Vol. 16, No. 1 (2017) 109-119



Revista Mexicana de Ingeniería Química

## PHYSICAL, BARRIER, MECHANICAL AND MICROSTRUCTURAL PROPERTIES OF Aloe vera-GELATIN-GLYCEROL EDIBLE FILMS INCORPORATED WITH Pimenta dioica L. MERRILL ESSENTIAL OIL

## PROPIEDADES FÍSICAS, DE BARRERA, MECÁNICAS Y MICROESTRUCTURALES DE PELÍCULAS COMESTIBLES A BASE DE GEL DE Aloe vera, GRENETINA Y GLICEROL INCORPORADOS CON ACEITE ESENCIAL DE Pimenta dioica L. MERRILL

N.L. Flores-Martínez<sup>1</sup>, A. Valdez-Fragoso<sup>2</sup>, H. Jiménez-Islas<sup>1</sup>, M.C. Pérez-Pérez<sup>1\*</sup>

<sup>1</sup> Posgrado en Ingeniería Bioquímica, Departamento de Ingeniería Bioquímica, Instituto Tecnológico de Celaya, Avenida Tecnológico y Antonio García Cubas S/N, Celaya, Guanajuato, 38010, México.

<sup>2</sup>Escuela de Ciencias e Ingeniería, Instituto Tecnológico y de Estudios Superiores de Monterrey, Av. Eugenio Garza Sada 2501

Sur, Monterrey, Nuevo León, 64849, México.

Received December 1, 2016; Accepted December 23, 2016

#### Abstract

Physical, barrier, mechanical and microstructural properties of an edible film based on *Aloe vera*, gelatin-glycerol with different concentrations of the essential oil of a pepper (*Pimenta dioica* L. Merrill) at 0, 0.5, 1.0, and 1.5% w / w were investigated. The optical properties showed low brightness and  $\Delta E$  of 59.6. In terms of water vapor permeability, the values ranged from  $2.54 \times 10^{-9} \pm 0.09$  to  $4.49 \times 10^{-9} \pm 0.3$  (g m/Pa s m<sup>2</sup>), due to the effect of decreased polarity in the structural matrix. In the case of the mechanical properties, an increase in the oil concentration significantly reduced the tensile strength, making the material more flexible (% E = 72 to 122), possibly due to the rearrangement of and the interaction between polysaccharides, proteins and plasticizers in the matrix. This behavior was also observed in the microstructural studies: the increased concentration of oil increased the roughness of the film (Ra = 0.222 to 2.12 mV). The results show that addition of *Pimenta dioica* oil to the film results in a film with increased WVP and resistant film qualities that favor the material for use in high-moisture food coating applications.

*Keywords*: edible films, *Aloe vera*, *Pimenta dioica* L. Merrill, physical and mechanical properties, microscopy techniques, optical properties.

#### Resumen

Se midieron las propiedades físicas, de barrera, mecánicas y microestructurales de una película comestible a base de gel de *Aloe vera*, grenetina y glicerol con diferentes concentraciones de aceite esencial de *Pimenta dioica* L. Merrill a 0, 0,5, 1,0 y 1,5% p/p. Las propiedades ópticas mostraron un brillo bajo y un  $\Delta E$  de 59.6. En términos de permeabilidad al vapor de agua, los valores oscilaron entre 2.54 ×10<sup>-9</sup> ± 0.09 y 4.49 ×10<sup>-9</sup> ± 0.3 (g m/Pa s m<sup>2</sup>), debido al efecto de la disminución de la polaridad en la matriz estructural. En el caso de las propiedades mecánicas, un aumento de la concentración de aceite redujo significativamente la resistencia a la tensión, haciendo que el material sea más flexible (% E = 72 a 122), posiblemente debido al reordenamiento y la interacción entre polisacáridos, proteínas y plastificantes en la matriz. El aumento de la concentración de aceite esencial de *Pimenta dioica* a la película (Ra = 0.222 a 2.12 mV). Los resultados muestran que la adición de aceite esencial de *Pimenta dioica* a la película, le confiere a ésta un aumento en la PVA, resistencia y flexibilidad, propiedades que favorecen su uso como recubrimientos comestibles.

Palabras clave: películas comestibles, Aloe vera, Pimenta dioica L. Merrill, propiedades físicas y mecánicas, técnicas de microscopía, propiedades ópticas.

<sup>\*</sup> Corresponding author. E-mail: cristina.perez@itcelaya.edu.mx

# **1** Introduction

Edible and biodegradable natural-polymer films offer alternatives to conventional packaging due to their excellent biodegradability, biocompatibility, edibility, and the range of their potential applications (Salmieri and Lacroix, 2006; Hernández-Carrillo et al., 2015; Rodriguez-Marín et al., 2016). They are applied as barriers to preserve and delay food deterioration (Sotelo-Boyás et al., 2015). Recently, researchers have developed edible films that enhance food properties such as color, texture, flavor and overall appearance (Abugoch et al., 2011; Bergo et al., 2010). Additionally, these films may operate as carriers for a range of functional ingredients, expanding their capabilities into active packaging, such as Aloe vera, gelatin, and the essential oil of Pimenta dioica L. Merril. Aloe vera gel films exhibit high water permeability; therefore, mixtures with diverse compounds (cellulose, gelatin, etc.) have been studied in order to improve water vapor and gas diffusion in the edible films (Saibuatong and Phisalaphong, 2010; Ramirez-Hernández et al., 2015).

Aloe vera is used as an antioxidant, antiinflammatory, digestion stimulant, activator of the immune system and an agent for healing. Aloe vera leaves contain a slimy tissue that stores water, which makes the leaves thick. This slimy, water-filled tissue has diverse names including pulp, mucilaginous tissue, mucilaginous gel, and parenchyma tissue. It is composed of degenerated cell organelles and a viscous liquid contained in the cells (Li et al., 2003). The mucilaginous gel contains water at a ratio of 99-99.5% and 0.56-0.66% polysaccharides and soluble sugars, followed by proteins (many of which are enzymes), amino acids, vitamins and anthraquinones (Boudreau and Beland, 2006; Liu et al., 2007). This gel has many applications as an ingredient in functional foods due to the biological activity of its components (Vega et al., 2005). The use of Aloe vera as an edible coating has been used in preharvest or postharvest treatments to maintain fruit quality attributes and to delay fungal decomposition in sweet cherries (Martínez et al., 2006), table grapes (Valverde et al., 2005; Serrano et al., 2006; Castillo et al., 2010), and nectarines (Navarro et al., 2011), with fairly satisfactory results with respect to the conservation of the sensory characteristics, control of respiration activity and moisture losses, and reduction of enzymatic browning and microbial growth. The Aloe vera coating maintained the firmness of kiwifruit slices (Benítez *et al.*, 2013), and microorganism growth was reduced by 50% in coated blueberries after 25 days of storage (Vieira *et al.*, 2016).

The addition of essential oils to edible films favors slower oxidation in foods and prevents microbial propagation. Some examples are edible films based on alginate and the essential oil of oregano (Benavides et al., 2012). Sotelo-Bóyas et al. (2015) report that chitosan nanoparticles added with thyme essential oil presented a significant inhibitory effect on the growth of Pectobacterium carotovorum. Romero-Bastida et al. (2011) used 1.5% cinnamon oil in banana films that presented an inhibitory effect on Staphylococcus aureus. Edible films rich in phenolic compounds formulated from orange and grapefruit peels (Hernández-Carrillo et al., 2015), and edible films based on fish protein and the essential oil of cloves (Teixeira et al., 2014); both studies reported microbial inhibition in gram-positive bacteria.

The selection criteria for edible films are often based on their barrier and mechanical properties, especially water vapor permeability (WVP) and elastic modulus. Due to the flexibility, antioxidant and antimicrobial properties enhanced with the incorporation of essential oils to edible films, the aim of this work was to evaluate the functionality of *Aloe vera*-gelatin-glycerol films with the incorporation of different concentrations of *Pimenta dioica* L. Merrill essential oil.

# 2 Materials and methods

# 2.1 Materials

Aloe vera gel was obtained from fresh Aloe vera leaves in the laboratory of Physicochemical and Alternative Packaging Materials (Instituto Tecnológico de Celaya, México) by scraping the outer epidermis. Gelatin Knox<sup>TM</sup> was provided by Con Alimentos S.A de C.V (Mexico City, Mexico), Glycerol ACS (catalog number 06441) was supplied by Productos Químicos de Monterrey, S.A de C.V. (Monterrey, N.L., Mexico), polyoxyethylene sorbitan monooleate (Tween 80) was provided by Hycel de México, S.A de C.V (Zapopan, Jal., Mexico). The extraction of essential oil was performed by hydrodistillation of Pimenta dioica L. Merrill (Locally known as "pimienta gorda" or "pimienta de Jamaica") harvested in Tacuapan, region located in the northern part of the state of Puebla, Mexico (Geographical coordinates 20°1'3"N - 97°31′25"W).



Fig. 1. Schematic representation of the cells and system used to determine the water vapor permeability.

#### 2.2 *Methods*

#### 2.2.1 Film preparation

Aloe vera mucilage was milled by adding 0.1% w/w of polyoxyethylene sorbitan monooleate, weighed and centrifuged at 2880 ×g for 40 minutes. The supernatant was separated, filtered, and placed in a circulator bath at  $40 \pm 2$  °C, and 2 % w/w gelatin and 0.4 % w/w glycerol were slowly added, with stirring over 20 minutes. Later, the essential oil was added and homogenized at 304.2 ×g for 3 minutes using a T25 digital Ultra Turrax disperser (IKA Works, Inc., Wilmington, USA). The concentrations of essential oil tested were 0.5, 1.0, and 1.5 % w/w. Finally, 8 mL of the filmogenic suspension was distributed in petri dishes (9 cm in diameter) and allowed to dry at room temperature for 48 h.

#### 2.2.2 Thickness

The film thickness (mm) was measured using a handheld digital micrometer (F1000/30-3, Käfer Messuhrenfabrik GmbH & Co., Villingen-Schwenningen, Germany) with a resolution of 0.001 mm. Measurements were performed at ten different film locations and the mean thickness value was used to calculate the permeability and mechanical properties of the films.

#### 2.2.3 Color properties

Color values of the films were determined using a colorimeter (CR-400, Minolta, Konica Ramsey, NJ, USA). The color scale was used for measuring color values: L\*, the lightness variable; a\*, from green to blue; and b\*, from yellow to red. The total color difference ( $\Delta E$ ) was calculated using Eq. (1):

$$\Delta E = \left(\sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}\right) \tag{1}$$

where  $\Delta L = L^*_{standard} - L^*_{sample}$ ,  $\Delta a = a^*_{standard} - a^*_{sample}$ and  $\Delta b = b^*_{standard} - b^*_{sample}$ . The standard plate (calibration plate  $L^* = 94.90 a^* = -0.45 b^* = 3.93$ ) was used as a standard. Five measurements were collected on each film, one at the center and four around the perimeter.

#### 2.2.4 Water vapor permeability

The water vapor permeability (WVP) of the films was determined gravimetrically, using a modified ASTM E00996-00 (ASTM, 2000a) procedure. The cup had an internal diameter of 3.5 cm and an external diameter of 4 cm and was 5 cm deep. The cup was filled with 27 mL of distilled water to generate a 100% relative humidity and was covered by the film. The cup was placed in a desiccator containing silica gel to provide 10-12% RH. Changes in the weight of the cup were measured to the nearest 0.018 mg. The tests were conducted in triplicate, and the water vapor permeability was calculated using Eq. (2):

$$WVP = \frac{\Delta m \cdot x}{A \cdot t \cdot \Delta P}; \left(\frac{g \cdot m}{Pa \cdot s \cdot m^2}\right)$$
(2)

where  $\Delta m$  = mass change over time (g); x = thickness (m); t = time (s); A = film area (m<sup>2</sup>) and  $\Delta P$  = partial vapor pressure difference of the atmosphere with silica gel and pure water (3167 Pa at 25 °C), as shown in Figure 1.

#### 2.2.5 Mechanical properties

All mechanical properties of the films were determined by a texture analyzer (TA-XT plus, Stable Micro Systems LTD, Surrey, UK), using a modified ASTM D882-00 (ASTM, 2000b; Rodriguez-Marín *et al.*, 2016) procedure. The films were cut into 1 x 8 cm strips for testing. The measurements were performed using a crosshead velocity of 0.5 mm s<sup>-1</sup> with an exposed area 1 x 6 cm for each film strip. Four strips were prepared from each film. Tensile stress was plotted versus elongation to give a stress-strain curve and Young's modulus is the ratio between the strength of a film and its deformation before it breaks.

At higher values of E, the film will be stiffer and more fragile (Gordon, 2013). The final tensile strength (TS, in MPa), elongation at break (%E) and elastic modulus (EM, in MPa) of the films were also reported.

# 2.2.6 Environmental scanning electronic microscopy (ESEM)

The microstructure of the films was observed using a scanning electron microscope (Philips XL-30, Andover, MA, USA) to visualize the overall morphology of the film surfaces. Films of 4 x 4 mm were fixed on the sample holder with double sided carbon tape, without a metallic conductive cover, and observed using the ESEM system. Micrographs were captured at 100x magnification in gray-scale using a voltage of 25 kV and stored in TIFF format. The cross-section of films was observed using a scanning electron microscope (JSM-6510LV, JEOL Ltd., Tokyo, Japan); the film samples were fractured, then mounted on the specimen holder with double sided adhesive carbon tape. After gold coating using a Denton Vacuum Desk V (HP) sputter coater (Denton Vacuum LLC, Moorestown, NJ, USA), the image was captured using an accelerating voltage of 30 kV.

#### 2.2.7 Atomic force microscopy (AFM)

AFM allows the analysis of the surface topography at nanometer levels and makes possible the creation of 3-D models showing the microstructure of the sample at minor scale. AFM (diMultimode V, Veeco, Santa Barbara, CA, USA) was used in the tapping mode with silicon probes (RTESP Bruker cantilevers) with a resonance frequency of 256 x 256 pixels. The roughness was calculated using the root square deviation of the heights (Rq, Eq. 4), and the arithmetic average of the absolute values of the height deviations (Ra, Eq. 5) of the edible film images using the software NanoScope Analysis 1.20 (Veeco, Santa Barbara, CA, USA).

$$Rq = \sqrt{\frac{\Sigma(Zi)^2}{N}}$$
(3)

$$Ra = \frac{1}{N} \sum_{i=1}^{N} |Zi| \tag{4}$$

In these equations, Zi is the height deviation from the mean of the heights, and N is the number of points in the image.

#### 2.2.8 Statistical analysis

The data were presented as the mean  $\pm$  standard deviation of each treatment. We performed a factorial design of experiments with a fully randomized design using analysis of variance (ANOVA) using SAS software (Version 8, Statistical Analysis System Institute Inc., Cary, NC, USA). Differences between the mean values of the measured film properties were compared by applying Tukey multiple range tests and a *p*-value < 0.05 was considered significant.

## **3** Results and discussion

#### 3.1 Water vapor permeability properties

Table 1 shows the average values of the thicknesses of the edible films. The thickness is directly proportional to the increase in concentration of the essential oil, and the values varied between 37 and 52  $\mu$ m. This behavior is due to intermolecular rearrangement of the matrix components by the incorporation of the essential oil, as has been described by Pelissari et al. (2009), with films produced with chitosan and the essential oil of oregano (Origanum vulgare L.), and Ghasemlou et al. (2013), with films formulated with corn starch and the essential oil of Zataria multiflora Boiss. Recently, Dashipour et al. (2015) confirmed this behavior in films made with carboxymethyl cellulose and the essential oil Zataria multiflora Boiss by observing that increasing the concentration of the essential oil in the edible films increased the material thickness.

The values of water vapor permeability ranged from  $2.54 \times 10^{-9} \pm 0.09$  to  $4.49 \times 10^{-9} \pm 0.3$  (g m / Pa s m<sup>2</sup>), a high permeability compared to other polymer matrices. The addition of the essential oil extends the structural matrix of the edible film; therefore, the water vapor permeability increased by 76%. Similar behavior was reported for edible films formulated with fish gelatin and the essential oil of bergamot (Ahmad *et al.*, 2012); in edible films based on pectin with lime essential oil (Sanchez-Aldana *et al.*, 2014) and in edible films based on fish gelatin and chitosan with oregano essential oil (Hosseini *et al.*, 2015). This could be due to the interaction that could be carried out between phenolic compounds present in the extract (Sanchez-Aldana *et al.*, 2014).

| vapor permeability (w v1) of Albe vera calole film. |               |                        |                                    |  |  |  |
|---|---------------|------------------------|------------------------------------|--|--|--|
| Films   | Essential oil | Thickness <sup>1</sup> | $WVP^{1} \ge 10^{-9}$              |  |  |  |
|   | (% w/w)       | (µm)                   | $(g \cdot m/Pa \cdot s \cdot m^2)$ |  |  |  |
| Control   | 0             | $37 \pm 3^{a}$         | $2.54 \pm 0.09^{a}$                |  |  |  |
| Allspice oil  | 0.5           | $44 \pm 5^{b}$         | $3.20 \pm 0.4^{b}$                 |  |  |  |
| Allspice oil  | 1.0           | $47 \pm 6^{b}$         | $3.75 \pm 0.2^{b}$                 |  |  |  |
| Allspice oil  | 1.5           | $52 \pm 4^{bc}$        | $4.49 \pm 0.3^{\circ}$             |  |  |  |

Table 1. Effect of *Pimenta dioica* essential oil concentrations (% w/w) on water vapor permeability (WVP) of *Aloe vera* edible film.

Means in same column for essential oil and control with different letters are significantly different at  $p \le 0.05$ .

 $^{1}$ Thickness and WVP data are mean values  $\pm$  standard deviations.

Table 2. Effect of *Pimenta dioica* essential oil concentrations (% w/w) on color parameters of *Aloe vera* edible film.

| Essential   | $a^*$                    | $b^*$                | $L^*$                           | $\Delta E^*$                  |  |
|---|--------------------------|----------------------|---------------------------------|-------------------------------|--|
| oil   |                          |                      |                                 |                               |  |
| (% w/w)   |                          |                      |                                 |                               |  |
| 0   | $1.170 \pm 0.01^{a}$     | $7.110 \pm 0.03^{a}$ | $79.080 \pm 0.001^{d}$          | $58.14 \pm 1.18^{a}$          |  |
| 0.5   | $1.477 \pm 0.01^{a}$     | $6.793 \pm 0.04^{a}$ | $79.140 \pm 0.026$ <sup>c</sup> | $61.04 \pm 0.71$ <sup>a</sup> |  |
| 1.0   | $1.370 \pm 0.20^{ab}$    | $7.013 \pm 0.37^{a}$ | $79.177 \pm 0.005^{b}$          | $59.84 \pm 1.80^{a}$          |  |
| 1.5   | $0.467 \pm 0.10^{\circ}$ | $6.037 \pm 0.04^{b}$ | $79.250 \pm 0.020^{a}$          | $59.61 \pm 1.10^{a}$          |  |
| $a^* b^* L^* \Delta E^*$ data are mean values $\pm$ standard deviations |                          |                      |                                 |                               |  |

Means in same column for control and essential oil with different letters are significantly different at p < 0.05.

Table 3. Effect of different concentrations (% w/w) of *Pimenta dioica* essential oil on mechanical properties of edible *Aloe vera* film.<sup>1</sup>

|  | i _ i                         |                          |                              |  |  |  |
|--|-------------------------------|--------------------------|------------------------------|--|--|--|
| Essential oil  | Tensile strength <sup>1</sup> | Elongation <sup>1</sup>  | Elastic modulus <sup>1</sup> |  |  |  |
| (% w/w)  | (MPa)                         | (%)                      | (MPa)                        |  |  |  |
| 0  | $11.72 \pm 1.01^{a}$          | $72.37\pm0.26^{a}$       | $166.78 \pm 0.52^{a}$        |  |  |  |
| 0.5  | $11.63 \pm 0.48^{b}$          | $57.86 \pm 0.00^{b}$     | $80.96 \pm 0.33^{b}$         |  |  |  |
| 1.0  | $10.27 \pm 0.70^{\rm bc}$     | $87.93 \pm 0.00^{\circ}$ | $34.13 \pm 0.49^{bc}$        |  |  |  |
| 1.5  | $10.08 \pm 0.62$ <sup>c</sup> | $122.56 \pm 0.00^{d}$    | $34.11 \pm 0.43^{\circ}$     |  |  |  |
| Means in same column for control and essential oil with different letters are significantly different at n |                               |                          |                              |  |  |  |

Means in same column for control and essential oil with different letters are significantly different < 0.05

<sup>1</sup>TS, E and WVP data are mean values ± standard deviations.

### 3.2 Color properties

Table 2 shows the color parameters L, a, and b and the total color difference  $(\Delta E)$  values for the Aloe vera film with added Pimenta dioica essential oil. The L\* (brightness) parameter showed a significant difference with a range of values from 79.080 to 79.250. The color values were not different. In general, the essential oil of pepper (Pimenta dioica L. Merrill) incorporated into the edible film formulated with the Aloe vera gel caused a slight change in the brightness of the material. This fact can be attributed to the gel extraction method, interactions between the components of the polymeric matrix and the content of anthraquinones in the gel. Anthraquinones are eliminated by separating the epidermis mucilage without damaging the vascular bundles by avoiding contact with air or light because otherwise an obscure appearance occurs (Hussain et al., 2008); similar behavior has been reported by Vieira et al. (2016) in edible films of chitosan and Aloe vera gel.

#### 3.3 Mechanical properties

Table 3 depicts the effect of the essential oil concentration on the mechanical properties of the films. It was observed the film with 1.5% w/w produced a higher percentage of deformation at 122.56%, while at 0.5% w/w only a 72.37% deformation is obtained. The Young's modulus also decreased, resulting in a film that was 80% more flexible than the control film. Mechanical properties of the films were characterized by measuring the tensile strength (TS) and elongation at break, which are key indicators of film strength and flexibility. Increased concentrations of the essential oil of pepper (Pimenta dioica L. Merrill) in films with Aloe vera decreased the tensile strength (TS) and simultaneously increased the flexibility of the film. This behavior has been reported by Ghasemlou et al. (2013) for films made with corn starch and Zataria multiflora essential oil and with edible films formulated with chitosan, fish gelatin and the essential oil of Origanum vulgare L.



Fig. 2. Scanning electron microscopic images obtained by ESEM (100x). (a) *Aloe vera*-Gelatin-Glycerol film; (b) *Aloe vera*-Gelatin-Glycerol-0.5% EO film; (c) *Aloe vera*-Gelatin-Glycerol-1% EO film and (d) *Aloe vera*-Gelatin-Glycerol-1.5% EO film.

In addition, Choi et al. (2016) tested edible films formulated with hydroxypropyl methylcellulose and oregano and bergamot essential oils, observing a similar trend. It has been reported that the incorporation of essential oils in a continuous polymeric matrix reduced its tensile strength due to structural discontinuities caused by the oil (Sanchez-Gonzalez et al., 2011.); which became flexible (Suppakul et al., 2003) with a decrease of Young's modulus (EM). The addition of essential oils in a continuous polymeric matrix reduced its tensile strength due to the structural discontinuities caused by the oil phase (Sanchez-Gonzalez et al., 2011), which in turn became more flexible (Suppakul et al., 2003), as the value of Young's modulus decreased. This behavior was also observed in this study.

#### 3.4 Film microstructure

A microscopic study of the structural arrangement of the different components in the dried film matrices contributes to better understanding of the physical,

mechanical, barrier and optical properties. Fig. 2 shows micrographs of the Aloe vera-gelatin-based films with and without the essential oil, which show appreciable differences as a function of the film composition. The effect of drying process and the structural interaction between biopolymers are also evident in ESEM images. The control film shows a smooth microstructure, while the films that incorporated the highest concentration of essential oils had a rougher film microstructure. The interaction of biopolymers in the mixture, i.e., the polysaccharide chains and the globular and amorphous matrix of Aloe vera, were reinforced structurally by the gelatin protein and glycerol, causing an increase in stiffness within the matrix. This behavior was also reported by Nonsee et al. (2011) in films of hydroxypropyl methylcellulose and clove essential oil. Similar behavior was also reported by Shojaee-Aliabadi et al. (2014) in films of  $\kappa$ -carrageenan and the essential oil of Mentha pulegium, and recently by Choi et al. (2016) in films with oregano essential oil.



Fig. 3. Scanning electron microscopic of cross-section obtained by SEM-LV (100x). (a) *Aloe vera*-Gelatin-Glycerol; (b) *Aloe vera*-Gelatin-Glycerol-0.5% EO film; (c) *Aloe vera*-Gelatin-Glycerol-1% EO film and (d) *Aloe vera*-Gelatin-Glycerol-1.5% EO film.

Fig. 3 shows cross-sectional micrographs of *Aloe vera*-gelatin based films, which exhibit significant differences as a function of film composition. A smooth and continuous microstructure was observed in the control film, while the emulsified films showed discontinuities associated with the essential oil droplets embedded in the polymeric matrix. At lower concentrations, emulsified films show a smaller oil-droplet size. However, a coarser microstructure was observed in the films that incorporated the highest concentration of essential oils because the higher lipid content favors the flocculation rate (Sánchez-González *et al.*, 2011).

## 3.5 Surface morphology

Atomic force microscopy (AFM) is a powerful tool for studying surfaces and has been used to provide qualitative and quantitative information regarding the topography of biopolymers at the nanometer scale that are often inaccessible by any other experimental techniques. AFM allows for the determination of roughness parameters that can be calculated for the film surface. According to the AFM images (Fig. 4), an increase in the essential oil concentration in the edible film modified the surface morphology with a rough appearance, while the control film exhibited a smoother surface. The presence of the essential oil appears to favor an increased surface roughness, which is probably due to the higher lipid content (Sánchez-González et al., 2011). These results agree with those reported by Shojaee-Aliabadi et al. (2014) for films prepared from *k*-carrageenan and essential oils (Zataria multiflora and Mentha pulegium). Other similar results were reported by Ma et al. (2012) for edible films of gelatin and olive oil and Hosseini et al. (2015) for edible films of chitosan and fish gelatin with oregano essential oil.



Fig. 4. AFM topography images of *Aloe vera*-Gelatin-Glycerol-EO pepper films. Letters a, b, c, and d in the AFM images represent 0, 0.5, 1.0, and 1.5% pepper oil, respectively. Ra: average roughness, Rq: root-mean square roughness. Values of the same parameter with same letters are not significantly different (p < 0.05).

# Conclusions

The *Aloe vera* films with the essential oil of pepper (*Pimenta dioica* L. Merrill) did not have a significant

effect on the total color difference but resulted in an increase in water vapor permeability. An increase in the essential oil concentration resulted in significantly lower tensile strength with a concurrent increase in the elongation at break, making the material more pliable. Microstructural studies allowed for the evaluation of the complexity, homogeneity and roughness of the films and changes were observed, as the concentration of oil increased the roughness of the film. The results obtained in this work suggest that *Aloe vera* films infused with pepper essential oil showed potential for use as active edible films, although further studies are necessary before using such films as an active packaging for food commodities.

## Acknowledgements

The authors gratefully acknowledge financial support from CONACyT and Tecnológico Nacional de México (TecNM). The authors also thank the Department of Food Industries of Instituto Tecnológico de Roque, the Department of Chemical Engineering of Instituto Tecnológico de Celaya, and Centro de Nanociencias y Micro y Nanotecnologías (CNMN) IPN for supporting the facilities for this research work and acknowledge the technical assistance from Department of Agroindustrial of Universidad Politécnica de Guanajuato.

## Nomenclature

- L\* lightness variable
- a\* green to blue
- b\* yellow to red
- C\* chroma
- x thickness, mm
- t time, s
- A film area,  $m^2$
- TS tensile strength, MPa
- %E elongation at break
- EM elastic modulus, MPa
- Rq roughness, root square deviation of the heights, mV
- Ra roughness, arithmetic average of the absolute values of the height deviations, mV
- Zi height deviation
- N number of points in the image
- WVP water vapor permeability
- RH relative humidity
- Greek symbols
- $\Delta E$  total color difference
- $\Delta m$  mass change over time, g

# References

- Ahmad, M., Benajkul, S., Prodpran, T., Agustini, T.W. (2012). Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leather jacket incorporated with essential oils. *Food Hydrocolloids* 28, 189-199.
- Abugoch, L.E., Tapia, C., Villamán, M.C., Yazdani-Pedram, M., Díaz-Dosque, M. (2011). Characterization of quinoa protein-chitosan blend edible films. *Food Hydrocolloids* 25, 879-886.
- ASTM. (2000a). Standard test method for water vapor transmission of materials. Designation: E00996-00. In ASTM annual of ASTM book standards (pp 907-914). Philadelphia: American Society for Testing and Materials.
- ASTM. (2000b). Standard test method for tensile properties of thin plastic sheeting. Designation: D00882-00. In ASTM annual of ASTM book standards (pp 160-168). Philadelphia: American Society for Testing and Materials.
- Benavides, S., Villalobos-Carvajal, R., Reyes, J.E. (2012). Physical, mechanical and antibacterial properties of alginate film: effect of the crosslinking degree and oregano essential oil concentration. *Journal of Food Engineering* 110, 232-239.
- Benitez, S., Achaerandio, I., Sepulcre, F., Pujolà, M. (2013). *Aloe vera* based edible coatings improve the quality of minimally processed "Hayward" kiwifruit. *Postharvest Biology and Technology* 81, 29-36.
- Bergo, P., Sobral, P.J.A. Prison, J.M. (2010). Effect of glycerol on physical properties of cassava starch films. *Journal of Food Processing and Preservation 34*, 401-410.
- Boudreau, M.D., Beland, F. A. (2006). An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe vera. Journal Environmental Science Health C* 24, 103-154.
- Castillo, S., Navarro, D., Zapata, P.J., Guillén, F., Valero, D., Serrano, M., Martínez-Romero, D. (2010). Antifungal efficacy of *Aloe vera* in vitro and its use as a preharvest treatment to maintain postharvest table grape quality. *Postharvest Biology and Technology 57*, 183-188.

- Choi, W.S., Singh, S., Suk-Lee, Y. (2016). Characterization of edible film containing essential oils in hydroxypropyl methylcellulose and its effect on quality attributes of "Formosa" plum (*Prunus salicina* L.). Food Science and Technology 70, 213-222.
- Dashipour, A. Razavilar, V., Hosseini, H., Shojaee-Aliabadi, S., German, J.B., Ghanati, K. (2015). Antioxidant and antimicrobial carboxymethyl cellulose films containing *Zataria multiflora* essential oil. *International Journal of Biological Macromolecules* 72, 606-613.
- Ghasemlou, M., Aliheidari, N., Fahmi, R., Shojaee-Aliabadi, S., Keshavarz, B., Cran, M.J., Khaksar, R. (2013). Physical, mechanical and barrier properties of corn starch films incorporated with plant essential oils. *Carbohydrate Polymers* 98, 1117-1126.
- Gordon, L.R. (2012). *Food Packaging: Principles and Practice*. Third Edition. Taylor & Francis Group, pp 93-95. Boca Raton, FL, USA.
- Hernández-Carrillo, J.G., Valdez-Fragoso, A., Welti-Chanes, J., Mújica-Paz, H. (2015). Tracing phenolic compounds through manufacturing of edible films from orange and grapefruit peels. *Revista Mexicana de Ingeniería Química 14*, 567-578.
- Hosseini, S.F., Reaei, M., Farahmandghavi, F. (2015). Bio-based composite edible films containing *Origanum vulgare* L. Essential oil. *Industrial Crops and Products* 67, 403-413.
- Hussain, A.I., Anwar, F., Sherazi, S.T.H., Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry* 108, 986-995.
- Li, J.Y., Wang, T.X., Shem, Z.G., Hu, Z.H. (2003). Relationship between leaf structure and aloin content in six species of *Aloe L. Acta Botanica Sinica* 45, 549-600.
- Liu, C.H., Wang, C.H., Xu, Z.Y., Wang, Y. (2007). Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. *Process Biochemistry* 42, 961-970.

- Ma, W., Tang, C.H., Yin, S.W., Yang, X.Q., Wang, Q., Liu, F., Wie, Z.H. (2012). Characterization of gelatin-based edible films incorporated with olive oil. *Food Research International* 49, 572-579.
- Martínez, L., Romero, N., Albuquerque, J.M., Valverde, F., Guillén, S., Castillo, D. (2006). Postharvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: A new edible coating. *Postharvest Biological Technology 39*, 93-100.
- Navarro, D., Huertas, M. Diaz-Mula, Guillén, F., Zapata, P.J., Castillo, S. (2011). Reduction of nectarine decay caused by *Rhizopus stolonifer*, *Botrytis cinerea* and *Penicillium difitatum* with *Aloe vera* gel alone or with the addition of thymol. *International Journal of Food Microbiology 151*, 241-246.
- Nonsee, K., Supitchaya, C., Thawien, W. (2011). Antimicrobial activity and the properties of edible hydroxypropyl methylcellulose based films incorporated with encapsulated clove (*Eugenia caryophyllata* Thunb.) oil. *International Food Research Journal 18*, 1531-1541.
- Pelissari, F.M., Grossmann, M.V.E., Yamashita, F., Pineda, E.A. (2009). Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil. *Journal of Agricultural and Food Chemistry 57*, 7499-7504.
- Ramírez-Hernández, A., Valera-Zaragoza, M., Aparicio-Saguilán, A. Conde-Acevedo, J. C. (2015). Thermal behavior of banana starch films with degraded polyethylene terephthalate. *Revista Mexicana de Ingeniería Química 14*, 513-521.
- Rodríguez-Marín, M.L., Álvarez-Ramírez, J., Bello-Pérez, L.A. (2016). Influence of storage time on mechanical properties of films made with montmorillonite/flour (unripe banana and rice) blends. *Revista Mexicana de Ingeniería Química 15*, 433-439.
- Romero-Bastida, C.A., Zamudio-Flores, P.B., Bello-Pérez, L.A. (2011). Antimicrobianos en películas de almidón oxidado de plátano: Efecto sobre la actividad antibacteriana,

microestructura, propiedades mecánicas y de barrera. *Revista Mexicana de Ingeniería Química 10*, 445-453.

- Saibuatong, O.A., Phisalaphong, M. (2010). Novo *Aloe vera* bacterial cellulose composite film from biosynthesis. *Carbohydrate Polymers* 79, 455-460.
- Salmieri, S., Lacroix, M. (2006). Physicochemical properties of alginate/ polycaprolactone-based films containing essential oils. *Journal of Agricultural and Food Chemistry* 54, 10205-10214.
- Sánchez-Aldana, D., Contreras-Esquivel, J.C., Nevárez-Moorillón, G.V., Aguilar, C.N. (2014). Caracterización de películas comestibles a base de extractos pécticos y aceite esencial de limón mexicano. *CyTA Journal of Food 13*, 17-25.
- Sánchez-González, L., Chafer, M., Hernandez, M., Chiralt, A., Gonzalez-Martinez, C. (2011). Antimicrobial activity of polysaccharide films containing essential oils. *Food Control 22*, 1302-1310.
- Serrano, M., Valverde, J.M., Guillén, F., Castillo, S., Martínez-Romero, D., Valero, D. (2006). Use of *Aloe vera* gel coating preserves the functional properties of table grapes. *Journal* of Agricultural and Food Chemistry 54, 3882-3886.
- Shojaee-Aliabadi, S., Hosseini, H., Mohammadifa, M.A., Mohammadi, A., Ghasemlou, M., Hosseini, S.M., Khaksar, R. (2014). Characterization of κ-carrageenan films incorporated plant essential oils with improved antimicrobial activity. *Carbohydrate Polymers* 101, 582-591.

- Sotelo-Boyás, M.E., Valverde-Aguilar, G., Plascencia-Jatomea, M., Correa-Pacheco, Z.N., Jiménez-Aparicio, A., Solorza-Feria, J., Barrera-Necha, L., Bautista-Baños, S. (2015). Characterization of chitosan nanoparticles added with essential oils. *In vitro* effect on *Pectobacterium carotovorum. Revista Mexicana de Ingeniería Química 14*, 589-599.
- Suppakul, P., Sonneveld, K., Miltz, J., Bigger, S.W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science* 68, 408-420.
- Teixeira, B., Marques, A., Pires, C., Ramos, C., Batista, I., Saraiva, J.A. (2014). Characterization of fish protein films incorporated with essential oils of clove, galic and origanum: physical, antioxidant and antibacterial properties. *LWT-Food Science and Technology 59*, 533-539.
- Valverde, J.M., Valero, D., Martínez-Romero, D., Guillén, F., Castillo, S., Serrano, M. (2005). Novel edible coating based on of *Aloe vera* gel to maintain table grape quality and safety. *Journal of Agricultural and Food Chemistry 53*, 7807-7813.
- Vega, A., Ampuero, N., Díaz, R. (2005). El Aloe vera (Aloe barbadensis Miller) como componente de alimentos funcionales. Revista Chilena Nutrición 32, 208-214.
- Vieira, J.M., Flores-López, M.L., Jasso de Rodríguez, D. Sousa, M.C., Vicente, A., Martins, J. (2016). Effect of chitosan-Aloe vera coating on postharvest quality of blueberry (Vaccinium corymbosum) fruit. Postharvest Biology and Technology 116, 88-97.