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Antimicrobial pectin-gellan films: effects on three foodborne pathogens in a meat medium, and selected physical-mechanical properties

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ABSTRACT

It is first reported the elaboration and characterization of films (F2) containing 1% (w/v) citrus pectin, 0.2% (w/v) gellan gum, 0.5% (w/v) glycerol, CaCl₂ 5 mM, ethylenediaminetetraacetic acid (EDTA) 0.05 M and 90 (Arbitrary Units)/mL of an antimicrobial concentrated supernatant (ACS) from fermentation culture broths of the lactic acid bacterium, Streptococcus infantarius. The functional films inhibited the growth of Listeria monocytogenes, Escherichia coli and Staphylococcus aureus in “Barbacoa” medium in 7-day cultures at 35°C. “Barbacoa” is a highly appreciated Mexican meat product. In contrast, the control cultures exhibited bacterial-growth up to 10⁻²⁻¹₀⁻⁸ (Colony-Forming Units)/g. An antimicrobial-activity synergy between ACS and EDTA was demonstrated. Some film-physical properties were modified by the EDTA-ACS incorporation [F2/control-film]: Young’s modulus (MPa), 1,394/707; elongation at break (%), 1.9/9.3; stress at break (MPa), 5.7/12.6; water vapor permeability (10⁻¹¹ g Pa⁻¹ s⁻¹ m⁻²), 3/20 and oxygen permeability (10⁻¹² g m Pa⁻¹ s⁻¹ m⁻²), 1.9/1.2.

Películas antimicrobianas de pectina-gelana: efectos sobre tres patógenos transportados por alimentos, en un medio cárnico, y propiedades físico-mecánicas seleccionadas

RESUMEN

Se reporta por primera vez la elaboración y caracterización de películas (F2) conteniendo 1% (p/v) pectina cítrica, 0.2% (p/v) goma gelana, 0.5% (p/v) glicerol, CaCl₂ 5 mM, ácido etilendiaminotetraacético (EDTA) 0.05 M, y 90 (Unidades Arbitrarias)/mL de sobrenadante concentrado de caldos de fermentación de la bacteria ácido láctica, Streptococcus infantarius, con actividad antimicrobiana (ACS). Las películas inhibieron el crecimiento de Listeria monocytogenes, Escherichia coli y Staphylococcus aureus en cultivos de 7 días a 35°C en medio “Barbacoa”. La Barbacoa es un producto cárnico Mexicano altamente apreciado. En contraste, los cultivos control sin película exhibieron crecimientos bacterianos hasta 10⁻²⁻¹⁻¹₀⁻⁸ (Unidades Formadoras de Colonias)/g. Se demostró un efecto antimicrobiano sinérgico entre ACS y EDTA. Algunas propiedades físico-mecánicas de las películas fueron afectadas por la inclusión de EDTA-ACS, como [F2/película control]: Módulo de Young (MPa), 1,394/707; elongación a la ruptura (%), 1.9/9.3; Esfuerzo a la ruptura (MPa), 5.7/12.6; permeabilidad al Vapor de Agua (10⁻¹¹ g Pa⁻¹ s⁻¹ m⁻²), 3/20 y Permeabilidad al Oxígeno (10⁻¹² g m Pa⁻¹ s⁻¹ m⁻²), 1.9/1.2.

Introduction

The research concerning the packaging for the conservation of food products is of worldwide interest due to implications on food safety, biomaterials applications and sustainability, among others (Campos, Gershenson, & Flores, 2010; Jabeen, Majid, Nayik, & Yildiz, 2015). Specifically, the research on the packaging of meat products encompasses studies concerning meat products from the minimal to the highly processed ones: fresh beef (Zinoviadou, Koutsoumanis, & Biladeris, 2010); pork meat hamburgers (Vargas, Albors, & Chiralt, 2011) and fresh white shrimps (Meenatchisundaram et al., 2016) among others. Furthermore, the antimicrobial packaging materials can effectively control the growth of spoilage and pathogenic microorganisms in the surface of meat products; in this sense, one alternative is the use of edible biopolymer films enriched with bacteriocins (Pattanayaiying, H-Kittikun, & Cutter, 2015; Salmieri et al., 2014). Bacteriocins are natural antimicrobial peptides synthetized by one bacterium species that are active against other bacteria species. After rigorous evaluations, these antimicrobials can be used as safe additives in food products for human consumption. This is the case of both bacteriocins, Nisin and Colicin (FDA, 2000, 2016). In order to increase the antimicrobial spectrum of bacterium inhibition, bacteriocins are frequently used in combination with other substances, like the chelating agent, ethylenediaminetetraacetic acid (EDTA), which contributes to make more permeable the bacterium outer membranes, resulting in an effective antimicrobial activity against Gram negative bacteria as well (Vaara, 1992). Furthermore, some biopolymers, like pectins, also exhibit antimicrobial activities that can contribute to a more effective functional films for food packaging (Calce et al., 2014; Jindal, Kumar, Rana, & Tiwary, 2013).

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The present article reports the main results concerning the elaboration and characterization of films of gellan gum mixed with citrus pectin, enriched with EDTA and antimicrobial concentrated supernatant (ACS) from fermentation culture broth of the lactic acid bacterium (LAB), Streptococcus infantarius, containing bacteriocin-like inhibitory substances (BLIS). The antimicrobial activity of the films was tested against Listeria monocytogenes, Escherichia coli and Staphylococcus aureus, growing in a medium based on Mexican “Barbacoa”, a highly appreciated meat product, that is usually prepared with lamb meat wrapped in agave leaves and cooked overnight in customized ovens (Natividad-Bonifacio et al., 2010). Also, some selected physical-mechanical properties of the films were determined (i.e. Young’s modulus, stress and elongation at break and water vapor and oxygen permeabilities). The development of bioconservation technologies for Mexican products, like “Barbacoa”, is of great interest for many reasons, including that concerning the food market (Rubio, Torres, Gutierrez, & Mendez, 2004).

Materials and methods

Biological specimens

The LAB, Streptococcus infantarius, originally isolated from Pozol, a traditional fermented food (Mendoza-Mendoza et al., 2013), was kindly provided by Dr. C. Wacher-Rodarte (School of Chemistry, UNAM, Mexico).

The bacterium indicators for testing the antimicrobial activity of the films were Listeria monocytogenes CFO-103, Escherichia coli ATCC-25922 and Staphylococcus aureus ATCC-25923, kindly provided by Dr. G. Diaz-Ruiz (School of Chemistry, UNAM, Mexico).

All bacterial strains were conserved at –80°C in 2 mL-vials containing 1 mL of 24 h old-culture broths of each bacterium mixed with 20% v/v glycerol. For this purpose, S. infantarius was grown in De Man-Rogosa-Sharpe broth, MRS (BD® DIFCO, France), although the other bacteria were grown in Brain Heart Infusion broth, BHI (Bioxon® México).

Antimicrobial additives

The ACS of S. infantarius was obtained according to Calderón-Aguirre et al. (Calderón-Aguirre et al., 2015). Briefly, reactivated S. infantarius-cells were cultured in MRS at 30°C for 6 h; then, the bacterial cells were removed by centrifugation. The supernatant was collected, adjusted to a pH of 6.5 and concentrated (60°C, 72 mbar) up to 40% of its initial volume. To inactive proteases, the ACS was heated at 110°C for 10 min; later, it was cooled and stored until use. The ACS exhibited an antimicrobial activity of 6,400 arbitrary units (AU)/mL, determined by the spot-on-the-lawn method (Nuñez, Tomillo, Gaya, & Medina, 1996), being this activity due to the presence of BLIS because its antimicrobial action was inhibited by proteases (i.e. Proteinase K (Invitrogen, U.S.A.); Peptidase and Trypsin (Sigma-Aldrich, U.S.A.); (Mimila-Méndez, 2017).

The chelating agent was EDTA disodium salt dehydrate (Sigma-Aldrich, U.S.A.).

Minimal inhibitory concentrations of the antimicrobial additives

The minimum inhibitory concentration (MIC) determination was performed by the broth microdilution method (CLSI, 2015). All combinations of ACS and EDTA at different concentrations (i.e. ACS: 0, 75, 90, 200 and 400 AU/mL; EDTA: 0, 1 × 10⁻³, 2 × 10⁻³, 4 × 10⁻³, 8 × 10⁻³, 0.02 and 0.05 M) were tested to find the MIC against L. monocytogenes, E. coli and S. aureus in BHI, with an initial inoculum of 4 × 10⁵–7 × 10⁶ Colony-Forming Units (CFU) per well, by triplicate, in microtiter plates (Corning® Costar®). The inoculated microdilution trays were incubated at 35°C for 20–24 h.

Film elaboration

Three film treatments (F1, F2 and F3) and the control (FC) were tested in this work. The film-forming solutions contained 1% (w/v) pectin (GENU® Pectin, DE = 36%, CP-Kelco, U.S.A.), 0.2% (w/v) low acyl Gellan gum (Kelcogel, CP-Kelco, U.S.A.), 0.5% (w/v) glycerol (Quimica Meyer, Mexico) and CaCl₂ (JT Baker, Mexico) 5 mM. The F1, F2, F3 and FC treatments contained the following EDTA (M)/ACS (AU/mL) ratios: 0.05/75, 0.05/90, 0.05/120 and 0/0, respectively. The biopolymers were dissolved separately in distilled water; later they were mixed together and heated at 60–65°C during 40 min; then, glycerol and CaCl₂ were added and temperature was increased up to 75°C. Mixing continued another 30 min; then, the corresponding quantities of the antimicrobial additives were added into the mixture. Once the film-forming solutions were already prepared, they were poured into Teflon® casts (EKCO®, 39.5 × 27 × 2 cm) and dried in an oven (Shel-Lab, 1380FX) at 35°C during 17 h.

The obtained films were then conditioned into a desiccator during 48 h at a relative humidity (RH) of 50–55% and 23°C. The average thickness of the films were determined by measurements at five points each film with a micrometer to the nearest 0.0001 mm (Truper, Mexico).

Film effects on the growth of Listeria monocytogenes, Escherichia coli and Staphylococcus aureus in selective media

L. monocytogenes, E. coli and S. aureus were grown in BHI at 35°C for 24 h; then, decimal dilutions of each bacterium culture were done with isotonic salt solution (1% (w/v) NaCl). 200 µL-samples of convenient dilutions of each bacterium culture were done with isotonic salt solution to inoculate the nearest 0.0001 mm (Truper, Mexico)
contained 4.5% (w/v) Barbacoa (bought in a traditional market in Tulancingo, Hidalgo; México) and 1.5% (w/v) bacto-agar (BD® DIFCO, France). A portion of 45 g of Barbacoa was thoroughly ground with a food processor (Oster® 3213, China), then mixed with 1 L of distilled water and 15 g of bacto-agar, maintaining constant agitation and heating, till boiling. Then the medium was sterilized (121°C for 2 h in an autoclave Tuttnauer, 3870ELV-D) and distributed into petri dishes until use.

The surface of "Barbacoa" plates, inoculated with bacteria, was covered with 6 cm-diameter circular films, previously sterilized with UV radiation during 24 h (12 h each side). The cultures were then incubated at 35°C during 7 days, taking samples at days 0, 1, 3, 5, and 7, by triplicate. Each sample (1 plate containing 5 mL of medium) was homogenized with 45 mL peptone water in a Seward Stomacher® 400 Circulator, at 300 rpm during 5 min. Viable cell counts were made involving decimal dilutions of the homogenate, then mixing 1 mL of diluted samples in BHI soft agar in plates (Interlux, 90 × 15 mm) to be incubated at 35°C during 24 h. Plates with F2 and FC films were tested, as well as bacterium inoculated plates without films (NF).

A bacterium growth curve was obtained for each experiment, being used to determine: (a) the initial viable cell count (X₀; CFU/g); (b) the maximum bacterium concentration (Xₘₐₓ; CFU/g); (c) the multiplication factor [(Xₘₐₓ/X₀), dimensionless) and (d) the maximum specific growth rate (µₘₐₓ; h⁻¹) calculated as the slope of the semi-logarithmic plot of the growth curve in the exponential growth phase (Liu, 2013).

**Mechanical characterization of the films**

Mechanical tests were done according to the ASTM D882-10 method (ASTM, 2010) in a Texture Analyser TA plus Lloyd. Samples were cut into "dog-bone" shape (Type M-I tension test specimen) according to the specifications of the standard ASTM D638M-93 (ASTM, 1993). After conditioning the samples during 48 h at 50–55% RH and 25°C, they were gripped into the texture analyzer with an initial separation of 0.05 m. The tensile tests were carried out at a cross head speed of 1 mm/s. At least 30 replicates per treatment were carried out to obtain the stress-Hencky strain curves of the samples through the force-distance data. Specimens that failed at the grip contact point were discarded. Young’s modulus (EM; MPa) was determined through the slope of the linear region of the stress-strain curves. The ultimate mechanical properties of the films, stress (σₚ₉ₕₘₐₓ; MPa) and elongation at break (εₚ₉ₕₘₐₓ; %) were determined in the rupture point (Calderón-Aguirre et al., 2015).

**Water vapor permeability of the films**

The water vapor permeability (WVP) of the films was determined with the ASTM E96-00 method (ASTM, 2000). Film disks, previously equilibrated at 53% RH and 25°C for 48 h, were mounted on permeation cells (aluminum cups) containing dried silica gel (0% RH); then cups were placed inside a cabinet that was equilibrated at 75 ± 2% RH and 23 ± 2°C during 24 h prior to WVP tests. The cups were weighed every hour during 8 h. The WVP was determined according to the procedure reported by Aguirre-Loredo, Rodríguez-Hernández & Chavarria-Hernández (2014). Four determinations were done per treatment.

**Oxygen permeability of the films**

The oxygen permeability (PO₂) of the films was determined in accordance with the ASTM D1434-82 method (ASTM, 1982) using a film-package permeability tester (Labthink VAC-V2, China). Films were conditioned at 50–55% RH during 48 h prior to be placed into the equipment chambers. The tests were performed at 25°C, using research-grade high-purity (99.998%) oxygen gas (34,161, INFRA® México). Four runs per treatment were carried out.

**Statistical analysis**

Data are presented as the mean ± standard deviation for each treatment. Results were analyzed for statistical significance using analysis of variance (ANOVA) followed by Tukey test (p < 0.05). Differences between pairs of means were assessed using t-test (p < 0.05) (SigmaPlot 12.5, SPSS Inc., USA).

**Results and discussion**

**Antimicrobial activity of the films**

First of all, the *S. infantarius*-ACS exhibited important activity against both Gram positive bacteria, *L. monocytogenes* and *S. aureus*, with no effects against the Gram negative bacterium, *E. coli*. The exhibited antimicrobial activity is due to the presence of BLIS involving molecules from 4 to 7 kDa of molecular weight, which can be inactivated by proteases (Mimila-Méndez, 2017); furthermore, the production of bacteriocins and BLIS by other bacterial strains which belong to the *Streptococcus bovis/Streptococcus equinus* complex has been reported. For example, *S. bovis* HCS produces the bacteriocin, bovicin HCS, which exhibits important antilisterial activity (Mantovani & Russell, 2003). Besides, some of this bacterial strains have been isolated from traditional fermented dairy products (Jans et al., 2013).

In the present work, in order to elaborate a film with antimicrobial activity against *L. monocytogenes*, *S. aureus* and *E. coli*, the metal chelator, EDTA, was added into the film-forming solutions to increase the bacterial sensitivity to the BLIS present in *S. infantarius*-ACS (Banin, Brady, & Greenberg, 2006). The determined MIC was a blend of ACS, 90 AU/mL, with EDTA, 0.05 M, which inhibited the growth of the three indicator bacteria (Figure 1).

There are reports of combinations of EDTA with antimicrobial agents, against both Gram positive and Gram negative bacteria. For example, Economou, Pournis, Ntzimani, and Savvidis (2009) reported the combination of 500–1,500 International Units of nisin with 50 mM EDTA, which affected the populations of the mesophilic bacteria, *Pseudomonas* sp., *Brochothrix thermosphaeta*, lactic acid bacteria and enterobacteriaceae during the storage of fresh chicken meat. Sinigaglia, Bevilacqua, Corbo, Pili, and Del Nobile (2008) used conditioning brines with 0.25 g/L lysozyme and 10–50 mM Na₂-EDTA during the storage of mozzarella cheese, reporting a significant inhibition of coliforms and Pseudomonadaceae. Furthermore, Banin et al. (2006) reported a synergic interaction of Gentamicin (10 mg/mL) with EDTA 50 mM, against *P. aeruginosa*.

The antimicrobial activity-films were elaborated with a constant EDTA concentration, 0.05 M, testing three levels of ACS (i.e. 75, 90 and 120 AU/mL), in a complex biopolymer matrix of low-methoxyl pectin and deacetylated gellan gum, involving a
gelation process greatly affected by the presence of calcium ions (Pérez-Campos, Chavarria-Hernández, Tecante, Ramírez-Gilly, & Rodríguez-Hernández, 2012; Thakur, Singh, & Handa, 1997). The Figure 2 presents the effects of the films on the growth of the three indicator bacteria in selective media. All bacteria grew well in control plates (C, bacterium inoculated media without films), where the counts were 105 CFU/plate and 78 CFU/plate for *L. monocytogenes* and *E. coli* after 2 days of incubation, respectively, although *S. aureus* exhibited 77 CFU/plate at the third day of incubation. In contrast, all plates with films (i.e., FC, F1, F2 and F3) did not exhibit any bacterial growth during a period of 30 days (Figure 2). The antimicrobial activity exhibited by the FC films, which contained no ACS nor EDTA, would rely on the contents of gellan gum and pectin. It has been reported the antimicrobial properties of several carbohydrate polymers (i.e., karaya gum, chitosan, algal polysaccharides (Ramawat & Mérillon, 2013)); specifically, pectins extracted from both apple peel (pristine and modified samples) and *Aegle marmelos* fruit, have exhibited antimicrobial activity against *E. coli* and *S. aureus* (Calce et al., 2014) and *Bacillus cereus* and *E. coli* (Jindal et al., 2013), respectively. Therefore, the bacterial growth inhibition exhibited by the FC films would be attributed to the pectin contents, being this antimicrobial activity associated to the uronic acid contents in the biopolymer (Jindal et al., 2013); furthermore, the pectic oligosaccharides have also been proposed as prebiotics with valuable antimicrobial properties (Gullón et al., 2013).

The Figure 3 presents the effects of the films on the growth of *L. monocytogenes*, *E. coli* and *S. aureus* in the “barbacoa” medium at 35°C. The three bacteria grew well in “barbacoa” medium plates with no films (Figure 3, triangle symbols) involving maximum specific growth rates, $\mu_{\text{max}}$ from 3.21 day$^{-1}$ (±0.13 h$^{-1}$) for *L. monocytogenes*, to 5.57 day$^{-1}$ (±0.23 h$^{-1}$) for *S. aureus*, with multiplication factors ($X_{\text{max}}/X_0$) from 1 × 10$^5$ times for *L. monocytogenes*, to 8.3 × 10$^5$ times for *S. aureus* (Table 1). The “barbacoa” medium (3.4% (w/v) muscle and 1% (w/v) total fat (Rubio et al., 2004)), supported vigorous bacterial growths with increases in the bacterial concentrations up to 5–6 log cycles. In analogous studies, Mansur, Park, and Oh (2016) reported the growth of *S. aureus* from 10$^5$ to 10$^8$ CFU/g in ham at 35°C, although Shekarforoush, Basiri, Ebrahimnejad, and Hosseinzadeh (2015) recorded the growth of *E. coli* from 10$^6$ to 10$^{8.8}$ CFU/g and *L. monocytogenes* from 10$^5$ to 10$^{0.5}$ CFU/g, in barbecue chicken at 20°C.

On the other hand, the FC films partially inhibited the growth of the three bacteria inoculated in “barbacoa”

**Figure 1.** Minimal inhibitory concentrations (MIC) of blends of antimicrobial concentrated supernatant (ACS) of *S. infantarius*-fermentations, and ethylenediaminetetraacetic acid (EDTA) for: (i) *Listeria monocytogenes*, (ii) *Escherichia coli*, and (iii) *Staphylococcus aureus* inoculated in Brain Heart Infusion broth (BHI) and incubated at 35°C for 24 h. The highest bacterial growth occurred in the treatment T1 (ACS, 0 AU/mL; EDTA, 0 M) and the MIC for the three indicators occurred in the treatment T2 (ACS, 90 AU/mL; EDTA, 0.05 M). C was the abiotic control.

**Figure 2.** Aspect of the growth of the indicator bacteria in selective media at 2 or 3, and 30 days of incubation at 25°C. (i) *Listeria monocytogenes* in Oxford medium; (ii) *Escherichia coli* in MacConkey agar, and (iii) *Staphylococcus aureus* in Baird Parker medium. Bacteria grew well in control plates (C) (bacterium-inoculated culture media with no films). No bacterial growth was recorded in plates with films (F1, F2 and F3). The antimicrobial activity exhibited by the FC films, which contained no ACS nor EDTA, would rely on the contents of gellan gum and pectin. It has been reported the antimicrobial properties of several carbohydrate polymers (i.e., karaya gum, chitosan, algal polysaccharides (Ramawat & Mérillon, 2013)); specifically, pectins extracted from both apple peel (pristine and modified samples) and *Aegle marmelos* fruit, have exhibited antimicrobial activity against *E. coli* and *S. aureus* (Calce et al., 2014) and *Bacillus cereus* and *E. coli* (Jindal et al., 2013), respectively. Therefore, the bacterial growth inhibition exhibited by the FC films would be attributed to the pectin contents, being this antimicrobial activity associated to the uronic acid contents in the biopolymer (Jindal et al., 2013); furthermore, the pectic oligosaccharides have also been proposed as prebiotics with valuable antimicrobial properties (Gullón et al., 2013).

**Figure 3.** Aspecto del crecimiento bacteriano en medio selectivo a los 2 o 3, y 30 días de incubación a 25°C. (i) *Listeria monocytogenes* en medio Oxford; ii) *Escherichia coli* en agar MacConkey, y iii) *Staphylococcus aureus* en medio Baird Parker. Las bacterias crecieron bien en las cajas control (C) (medio de cultivo inoculado y sin película). Las cajas con película F2 (película biopolimérica base con gelana y pectina, ASC, 90 AU/mL, y EDTA, 0.05 M) no exhibieron crecimiento bacteriano, siendo el mismo resultado para los tratamientos F1, F3 y FC (no mostrados).
medium (Figure 3, diamond symbols), involving low \( \mu_{\text{max}} \) values (Table 1). In fact, the achieved bacterial concentrations were from 2.67 \( \times 10^2 \) CFU/g for \( S. \) aureus, to 1.6 \( \times 10^3 \) CFU/g, for \( L. \) monocytogenes, associated with multiplication factors from 1.8 \( \times 10^1 \) to 8.3 \( \times 10^1 \) times, respectively, involving increases in the bacterial concentrations of only 1 log cycle during the experiments. The partial antimicrobial activity exhibited by the FC films would be attributed to the presence of pectins (Jindal et al., 2013).

Furthermore, an outstanding antimicrobial activity was exhibited by the F2 films against the three tested bacteria which did not grow during the cultures; even more, from the day 1 of experiments, the viable cell counts were minor than 1 CFU/g (Figure 3, circle symbols). This bacterial inhibition would imply a synergy of the individual antimicrobial-activity contributions of ACS, EDTA and pectins, present in the bioactive F2 films. In an analogous study, Sivarooban, Hettiarachchy, and Johnson (2008) reported a maximum antimicrobial activity of soy protein films enriched with grape seed extract (1%), nisin (10,000 IU/g) and EDTA (0.16% w/w), which reduced the populations of \( L. \) monocytogenes, \( E. \) coli and \( S. \) aureus, in 3, 2 and 1 log (CFU/mL), respectively, in contact periods of 1 h at 25°C. In other study concerning the antimicrobial activity of whey-protein-isolate films enriched with nisin (6,000 IU/g), Johnson (2008) reported an \( E. \) coli O157:H7-growth inhibition of 4.6 log cycles in turkey Frankfurters inoculated with 6.15 log (CFU/g), after a storage of 28 days at 4°C.

Table 1. Growth parameters of Listeria monocytogenes, Escherichia coli and Staphylococcus aureus, in “Barbacoa” medium at 35°C.

![Image](https://example.com/image1.png)

**Figure 3.** Growth kinetics of the indicator bacteria (CFU/g) at 35°C in “Barbacoa” medium. (i) Listeria monocytogenes; (ii) Escherichia coli, and (iii) Staphylococcus aureus. Treatments were bacterial inoculated plates with: (a) No films (NF); (b) FC films (base biopolymer films containing pectin and gellan gum), and (c) F2 film (base biopolymer films, plus ACS, 90 AU/mL, and EDTA, 0.05 M). Runs were done by triplicate. Error bars are standard deviations, and same letters indicate no statistical differences on the basis of Tukey’s means comparison, \( p < 0.05 \).

Table 1. Parámetros del crecimiento de Listeria monocytogenes, Escherichia coli y Staphylococcus aureus, en medio “barbacoa” a 35°C.

| Indicator                   | Plate without film | Plate with FC film |
|----------------------------|--------------------|--------------------|
|                            | Maximum specific growth rate (\( \mu_{\text{max}} \) day\(^{-1} \)) | Multiplication factor (\( X_{\text{max}}/X_0 \), dimensionless) | Maximum specific growth rate (\( \mu_{\text{max}} \) day\(^{-1} \)) | Multiplication factor (\( X_{\text{max}}/X_0 \), dimensionless) |
| Listeria monocytogenes      | 3.21 (0.97)*       | 1 \( \times 10^{-1} \) (\( 1.4 \times 10^{-1} \)) | 0.24 (0.88)*       | 8.3 \( \times 10^{-1} \) (\( 2.5 \times 10^{-1} \)) |
| Escherichia coli            | 5.51 (1.00)*       | 9.8 \( \times 10^{-1} \) (\( 1.1 \times 10^{-1} \)) | 0.21 (0.87)*       | 2.4 \( \times 10^{-1} \) (\( 1.5 \times 10^{-1} \)) |
| Staphylococcus aureus       | 5.57 (0.97)*       | 8.3 \( \times 10^{-1} \) (\( 5.9 \times 10^{-1} \)) | 0.20 (0.86)*       | 1.8 \( \times 10^{-1} \) (\( 2.6 \times 10^{-1} \)) |

*Regression coefficient, \( r^2 \).
*Coefficient de regresión, \( r^2 \).
Table 2 presents the values of WVP of the FC and F2 films. Mean values ± standard deviation.

| Properties                              | FC                      | F2                      |
|-----------------------------------------|-------------------------|-------------------------|
| Young's modulus (MPa)                   | 706.55 ± 47.75          | 1393.72 ± 144.24        |
| Elongation at break (%)                 | 9.26 ± 1.97             | 1.86 ± 0.39             |
| Stress at break (MPa)                   | 12.58 ± 1.28            | 2.88 ± 0.80             |
| Water vapor permeability, WVP \(10^{-11} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}\) | 19.8 ± 0.42             | 1.23 ± 0.24             |
| Oxygen permeability, \(\text{PO}_2\) \(10^{-12} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}\) | 2.63 GPa, %E\(_{\text{max}}\) = 3\% (Jin, Liu, Zhang, & Hicks, 2009), chitosan-eugenol and chitosan-cinnamon essential oil (EM = 1,660–1,460 MPa, %E\(_{\text{max}}\) = 6–8\%) (Va1encia-Sullca et al., 2016) and antibacterial gelatin films reinforced with metallic nanoparticles (EM = 2.30–2.63 GPa, %E\(_{\text{max}}\) = 8.3–9\%) (Shankar, Jaiswal, Selvakannan, Ham, & Rhim, 2016).


dosterone \(2.88 \times 10^{-11}\) g m Pa\(^{-1}\) s\(^{-1}\) m\(^{-2}\). The WVP was affected by the incorpora-

Mechanical characterization

The selected physical-mechanical properties of both bioactive (F2) and control (FC) films are shown in the Table 2. The mechanical parameters were statistically significant different \((p < 0.001)\). The incorporation of EDTA and ACS to the films led to increase the Young’s modulus (EM), F2 showed more resis-
tance to axial deformation than FC. Accordingly, F2 was almost five times less extensible than FC, and the stress value at the end of the stretching of the FC was higher than that of the F2 film. The high stiffness of the bioactive film can be attributed to the development of a heterogeneous film structure, presum-
ably due to the formation of crystals composed of calcium and H\(_2\)EDTA\(^{-2}\) ions and water molecules (Zabel, Poznyak, & Pawlowski, 2006), which could be formed during either drying or storage process of the films. This effect was not expected, CaCl\(_2\) was added to film-forming solution to promote gellan and pectin gelation and yield biopolymer matrices with higher degree of cross-linking and therefore, less gas-permeable films. High values of mechanical properties have been also reported for other bioactive films; for instance, pectin-polyactic acid-
nisin films \((\text{EM} = 2,590 \text{ MPa}, \%\text{E}\(_{\text{max}}\) = 3\%) (Jin, Liu, Zhang, & Hicks, 2009), chitosan-eugenol and chitosan-cinnamon essen-
tial oil \((\text{EM} = 1,660–1,460 \text{ MPa}, \%\text{E}\(_{\text{max}}\) = 6–8\%) (Valencia-Sullca et al., 2016) and antibacterial gelatin films reinforced with metallic nanoparticles \((\text{EM} = 2.30–2.63 \text{ GPa}, \%\text{E}\(_{\text{max}}\) = 8.3–9\%) (Shankar, Jaiswal, Selvakannan, Ham, & Rhim, 2016).

Water vapor permeability

The Table 2 presents the values of WVP of the FC and F2 films. The results obtained were statistically significant dif-
dent \((p < 0.001)\). The WVP was affected by the incorpora-
tion of antimicrobial additives, mainly EDTA, F2 film was near 85% less WVP than FC. This decrease could be explained by the structural modifications arose in the F2 network by the development of crystal aggregates of EDTA-calcium. This assumption is based on the observations reported in studies concerning films containing nanoparticles (nano-clays, nano-
fibers, nanowhiskers (Sanchez-Garcia, Lopez-Rubio, & Lagaron, 2010)). These studies have attributed the reduction in WVP of nanocomposite films to the high nanodispersion of the particles across the matrix, the high crystallinity and the good interfacial adhesion in the nanobiocomposites. However, it has also been reported that at high concentra-
tion of nanoparticles, the WVP increases due to the forma-
tion of filler agglomeration, which usually results in the creation of preferential paths for the permeants to diffuse faster. Thus, the good dispersion of fillers into the biopoly-
mer matrix as well as the amount and nature of the plasti-
cizers could be relevant aspects to consider to enhance the water barrier properties of the films.

Furthermore, in the present study, the films did confer a dehydration-protection to the tested agar media, due to its WVP properties. The agar plates with no films were sponta-
neously dried during the experiments; in contrast, the agar plates with films remained without apparent signs of desic-
cation (Figure 2). This properties are important for the con-
servation of processed foods.

Oxygen permeability

The oxygen permeability \((\text{PO}_2\)\) of the films was not affected by the presence of EDTA and ACS \((\text{Table 2})\). There was not a statistically significant difference between the oxygen perme-
ability of the two films, F2 and FC \((p = 0.358)\). These values were lower than the corresponding WVP ones. The hydrophilic nature of the pectin-gellan films, their low plast-
icization and microstructural organization, provided a good barrier to oxygen diffusion and this could have enhanced the antimicrobial activity of the films.

Conclusions

It is reported the elaboration and characterization of func-
tional films containing citrus pectin, gellan gum, glycerol, CaCl\(_2\), EDTA and ACS from fermentation culture broths of S. infantarius. The bioactive films exhibited inhibitory effects against L. monocytogenes, E. coli and S. aureus, inoculated in both selective media (Oxford, MacConkey and Baird Parker, respectively) and in “Barbacoa” medium (this last to mimic “Barbacoa”, an important Mexican meat product). The recorded antimicrobial activity would be attributed to a synergic interaction of ACS, EDTA and Pectin. On the other hand, the mechanical properties of the bioactive films were influenced by the EDTA contents, giving stronger and less extensible films than the controls with no EDTA; these effects might be attributed to the formation of EDTA-calcium crystals into the biopolymer matrix. These crystals also enhanced the water barrier properties of the bioactive films. Notwithstanding more research must be done to improve the mechanical properties of the films without affecting the antimicrobial activity.

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