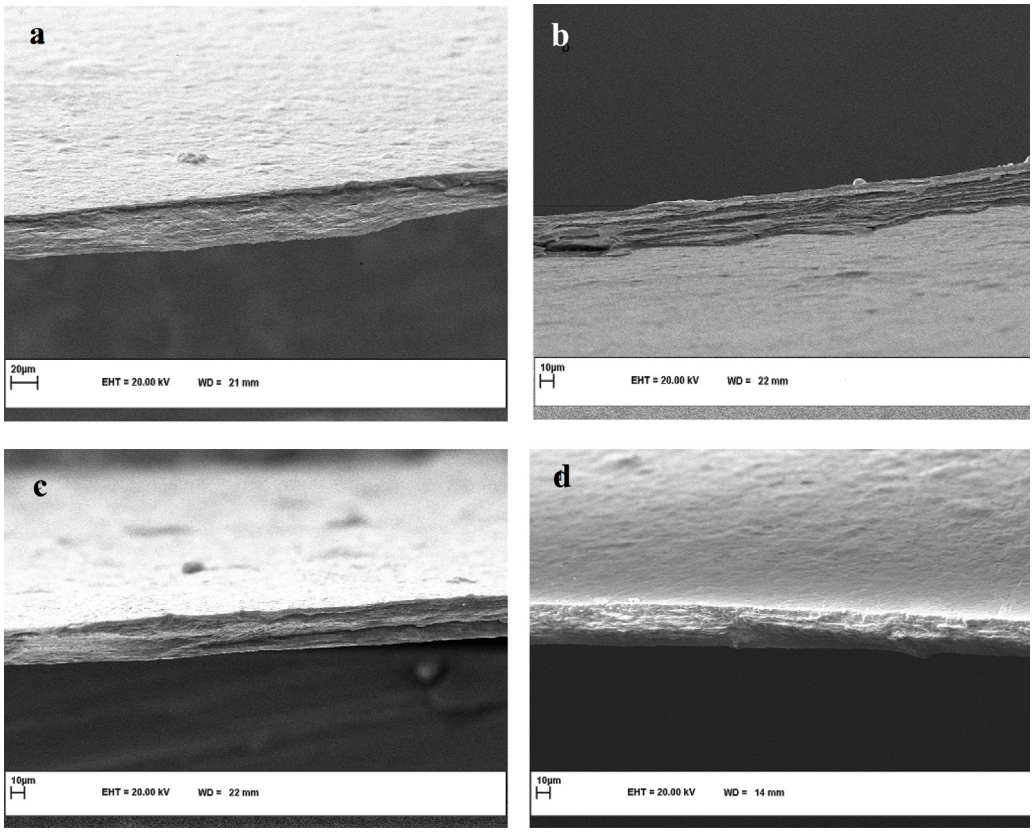

Fig. 2. Scanning electron micrograph of cross-section of LPSG films (magnification 1000) – a: 40%; b: 50%; c: 60%; d: 70% glycerol content.

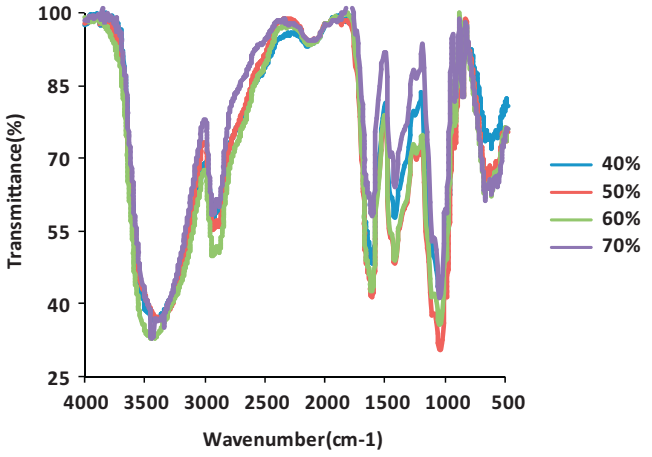

Fig. 3. FTIR spectra of LPSG films, with different glycerol content.

glycerol avoids cracking of LPSG films during handling and storage, but, on the other hand, increases water vapor permeability of the films. Similar effect has been previously observed for the surface of kefiran (Ghasemlou et al., 2011) and Cress seed gum (Jouki et al., 2013) films plasticized with glycerol.

3.8. FTIR

To confirm the cross-linking reaction, analysis of FTIR was carried out (Alves et al., 2011). When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectra peaks (Guan, Liu, Zhang, & Yao, 1998). Fig. 3 shows the FTIR spectra of the LPSG films containing 40, 50, 60 and 70% of glycerol. Comparing the spectra, it can be concluded that when the glycerol concentration increased, changes occurred in the characteristic peaks of LPSG based film. The broad band ranging between 3500 and 3100 cm^{-1} is assigned to O—H stretching vibration of hydroxyls and bound water (Synytsya, Copikova, Matejka, & Machovic, 2003). Meanwhile, the broad band around 2800–3000 cm^{-1} is attributed to C—H stretching vibration (Yuen, Choi, Phillips, & Ma, 2009). When the FTIR spectra containing different glycerol concentrations are compared, it can be seen that with increasing glycerol content, peaks at 3421.4 and 2924.62 cm^{-1} shifted slightly toward the higher wavenumber (Fig. 3). As can be seen from Fig. 3, increasing glycerol content up to 60%, exhibited partial differences in frequency range compared with the films with lower proportions of added glycerol, changing the peak from 2924.62 to 2934.61 cm^{-1}. These peaks also present some displacement, changing from 3421.4 to 3438.8 cm^{-1}. Therefore, the hydrogen associate functional group such as (—CH, —OH) is believed to be a more reliable indicator to clarify its relevant interaction of glycerol with LPSG. These results indicate that the addition of glycerol promoted the hydrogen bonding interactions among LPSG and glycerol.

Further, the peak at 1613 cm^{-1} depicts the stretching zone of C=O, whereas the band at 1416 cm^{-1} is due to the symmetrical —COO stretching vibration (Nakanishi, 1962). Increase in glycerol content leads to a shift of the peak 1613.13 cm^{-1} (presented in LPSG films with 40% glycerol), to peak bands of 1613.10, 1611.32 and 1609.17 cm^{-1} for LPSG films with 50, 60 and 70% of glycerol, respectively.

The peaks around 1200–800 cm^{-1} was due to the symmetric stretching vibrations of the alcoxyl group (C—O—C), resulting mainly from the glycerol presence (Jamróz et al., 2007). The results indicated that increasing glycerol concentration also increased the peak around 1038.67 cm^{-1}. Bergo and Sobral (2007) reported that the displacements were related to the possible interactions that arise between the plasticizer and the film structure.

Furthermore, a gradual increase in glycerol content led to an increase in intensity of the bands in that spectral region, for LPSG films. This means that the number of free hydroxyl groups of glycerol increased, thus becoming available to bind water molecules that may contribute to increase the moisture content of films formulated with more concentrated glycerol.

4. Conclusion

The results obtained in this study showed that LPSG could be used as a new film-forming material. Preliminary experiments showed that preparation of LPSG films without plasticizer addition is impossible. Therefore, blends of LPSG and glycerol were prepared by mixing solutions of LPSG (0.6%, w/v) and glycerol (40%, 50%, 60% and 70%, w/w). Glycerol had an essential role in making flexible films and also affected physical, barrier, mechanical and microstructure properties of LPSG films. The increase of glycerol concentration improved the mechanical and thermal properties of LPSG films. The authors believe that this new biopolymer is useful for industrial utilization possibly as a film-forming and binding agent. However, LPSG films were characterized by high WVP, moisture content, moisture adsorption and water solubility. Hence, further research is needed in attempts to improve these films, besides ascertaining the impact of using such films for packaging a wider variety of food products.

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