Vol. 19, No. 2 (2020) 983-996
Revista Mexicana de Ingeniería Química

Physico-mechanical, barrier and antimicrobial properties of linseed mucilage films incorporated with *H. virginiana* extract

Propiedades físico-mecánicas, de barreras y antimicrobianas de películas de mucilago de linaza incorporadas con extracto de *H. virginiana*

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Received: September 20, 2019; Accepted: November 14, 2019

Abstract

Increased interest in providing safe food with excellent quality and shelf-life has resulted in increased efforts toward developing new bio-based packaging materials. The objectives of this study were to develop and characterize films based on linseed mucilage (LM) at concentrations of 2.0%, 2.5%, and 3.0% and the further development of antimicrobial films (AFs) incorporating *Hamamelis virginiana* (Hv) extract. The films with the greatest LM concentration was selected as the best formulation based on its mechanical properties, water vapor permeability and moisture sensitivity. Moreover, the antimicrobial activities of Hv extract against foodborne pathogens were evaluated. Minimum inhibitory concentrations were 1.18 mg mL^{-1} for *L. monocytogenes* and 2.37 mg mL^{-1} for *S. Typhi, S. aureus*, and *E. coli*. Finally, AFs were developed by incorporating Hv extract at 2.37 mg mL^{-1} into a base of 3.0% LM, increasing elongation at break, antioxidant activity to 80.56%, moisture sensitivity, and antimicrobial activity (increasing inhibition zones to 19.50 – 22.50 mm). It also decreased tensile strength, maximum force, and water vapor permeability. These results suggest that AFs based on LM with Hv extract have sufficient properties for a potential packaging material.

Keywords: Mucilage, *Hamamelis virginiana*, antimicrobial activity, antimicrobial films, physico-mechanical properties.

Resumen

El creciente interés en proporcionar alimentos seguros con excelente calidad y vida útil ha resultado en un incremento en los esfuerzos hacia el desarrollo de nuevos materiales de empaque de base biológica. Los objetivos de este estudio fueron desarrollar y caracterizar películas a base de mucílago de linaza (LM) en concentraciones de 2.0%, 2.5% y 3.0% y adicionalmente desarrollar películas antimicrobianas (AFs) que incorporen un extracto de *Hamamelis virginiana* (Hv). La película con la mayor concentración de LM fue seleccionada como la mejor formulación en función de sus propiedades mecánicas, permeabilidad al vapor de agua y sensibilidad a la humedad. Por otra parte, se evaluó la actividad antimicrobiana del extracto de Hv contra patógenos transmitidos por los alimentos. Las concentraciones inhibitorias mínimas fueron 1.18 mg mL^{-1} para *L. monocytogenes* y 2.37 mg mL^{-1} para *S. Typhi, S. aureus* y *E. coli*. Finalmente, se desarrollaron películas incorporando el extracto de Hv en 2.37 mg mL^{-1} en una base de 3.0% de LM, aumentando la elongación, la actividad antioxidante a 80.56%, la sensibilidad a la humedad y la actividad antimicrobiana (incrementando las zonas de inhibición a 19.50 - 22.50 mm). Esto también disminuyó la resistencia a la tensión, la fuerza máxima y la permeabilidad al vapor de agua. Estos resultados sugieren que las AFs a base de LM con extracto de Hv tienen propiedades adecuadas para un material potencial de empaque.

Palabras clave: Mucílago, *Hamamelis virginiana*, actividad antimicrobiana, películas antimicrobianas, propiedades físico-mecánicas.

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https://doi.org/10.24275/rmiq/Bio872
issn-e: 2395-8472

1 Introduction

The use of films based on biodegradable materials has increased because of rising interest in reducing the use of petroleum-based synthetic materials currently widely used in the packaging industry and associated serious environmental problems (Bautista-Baños *et al*., 2018; Capitani *et al*., 2016; Hopkins *et al*., 2015; Sorrentino *et al*., 2007). On the other hand, the growing interest in providing safe foods of high quality and long shelf-life has increased efforts developing new bio-based packaging materials (Sorrentino *et al*., 2007; Oms-Oliu *et al*., 2010; Hernández-Carrillo *et al*., 2019).

Films are preformed thin matrices based on an edible material such as polysaccharides (e.g. starch, chitosan, alginate, carragenan, pectin, or mucilages), proteins (e.g. whey, triticale, hake, or fish proteins), lipids (e.g. carnauba, beeswax, or fatty acids, among others), or blends of these materials (Teixeira *et al*., 2014; Pires *et al*., 2013, Aguirre *et al*., 2013; Fakhouri *et al*., 2015; Sharma *et al*., 2017). They are applied to foodstuffs and function to protect them from microbiological, physicochemical, and mechanical damage (Fakhouri *et al*., 2015; Falguera *et al*., 2011). One of the most important features of these materials is their ability to carry active compounds such as flavorings, nutraceuticals, antibrownings, antioxidants, and antimicrobial agents, among others (Falguera *et al*., 2011; Rojas-Grau *et al*., 2009; Jouki *et al*., 2014).

Several studies have shown that the incorporation of natural compounds such as essential oils or plant extracts [e.g. oregano (Jouki *et al*., 2014; Seydim *et al*., 2006), cinnamon, clove (Veena *et al*., 2015), garlic (Teixeira *et al*., 2014), or thyme (Pires *et al*., 2013), among others] into active packaging could be a potential alternative for improving the quality and extending the shelf-life of foodstuffs. The antimicrobial activities of these compounds reduce the development of spoilage microorganisms [e.g. *Aspergillus niger* and *Penicillium digitatum* (Avila-Sosa *et al*., 2012)] and reduce the risk of contamination by foodborne pathogens [e.g. *S. aureus, L. monocytogenes, E. coli*, and *Salmonella* spp. (Pires *et al*., 2013; Sharma *et al*., 2017; Benavides *et al*., 2012; Morsy *et al*., 2014; Alboofetileh *et al*., 2014). Also, these compounds provide antioxidant activities because they are rich in phenolic compounds (Pires *et al*., 2013; Shojaee *et al*., 2014), among other properties. Recently, interest has focused on the development of films based on mucilages from nopal cactus (Espino-Díaz *et al*., 2010), quince seed (Jouki *et al*., 2014), and chia (Capitani *et al*., 2016; Dick *et al*., 2015). Jouki *et al*. (2014) developed antimicrobial films (AFs) based on quince seed mucilage and oregano essential oil; these films exhibit antioxidant (45.09% – 61.03%) and antimicrobial activities against pathogens such as *S. aureus, L. monocytogenes, Y. enterocolitica, S. putrefaciens, S. typhimurium*, and *P. aeruginosa*. In addition, the AFs presented acceptable mechanical properties (elastic modulus, tensile strength, and elongation at break) and permeability (for oxygen and water vapor), making them suitable for use as active packaging.

Linseed (*Linum usitatissimum*) mucilage (LM) is a heterogeneous polysaccharide consisting of rhamnogalacturonan I (the acidic fraction which has terminal residues of L-galactose attached at the O-3 position of the rhamnosyl residues) and arabinoxylan [the neutral fraction which presents double branches of terminal L-arabinosyl units at the O-2 and O-3 positions along the xylan backbone (Naran *et al*., 2008)]. This polymer has been used in various applications such as thickening, emulsifying, stabilizing, and recently as a film and edible coating (Wang *et al*., 2012; Li *et al*., 2011; Salamanca *et al*., 2011; Tee *et al*., 2016; Treviño-Garza *et al*., 2017). Reportedly, LM can potentially be an alternative packaging material and an edible coating for foods.

Hamamelis virginiana (Hv; witch hazel) is a deciduous shrub of the *Hamamelidaceae* family, native to North America. Herbal substances from this plant (the folium, leaf, and bark) have anti-inflammatory, antiviral, and antibacterial activities, among other properties (European Medicines Agency, EMEA, 2009; World Health Organization, WHO, 2004; Pereira *et al*., 2014). The main constituents of Hv are tannins (up to 10% and 12%; bark and leaf, respectively). Cortex tannins consist mainly of hamamelitannin (up to 65%) while folium tannins consist of a mixture of gallic acid (10%), hydrolysable hamamelitannin (1.5%) and condensed proanthocyanidins (88.5%) (EMEA, 2010; WHO, 2004). Brantner *et al*. (1994) found that an aqueous extract of the leaves of Hv provide antibacterial activity against *E. coli, S. aureus, B. subtilis*, and *E. faecalis*. On the other hand, Pereira *et al*. (2014) suggested that an alcoholic extract of Hv can be utilized as a potential inhibitory agent against multi-drug resistant strains of *S. aureus* and attributed such activity to the presence of hydrolyzable tannins

(hamamelitannins). Recent studies have reported antimicrobial activity of Hv extract against *S. aureus, P. aeruginosa*, and *C. albicans* (Solís-Arévalo *et al*., 2019). Moreover, Hv antiviral activity also has been reported against herpes simplex virus, human immunodeficiency virus (Laganà *et al*., 2019), influenza A, and human papillomavirus (Theisen *et al*., 2014). Interestingly, Hv fractions composed by gallotannins and condensed tannins (80% and 20%, respectively; 0.03 mg mL^{-1}) and fractions with low galloylation degree (0.15 - 0.25 gallate group / molecule; 0.1 mg mL^{-1}) have been used as functional ingredients for preventing lipid oxidation in muscle-based seafood (González *et al*., 2010; Pazos *et al*., 2010).

Linseed mucilage is considered to be a potential alternative for packaging material and as an edible coating for foods, and it can be used with extracts of Hv to develop an AFs. Therefore, the objectives of this study were: a) to develop and characterize films of LM based on their physical, mechanical, barrier, solubility, antioxidant, and antimicrobial properties; b) to select the film with the best properties for incorporating into AFs; and c) to develop and characterize these AFs based on LM incorporated with Hv extract.

2 Materials and methods

2.1 Edible film compounds

Linseed was obtained from a local market. Glycerol (99.5% purity) was purchased from Analytika®, and an ethanolic fluid extract of Hv (leaves 100%; 152 mg mL^{-1}, Lot 18067024) was acquired from Pacalli, S. de R.L. de C.V, Mexico.

2.2 Antimicrobial activity of H. virginiana extract

2.3 Preparation of inoculums

Listeria monocytogenes (ATCC 19114), *S. Typhi* (ATCC 19430), *S. aureus*, and *E. coli* (provided by Laboratorio de Bioquímica y Genética de Microorganismos LABGEM-FCB-UANL) were cultured on 10 mL brain heart infusion (BHI) broth (BD Bioxon®) for 16 h at 37 °C. After incubation, bacterial cells were harvested by centrifugation (2800 g for 10 min) and then diluted in BHI broth to achieve a concentration of 1 x 10^8 cells mL^{-1} for each microorganism (Iturriaga *et al*., 2012; Alegre *et al*., 2010).

2.3.1 Antimicrobial activity by the agar diffusion method

Antimicrobial activity assays were performed following literature methods (Alboofetileh *et al*., 2014; Sharma *et al*., 2017) with some modifications. On agar plates with BHI agar (20 mL), 0.1 mL of each inoculum was spread and allowed to embed. Wells 10 mm in diameter were made (3 per plate), and 0.1 mL Hv extract (152 mg mL^{-1}) was placed in each well. Plates were incubated for 24 h at 37 °C, and the inhibition zones were measured (n = 3).

2.3.2 Determination of minimum inhibitory concentration

Determinations of minimum inhibitory concentrations (MICs) were carried out according to literature methods (Kolarević, *et al*., 2016; Kim *et al*., 1995) with modifications. Brain heart infusion broth (150 μL) was placed in sterile 96-well plates, then 150 μL Hv extract was added to the first vertical row, and two-fold dilutions were performed in the horizontal rows to obtain concentrations ranging from 76.13 mg mL^{-1} to 0.14 mg mL^{-1}. Subsequently, 10 μL inoculum (1 x 10^8 cells mL^{-1} of each strain) were placed in each well, and the microplates were incubated for 24 h at 37 °C. Minimum inhibitory concentrations were determined by measuring the turbidity (bacterial growth) using a microplate reader (EZ Read 2000; Biochrom) at 625 nm (n = 3).

2.4 Extraction of mucilage

Linseed mucilage was extracted via ethanolic precipitation as described in the literature (Treviño-Garza *et al*., 2017). Briefly, linseeds were placed in distilled water (30 g in 100 mL) with constant stirring (250 rpm, 25 °C) for 2 h and then were removed using a strainer. Mucilage was precipitated by adding 200 mL ethanol (96%) to the aqueous suspension (100 mL) and solids were extracted by centrifugation (1500 g for 20 min). The obtained mucilage was dried at 70 °C for 24 h and pulverized.

Table 1. Composition (%) of the film forming solutions.

Formulation	LM	Glycerol	Hv
LM2.0	2	0.5	-
LM2.5	2.5	0.5	-
LM3.0	3	0.5	-
*LM3.0Hv	3	0.5	0.237

Note: LM, linseed mucilage; Hv, *H. virginiana* extract (0.237% = 2.37 mg mL^{-1}).

2.4.1 *Preparation of film-forming solutions*

Three film-forming solutions based on LM (2.0%, 2.5% and 3.0%) and glycerol (0.5%) were prepared (Table 1); concentrations were established based on previous experiments (data no shown). The compounds were dissolved in distilled water with constant stirring to obtain homogeneous solutions (550 rpm and 25 ± 2 °C for ~ 1 h).

2.4.2 *Elaboration of films*

Film-forming solutions (20 mL each) were poured onto acrylic plates (5 cm x 10 cm) and extended using a stainless-steel blade. They were dried at room temperature (25 ± 2 °C) for 24 h, and the films were subsequently recovered and stored (50 ± 2% relative humidity), prior to evaluations (Espino-Díaz *et al*., 2010; Jouki *et al*., 2014).

2.5 *Characterizations of films*

2.5.1 *Film thickness*

Thickness was measured using a micrometer (Model 293; Mitutoyo) on five parts of the film. An average thickness was calculated (Capitani *et al*., 2016; Jouki *et al*., 2014; Espino-Díaz *et al*., 2010). Determinations were conducted in triplicate.

2.5.2 *Opacity*

Opacity (n = 3) was determined as described in the literature (Teixeira *et al*., 2014; Fakhouri *et al*., 2015). Briefly, measurements (in triplicate) were carried out using a colorimeter (171 Colorflex® EZ; HunterLab). Opacity values were calculated: opacity (%) = (opacity of the film against a black background / opacity of the film against a white background) x 100.

2.5.3 *Water solubility*

Samples (n = 5), cut into 3 cm x 3 cm pieces and 50 mL distilled water were placed in a flask and slowly stirring until complete dissolution was reached. The solubilizing time of the films was determined (Fakhouri *et al*., 2015; Jouki *et al*., 2014).

2.5.4 *Moisture sensitivity*

Moisture content was determined by the mass loss. Films (3 cm x 3 cm; n = 5) were weighed and placed under controlled conditions of humidity and temperature (25 °C, 70% and 90% relative humidity) for 24 h. The samples were dried (70 °C for 24 h) and weighed to determine their final dry weights (Capitani *et al*., 2016; Fakhouri *et al*., 2015; Jouki *et al*., 2014). Moisture content was then determined according to the following formula: Moisture (%) = [(initial film weight) − (final film weight)] / (initial film weight) x 100.

2.5.5 *Water vapor permeability*

Water vapor permeability (WVP; n = 4) was determined using ASTM E96-95 with modifications (Fakhouri *et al*., 2015; ASTM, 1995). Samples were placed in 2 g calcium chloride in plastic capsules which were then sealed to ensure the transmission of water vapor only through the exposed area. The capsules were placed in a glass chamber containing a solution saturated with magnesium nitrate (90% relative humidity and 25 °C). The capsules were weighted at 24-h using an analytical balance (AB204; Mettler Toledo). The gain in mass of the calcium chloride is related to the amount of water that migrates through the film. The WVP was determined: WVP (g H_2O mm^{-2} h^{-1}) = [(final capsule weight − initial capsule weight) / (mm^2)]/ (h).

2.5.6 *Mechanical properties*

Tensile strength (TS), elongation at break (EB), maximum force (MF), and elastic modulus (EM) were determined using an ASTM standard method (American Society for Testing and Material; D882-91) with modifications (Capitani *et al*., 2016; Jouki *et al*., 2014; ASTM, 1996), using a Universal Machine (AGS-X; Shimadzu). Films (30 mm x 30 mm) were clamped in grips 35 mm apart which moved at a speed of 20 mm min^{-1}. All measurements were made in triplicate (n = 3).

2.5.7 *Free radical scavenging assay*

Antioxidant activity was determined as described in the literature (Teixeira *et al*., 2014; Jouki *et al*., 2014) with slight modifications. Briefly, films (1 cm x 1 cm; $n = 3$) were placed and dissolved in 1 mL of a 0.1 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich Co. (St. Louis, MO) for a period of 20 min in darkness at 25 °C. Subsequently, the samples were centrifuged (2800 g for 10 min), and 300 μL of the supernatant was placed in a 96-well plate. Absorbance was read in a microplate reader (EZ Read 2000) at 520 nm. Finally, percentage of DPPH radical scavenging activity was calculated. Ascorbic acid solution (1.0 mg mL^{-1}; radical scavenging activity= 85.59 ± 0.68%) and Hv extract (radical scavenging activity= 78.86 ± 0.24%) were used as positive controls. DPPH radical scavenging activity (%) = (absorbance of DPPH solution − absorbance of sample / absorbance of DPPH solution) x 100.

2.6 *Development of AFs*

Based on the properties of the film (higher TS, MF, EB and, lower permeability and moisture sensitivity), LM3.0 was selected to incorporate it into the AFs. The Hv extract concentration (2.37 mg mL^{-1}, corresponding to the MIC for the most pathogens evaluated) was added to the film-forming solution, resulting in LM3.0Hv (Table 1), and AFs were developed and analyzed as described above (sections 2.5 and 2.6). The results of characterization analysis of the AFs were included in figures and tables together with the films and marked with an asterisk.

2.7 *Antimicrobial activity assays of AFs*

The AFs produced from LM3.0Hv were cut into 10-mm diameter circles and placed in Petri dishes containing BHI agar (20 mL) with previously-inoculated bacterial suspensions (0.1 mL, 1 x 10^8 cells mL^{-1} *L. monocytogenes, S. aureus, S. Typhi*, and *E. coli*). The plates were then incubated at 37 °C for 24 h, and inhibition zones were measured in mm (Pires *et al*., 2013; Sharma *et al*., 2017; Matan *et al*., 2012). Controls were films without extract (LM3.0). Tests were conducted in triplicate.

2.8 *Statistical analyses*

All experimental data were subjected to analysis of variance. Differences between treatments were determined using the Tukey test ($P \leq 0.05$) via SPSS software version 19.0. For comparisons of efficacy between LM3.0 and LM3.0Hv films, experimental data were subjected to the Student *t* test.

3 Results and discussion

3.1 *Antimicrobial properties of H. virginiana extract*

The Hv extract showed antimicrobial activity against the pathogens evaluated, agreeing with previous reports (Pereira *et al*., 2014; Brantner *et al*., 1994). *L. monocytogenes* showed the greatest sensitivity ($P < 0.05$) with inhibition zones of 19.00 ± 1.00 mm. No significant differences ($P > 0.05$) were found among the inhibition zones of *S. aureus, E. coli*, and *S. Typhi*, whose values ranged from 9.66 to 13.66 mm (Table 2). The MIC of Hv extract was similar for *S. aureus, S. Typhi*, and *E. coli* (2.37 mg mL^{-1}). *L. monocytogenes* was the most sensitive bacterium, showing a MIC of 1.18 mg mL^{-1}. These values are greater than those reported by Brantner *et al*. [(1994); 0.4 mg mL^{-1} for *E. coli* and *S. aureus*].

Table 2. Antimicrobial properties of *H. virginiana* extract against foodborne pathogens.

	***Diameter of inhibition zone (mm)**	****MIC) (mg mL-1)**
L. monocytogenes	19.00 ± 1.00^b	1.18 ± 0.00^a
S. aureus	9.66 ± 0.57^a	2.37 ± 0.00^b
S. Typhi	13.66 ± 3.21^a	2.37 ± 0.00^b
E. coli	10.00 ± 1.00^a	2.37 ± 0.00^b

Note: Values are given as means ± standard deviations ($n = 3$). MIC, minimum inhibitory concentration. ^{a,b}Values within a column with a common superscript letter are not significantly different ($P \geq 0.05$).

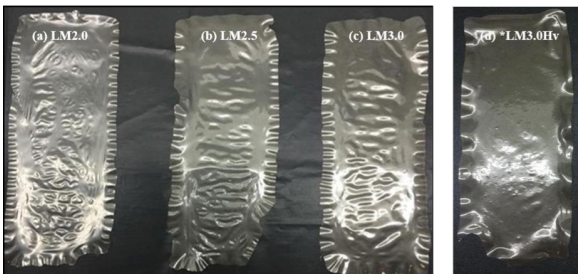

Fig. 1. Representative images of films based on linseed mucilage (LM) at concentrations of (a) 2.0%, (b) 2.5%, (c) 3.0%, and (d) 3.0% with *H. virginiana* (Hv) extract, forming an antimicrobial film.

The difference can be attributed to the changes in chemical composition, concentration, and/or the portion of the active compound used (such as hamamelitannin), which consequently depends on the part of the plant, season of harvesting, processing, storage, and other factors (Teixeira *et al*., 2014; Jouki *et al*., 2014; Alboofetileh *et al*., 2014).

3.2 Production of films

Extraction of LM yielded 6.0% ± 0.38%, similar to that reported by Fedeniuk *et al*. (1994), yet Fekri *et al*. (2008), obtained yields of 8.6%. The differences could be associated with seed variety and extraction method.

The films were produced successfully (Fig. 1.) as homogeneous, slightly opaque, thin, and flexible films obtained from LM (LM2.0, LM2.5, LM3.0 and LM3.0Hv). These characteristics are similar to those reported by other researches (Salamanca *et al*., 2011; Tee *et al*., 2016).

3.3 Characterization of films

3.3.1 Film thickness and opacity

Edible film thickness increased significantly ($P < 0.05$) as the mucilage concentration increased [Fig. 2a; (Capitani *et al*., 2016; Wang *et al*., 2011)]. The thinnest films were produced using LM2.0 (0.051 ± 0.009 mm). Films produced using LM2.5 and LM3.0 were 0.067 ± 0.003 mm and 0.090 ± 0.002 mm, respectively. Film thicknesses produced using LM2.0 are similar to those previously reported by Tee *et al*. (2016); however, those produced using LM2.5 and LM3.0 were greater as a result of the mucilage concentration. On the other hand, LM3.0Hv AFs were thicker than those produced using the precursor (LM3.0).

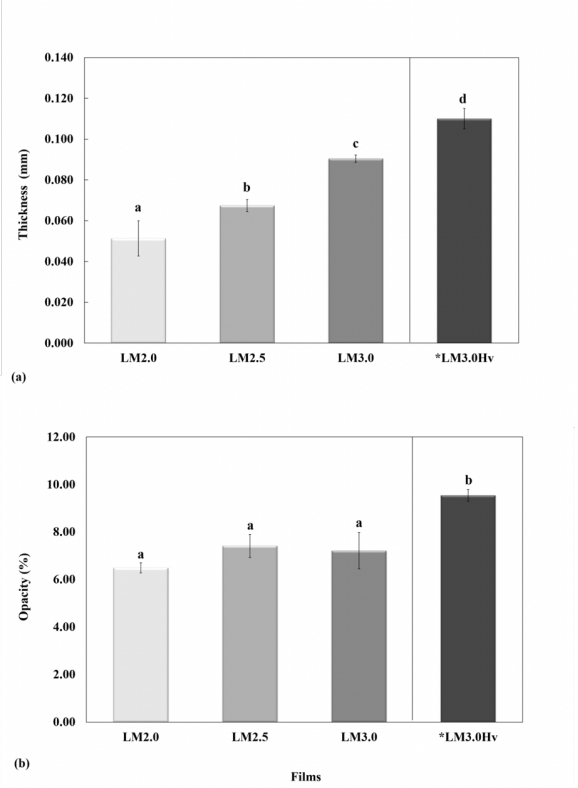

Fig. 2. (a) Thicknesses and (b) opacities of films based on linseed mucilage (LM) at various concentrations (2.0%, 2.5%, and 3.0%) and antimicrobial films produced from 3.0% linseed mucilage incorporated with *H. virginiana* (Hv) extract. Vertical bars represent standard deviations ($n = 3$).

The incorporation of essential oils such as oregano (Benavides *et al*., 2012), clove, cinnamon (Veena *et al*., 2015), and *Z. multiflora Boiss* (Shojaee-Aliabadi *et al*., 2014) increases film thickness. According to Benavides *et al*. (2012), the increase in AFs thickness can be associated with a higher solids content in the film-forming solution.

Opacities of films produced using LM2.0, LM2.5, and LM3.0 did not differ ($P > 0.05$) based on LM concentration (6.49% – 7.41%; Fig. 2b). On the contrary, at higher concentrations (up to 70%), Salamanca *et al*. (2015) reported increased films opacity. Moreover, the incorporation of Hv extract into the LM3.0 matrix increased this parameter from 7.21% to 9.54% ($P < 0.05$); this behavior can be attributed to the color of the Hv extract (greenish-brown) that reduces transparency, increasing the opacity of the AFs. Using κ-carrageenan AFs incorporated with *Z. multiflora Boiss* also exhibited

similar values (Shojaee-Aliabadi *et al*., 2014). Teixeira *et al*. (2014) reported much higher values (AFs based on fish proteins with clove, garlic, or origanum) as did Pires *et al*. [(2013); AFs based on hake protein with citronella, coriander, thyme, or tarragon]. The incorporation of essential oils or extracts from plants such as oregano, clove, tea tree, coriander, mastic, thyme, laurel, rosemary, sage (Fernández-Pan *et al*., 2012), cinnamon (Mahdi *et al*., 2010), or oregano (Jouki *et al*., 2014) changes the native color of the films, decreases transparency, and increases opacity.

3.3.2 Solubility, moisture sensitivity and WVP

All three films were 100% water soluble after 24 h, a characteristic also observed for LM3.0Hv AFs (data not shown). In general, our films were more soluble than those based on chia mucilage (Capitani *et al*., 2016; Dick *et al*., 2015). These higher levels of solubility can be attributed to the hydrophilic nature of the film components [a hetero-polysaccharide (branched mucilage chemical structure) and glycerol] stemming from the interaction of water molecules with polar groups in the polymeric matrix (Capitani *et al*., 2016; Dick *et al*., 2015; Salamanca *et al*., 2011).

Because of the hydrophilic nature of the LM films, they can absorb water when stored in high moisture conditions (Hopkins *et al*., 2015); this process leads to swelling, changes in the structure, and loss of integrity, resulting in a less dense structure where mobile regions with greater inter-chain distances and micro perforations form of the polymeric network (Dick *et al*., 2015; Salamanca *et al*., 2011). Regarding moisture sensitivity, although the LM films were completely soluble in water, they tolerated storage at high moisture concentrations. Moisture content ranged from 5.89% to 9.7% for LM films stored at 70% HR and was inversely related to LM concentration (Fig. 3a). Moreover, moisture content of LM films stored at 90% RH was higher and fluctuated between 9.80% and 10.69%, not significantly different ($P > 0.05$) from those given other treatments (Fig. 3b). On the other hand, in general, the moisture content of LM3.0Hv AFs was significantly higher ($P < 0.05$; 10.76 - 11.18% at 70, and 90% RH, respectively) compared with its precursor LM3.0. These findings agree with data reported by Pires *et al*. (2013) and Jouki *et al*. (2014). Essentials oils were found to increase the water solubility of films. According to Aguirre *et al*. (2013), the solubility and moisture sensitivity of the films are directly related to its structural properties and the presence of phenolic compounds.

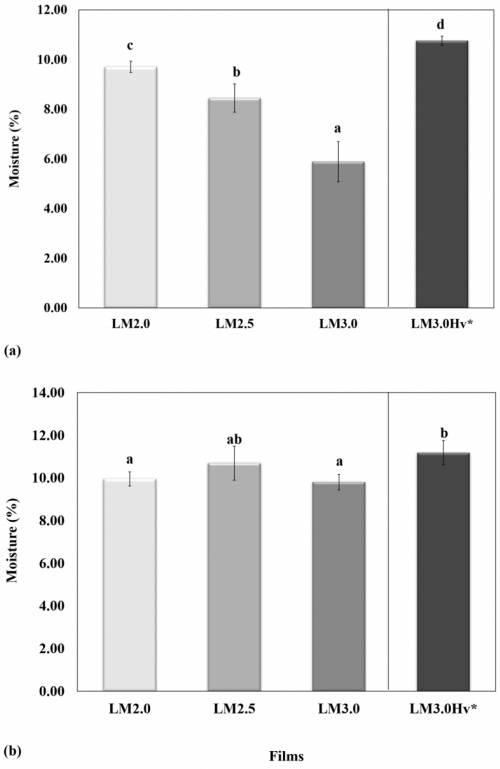

Fig. 3. Moisture content of films based on linseed mucilage (LM) at various concentrations (2.0%, 2.5%, and 3.0%) and antimicrobial films produced from 3.0% linseed mucilage incorporated with *H. virginiana* (Hv) extract and subsequently stored for 24 hours at (a) 25 °C and 70% relative humidity and (b) 25 °C and 90% relative humidity. Vertical bars represent standard deviations ($n = 5$).

The increase in these parameters could be the result of the Hv extract reducing the interaction capacity of the polymer matrix due to its phenolic nature (presence of −OH groups).

An important parameter to evaluate in films is the WVP because it is related to food spoilage (Salamanca *et al*., 2011). As shown in Fig. 4, WVP values ranged between 1.62 x 10-5 and 1.90 x 10-5 g mm^{-2} h^{-1} ($P > 0.05$) for films produced using LM2.0, LM2.5, and LM3.0. Differences in WVP values among the various LM films could be related to the concentration of mucilage in the film forming solution (a higher concentration would render greater water retention and less migration through the polymeric network) and to film thickness.

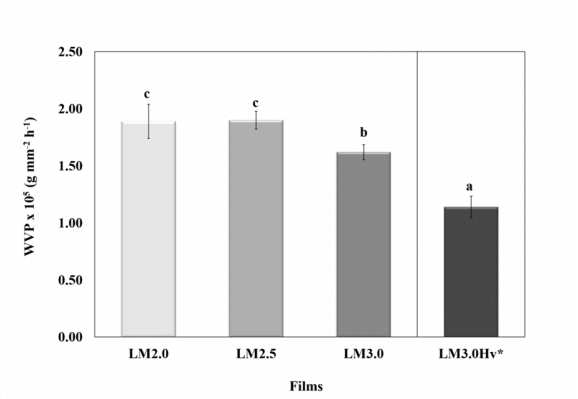

Fig. 4. Water vapor permeability of films based on linseed mucilage (LM) at various concentrations (2.0%, 2.5%, 3.0%) and antimicrobial films produced from 3.0% linseed mucilage incorporated with *H. virginiana* (Hv) extract. Vertical bars represent standard deviations (n = 4).

A thicker film can further reduce water mobility through the polymeric network of the film (Hopkins *et al*., 2015). In addition, according to Salamanca *et al*. (2011), WVP can be associated to the sorption of water by the polymer matrix (mucilage), resulting in swelling, changes in conformation, and formation of micro cavities that allow greater mobility of the polymeric chain, hence increasing film permeability. In general, the addition of Hv extract significantly decreased ($P < 0.05$) the WVPs of AFs, which produced lower values than their precursor films (1.14×10^{-5} g mm^{-2} h^{-1}; Fig. 4). Similar effects were reported by other researches (Teixeira *et al*., 2014; Pires *et al*., 2013; Benavides *et al*., 2012; Shojaee-Aliabadi *et al*., 2014; Ojagh *et al*., 2010) who found that essential oils, extracts, and other compounds can affect the hydrophilic-hydrophobic ratios within AFs, in turn affecting water vapor transfer processes. Also, the low WVP values found for LM3.0Hv relative to its precursor film can be attributed to its greater thickness (0.110 mm vs. 0.090 mm; Fig. 2). In contrast, Jouki *et al*. (2014) reported that WVP was not affected when quince seed mucilage films were incorporated with oregano oil.

3.3.3 Mechanical properties

Mechanical properties are important parameters for determining and predicting characteristics, behaviors, and usage fields of packaging materials (Shojaee-Aliabadi *et al*., 2014). Mechanical properties were significantly affected ($P < 0.05$) by LM concentration. All measured mechanical properties (TS, MF, and EB) increased significantly ($P < 0.05$) with mucilage concentration. Without additives, TS ranged between 13.61 and 17.30 MPa, MF between 37.60 and 73.60 N, and EB between 1.62 and 2.76%, each increasing with increasing mucilage concentration (Table 3). According to Li *et al*. (2009), mechanical properties such as TS improved when flaxseed mucilage was increased in starch-based films. Our values for TS for all the LM films were greater than those reported by Tee *et al*. (2016), who studied films produced from flaxseed mucilage and glycerol (1 to 5%). Nevertheless, LM2.0 produced films with TSs similar to those reported for 0% glycerol. In addition, LM films produced TS values that were similar to those reported for chia mucilage films (Capitani *et al*., 2016) and greater than those reported for nopal mucilage films (Espino-Díaz *et al*., 2010). Furthermore, our values for MF were greater than those reported for films produced using soy protein isolate/flaxseed oil (Hopkins *et al*., 2015). In addition, our values for EB (1.62% − 2.76%) were lower than those reported by Tee *et al*. (2016). Differences can be attributed to the added glycerol. This plasticizer reduces the inter-chain interactions of the mucilage by increasing their mobility, providing greater flexibility in the films (Tee *et al*., 2016).

Table 3. Mechanical properties of EFs based on linseed mucilage.

	TS (MPa)	MF (N)	EB (%)	EM (MPa)
LM2.0	13.61 ± 2.03^{a}	37.60 ± 1.93^{a}	1.62 ± 0.27^{a}	506.92 ± 43.72^{b}
LM2.5	16.59 ± 0.30^{ab}	54.60 ± 2.77^{b}	2.01 ± 0.05^{a}	636.96 ± 66.39^{cd}
LM3.0	17.30 ± 0.90^{b}	73.60 ± 0.19^{c}	2.76 ± 0.09^{a}	610.01 ± 25.20^{c}
***LM3.0Hv**	7.18 ± 0.86^{a}	37.92 ± 3.39^{a}	4.14 ± 0.75^{b}	126.11 ± 10.04^{a}

Note: Values are given as means ± standard deviations (n = 3). EFs, edible films; LM, linseed mucilage; TS, tensile strength; MF, maximum force; EB, elongation at break; EM, elasticity modulus. ^{a,b}Values within a column with a common superscript letter are not significantly different (P ± 0.05).*Antimicrobial EFs incorporated with *H. virginiana* extract.

Finally, the values for EM were 506.92 MPa, 610.01 MPa, and 636.96 MPa for LM2.0, LM2.5, and LM3.0, respectively. The latter two did not differ ($P > 0.05$; Table 3). In general, all LM films exhibited higher values than those reported by Jouki *et al*. (2014) [quince mucilage films] and Tee *et al*. (2016). High concentrations of glycerol increased films elasticity, but simultaneously decreased TS and EM (Wang *et al*., 2011; Tee *et al*., 2016).

Addition of Hv extract to films significantly ($P < 0.05$) affected their mechanical properties (Table 3). Both TS and MF were reduced when LM3.0Hv was used rather than LM3.0 (from 17.30 MPa to 7.18 MPa and from 73.60 N to 37.92 N, respectively). In addition, EM decreased ($P < 0.05$) from 610.01 MPa to 126.11 MPa. On the other hand, EB values increased significantly (2.76% to 4.14%, $P < 0.05$) with the addition of Hv extract. These results agree with those of Benavides *et al*. (2012), Shojaee-Aliabadi *et al*. (2014), and Jouki *et al*. (2014). They found that the essential oils *Z. multiflora Boiss, M. pulegium* (Shojaee-Aliabadi *et al*., 2014), and oregano (Jouki *et al*., 2014; Benavides *et al*., 2012) decreased both TS and EM but increase EB when AFs were based on κ-carrageenan, alginate, and quince seed mucilage, respectively. The incorporation of essential oils provides a plasticizing effect that changes the interaction forces in the polymeric matrix, facilitating the mobility of the chains (Shojaee-Aliabadi *et al*., 2014; López-Hernández *et al*., 2018). According to Arcan *et al*., (2011), the positive effect of the most plasticizers on the flexibility of the films can be attributed to their hydroxyl groups (OH), which form H-bonding with the polymers; this contributes to an increase in the mobility and elongation of the polymers chains in the film matrix. The main constituents of the Hv (phenolic compounds such as gallic acid, hamamelitannin, and condensed proanthocyanidins) possess a higher number of hydroxyl groups in their chemical structure. Therefore, the interaction of these hydroxyl groups with the LM increases the free volume of the film matrix and EB, reducing TS and EM of the AFs (Arcan *et al*., 2011).

Compared with values reported by Jouki *et al*. (2014), our LM3.0Hv AFs produced lower TS and EB values; however, they produced greater EM values. Differences can be attributed to film processing methods, film thickness, film composition (type of polymer, plasticizer, and active agents, among others), and interactions between the polymeric matrix and the essential oil (Teixeira *et al*., 2014; Pires *et al*., 2013; Benavides *et al*., 2012; Ojagh *et al*., 2010). Interestingly, the LM3.0Hv AFs produced TS and EB values similar to those reported by Benavides *et al*. (2012).

3.3.4 *Free radical scavenging assay*

No significant differences ($P > 0.05$) in antioxidant activity were found between the three films (4.46% – 4.65%; Fig. 5). This slight activity might be associated with the content of natural phenolic compounds present in linseed mucilage (20.22 ± 0.26 mg gallic acid g^{-1}, data no shown) such as lignans, phenolic acids, flavonoids, phenylpropanoids, and tannins (Kasote *et al*., 2013; Bouaziz *et al*., 2016). In contrast, a significant difference ($P < 0.05$) was found between the antioxidant activity of LM3.0Hv and its precursor film (80.56% ± 1.69% vs. 4.65% ± 0.32%). This increase can be attributed to the phenolic components of Hv extract (122.84 mg gallic acid g^{-1} sample, data no shown) such as tannins (e.g. gallotannins), flavonoids (e.g. quercetin, quercitrin and kaempherol), phenolic acids (e.g. gallic and caffeic acids), and catechins (e.g. gallocatechin, epicatechin and epigallocatechin gallate), among others (EMEA, 2009), which provide antioxidant activity to the films. Compared with other AFs, our LM3.0Hv AFs showed higher antioxidant activities (80.56 ± 1.69%) than those developed by other researches (DPPH radical scavenging activities with values of 45.09 – 72.00% according to Jouki *et al*., 2014; Pires *et al*., 2013 and Teixeira *et al*., 2014).

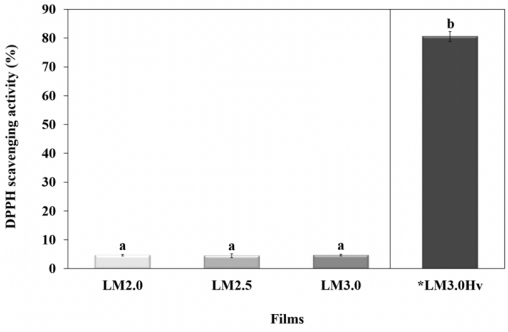

Fig. 5. (a) Antioxidant activities of films based on linseed mucilage (LM) at various concentrations (2.00%, 2.5%, and 3.0%) and antimicrobial films produced from 3.0% linseed mucilage incorporated with *H. virginiana* (Hv) extract ($n = 3$). DPPH radical scavenging activity of Hv extract = 78.86 ± 0.68%.

However, they produced activities similar to those produced by Shojaee-Aliabadi *et al*., (2014). In general, the antioxidant properties of AFs are associated with the content and composition of the various bioactive agents in the extract or essential oil (Pires *et al*., 2013; Jouki *et al*., 2014; Oussalah *et al*., 2004; Ruíz-Navajas *et al*., 2013).

3.4 Antimicrobial effectiveness of AFs

Our LM3.0 films did not show antimicrobial activities against the pathogens evaluated (data no shown). However, by incorporating Hv extract into the polymer matrix, the films LM3.0Hv were provided with antimicrobial activities that were similar ($P > 0.05$) for each of the four pathogenic microorganisms evaluated (diameters between 19.50 and 22.50 mm; Figs. 6).

Compared to the antimicrobial activities of AFs developed from quince seed mucilage/oregano (Jouki *et al*., 2014) or chitosan/cinnamon essential oil (Ojagh *et al*., 2010), our LM3.0Hv AFs showed lower antimicrobial activities. Nonetheless, they showed greater activities compared to AFs based on pectin/cinnamon or on whey protein/cinnamon (Sharma *et al*., 2017). Overall, the effectiveness of these active films depends on the bacterial isolate, extract or essential oil (chemical composition), action mechanisms (e.g. synergistic interactions) of the various active agents, and it is also related to the interactions between these agents and the various polymeric matrices (Pires *et al*., 2013; Jouki *et al*., 2014; Oussalah *et al*., 2004; Ruíz-Navajas *et al*., 2013).

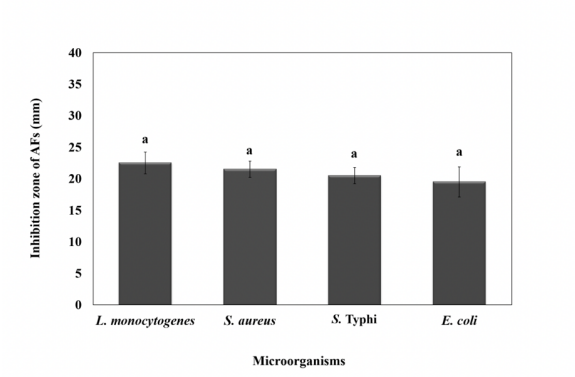

Fig. 6. Antimicrobial activities of antimicrobial films (LM3.0Hv) against foodborne pathogens (n = 4). Vertical bars represent standard deviations.

Conclusions

Homogeneous, slightly opaque and flexible films were produced from LM2.0, LM2.5, and LM3.0. The mucilage concentration significantly affected the WVP, water solubility, and physico-mechanical properties of LM films with the exception of opacity. Of the three, LM3.0 provided a film with superior TS, MF, EB, thickness, moisture resistance, and WVP properties. Therefore, it was selected for developing the AFs. The Hv extract added antimicrobial activity against foodborne pathogens; *L. monocytogenes* was the most sensitive strain (MIC = 1.18 mg mL^{-1}) relative to *S. Typhi, S. aureus*, and *E. coli* (MIC = 2.37 mg mL^{-1}). The incorporation of Hv extract into LM3.0 to produce LM3.0Hv resulted in modified films properties. It increased certain parameters, such as EB, thickness, opacity, antioxidant activity, water solubility, and antimicrobial activity. Nevertheless, it decreased certain other parameters, such as TS, MF, EM, and WVP. Finally, our findings showed that AFs based on LM with Hv extract could be a good alternative as antimicrobial packaging material for high oxidative and microbiological-sensitivity foods. Nonetheless, it is necessary to continue with this research in order to improve some properties of the films such as moisture sensitivity and solubility, since these conditions could limit its use in a wide variety of foods.

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