Journal of Agriculture and Food Research 19 (2025) 101555

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Development and characterization of active gelatin-chitosan packaging incorporated with guava leaf extract for extending meat shelf life

Enrique Daniel Archundia Velarde [a], María Dolores Mariezcurrena Berasain [b,**], Raúl Alberto Morales Luckie [c], Dora Iliana Medina Medina [d], Maximilian Lackner [e], Gisela Velázquez Garduño [a], Abdelfattah Zeidan Mohamed Salem [f,*]

[a] Universidad Tecnológica del Valle de Toluca, Carretera del Departamento del D.F. km 7.5, 52044, Santa María Atarasquillo, Mexico
[b] Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Instituto Literario N°. 100, Toluca, Estado de México, Mexico
[c] Centro Conjunto en Investigación en Química Sustentable UAEM-UNAM, Carretera Km. 14.5, Unidad San Cayetano Toluca, Estado de México, Mexico
[d] Instituto Tecnológico y de Estudios Superiores de Monterrey) Carretera Lago de Guadalupe Km.3.5 Atizapán de Zaragoza Col. Margarita Maza de Juarez, Atizapán de Zaragoza, Mexico
[e] Department of Industrial Engineering, University of Applied Sciences Technikum Wien, Hoechstaedtplatz 6, 1200, Vienna, Austria
[f] Facultad de Medicina y Veterinaria, Universidad Autónoma del Estado de México, Toluca, Instituto Literario # 100, Estado de México, Mexico

ARTICLE INFO

Keywords:
Biopolymeric films
Chitosan
Guava leaf extract
Meat
Packaging

ABSTRACT

Biopolymer films (biofilms) were evaluated for suitability in meat packaging applications. Polyesters, proteins, and carbohydrates are among the most commonly utilized materials for producing bioplastics due to their mechanical properties, which closely resemble those of conventional plastics. However, these properties are significantly influenced by the film's composition, molecular weight, solvent type, pH, component concentration, and processing temperature. Conventional plastic films, including microplastics, are non-bioactive and contribute to persistent pollution. This underscores the importance of developing novel materials that incorporate bioactive plant compounds to endow plastic films with antimicrobial and antioxidant functionalities. In this study, two gelatin-chitosan films were fabricated: one without any extract and another incorporating guava leaf extract. These films were characterized to determine their optical, mechanical, morphological, and thermal properties. Additionally, a microbiological analysis was conducted to assess the impact of polymeric biofilm packaging on the shelf life of beef. Both films exhibited favorable tensile strength values (24.74–27.12 MPa), high transparency (0.79–1.18), and effective barrier properties against water vapor (8.95–9.29 $\times$ 10^{-8} g·mm·Pa^{-1} h^{-1} m^{-2}) and oxygen (9.7–9.10 $\times$ 10^{-18} m^3·m^{-2} s^{-1} Pa^{-1}). During storage, the biofilm containing guava leaf extract demonstrated a reduction in microbial growth, and this enhanced the beef's color stability. In conclusion, biopolymeric films incorporating guava leaf extract demonstrated notable antioxidants and antimicrobial properties, effectively inhibiting microbial growth, preserving the physical quality of beef, and significantly extending its shelf life under refrigerated conditions.

1. Introduction

Bovine meat is a nutritionally complete food for human consumption. In addition to being a source of proteins and fats, it contains vitamins and minerals, including all essential amino acids, making it an excellent food with high biological value [1]. However, beef and meat, in general, are highly susceptible to degradation caused by oxygen and microorganisms, compromising their quality and safety. To mitigate this, meat is typically packaged in plastic containers designed to store, display, and preserve the product. These containers extend the shelf life of meat by providing structural and mechanical integrity, impermeability to water and oxygen, and acting as a barrier against microorganisms [2].

Despite its advantages, the extensive use of plastic packaging has led to severe environmental challenges due to its limited or nonexistent biodegradability. This has resulted in significant ecological concerns,

* Corresponding author.
** Corresponding author.
E-mail addresses: nekkane16@hotmail.com (M.D. Mariezcurrena Berasain), salem@uaemex.mx (A.Z. Mohamed Salem).

https://doi.org/10.1016/j.jafr.2024.101555
Received 28 February 2024; Received in revised form 26 November 2024; Accepted 26 November 2024
Available online 2 December 2024

including threats to marine and terrestrial biodiversity, as well as contributions to atmospheric pollution [3]. These issues arise from plastic waste accumulating in natural environments and the proliferation of micro- and nanoplastics. Moreover, plastic packaging poses potential health risks. Harmful substances such as monomers, oligomers, plasticizers, synthetic antioxidants, additives, colorants, and nano- or microplastics can migrate from the packaging material into food, especially when exposed to heat. This migration can compromise the aroma, flavor, and safety of the packaged product, raising concerns about its overall quality and health implications [4].

To address these challenges, there is growing market demand for eco-friendly food packaging solutions that offer the same or even better functionality as conventional plastics while reducing environmental impact, particularly concerning carbon emissions. Currently, food packaging options include flexible films, rigid films, and coatings [5]. Biodegradable packaging has emerged as a promising alternative due to its ability to decompose into carbon dioxide, biomass, methane, and water during mineralization after disposal. This process can occur under aerobic (oxygen-rich) or anaerobic (oxygen-free) conditions, rendering such biobased and biodegradable packaging compostable [6].

The physicochemical properties of bioplastics are influenced by numerous factors, including the composition and molecular weight of the raw materials (e.g., proteins, carbohydrates, and polyesters), the type of solvent, pH, component concentration, processing temperature, and the concentration of plasticizers or other additives [7,8]. These factors significantly impact the final packaging material's mechanical and optical properties, gas retention/barrier properties (e.g., oxygen and carbon dioxide), and structural resistance to water and microbial degradation [9].

Chitosan (β-(1–4)-2-acetamido-d-glucose and β-(1–4)-2-amino-d-glucose) [10] and gelatin are widely used polymeric base materials for producing bio-based films due to their favorable physicochemical properties. Chitosan, a carbohydrate soluble in acidic solutions, is derived from chitin, extracted from the exoskeletons of crustaceans or fungal cell walls through deacetylation in an alkaline medium [11,12]. It is an abundant and cost-effective polysaccharide with a semi-crystalline structure. Chitosan exhibits antimicrobial and properties and is biodegradable and safe for consumption [13,14]. Gelatin, on the other hand, is a partially hydrolyzed derivative of collagen that is obtained from animal connective tissues, bones or fish and insect byproducts [15]. Biopolymeric films made from gelatin and chitosan exhibit excellent optical properties (e.g., transparency) and mechanical flexibility. They also provide effective barriers against oxygen and water and serve as a suitable base for incorporating bioactive compounds [16]. The properties of gelatin depend on its source, the type of collagen, amino acid composition, and the presence of lower-molecular-weight proteins [17].

Although chitosan and gelatin are not naturally occurring mixtures, they can be combined to produce composite films for food packaging. These films are of particular interest due to their inherent properties, including biodegradability, edibility, film-forming capability, mechanical strength, hydrophobicity, color stability, barrier efficiency, and thermal performance, all aligning with food industry requirements [18]. Furthermore, the functional properties of these films can be enhanced by incorporating plant extracts or essential oils with antimicrobial or antioxidant properties, resulting in "active biopolymer films (biofilms)". Active packaging represents a novel category of materials that incorporate and integrate active compounds to release or absorb substances into or from the packaged food or its environment. Active compounds are classified into two categories: scavengers, which remove undesirable substances from the food environment, and emitters, which release beneficial substances into the food or its headspace, providing long-term antioxidant and/or antimicrobial protection [19]. Commonly used bioactive compounds include extracts from spices such as black pepper, rosemary, garlic, clove, cinnamon, thyme, oregano, and lavender [20]. However, the high demand for these spices in the food, pharmaceutical, and chemical industries makes their use as bioactive components cost-prohibitive. A more economical alternative is the agro-industrial side and waste streams, which often possess similar bioactive properties.

In a study conducted in 2019, various agro-industrial residues were analyzed, and different hydroalcoholic concentrations were evaluated. The findings revealed that guava leaf extract exhibited remarkable antimicrobial and antioxidant properties at a hydroalcoholic ratio of 50/50 (v/v). The extract showed broad-spectrum efficacy by effectively inhibiting Gram-positive bacteria (*Listeria monocytogenes* ATCC 19115, *Bacillus subtilis* ATCC 662, *Enterococcus* sp., *Staphylococcus* sp.) as well as Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella enterica serotype* Enteritidis ATCC 13076, *Klebsiella* sp., *Pseudomonas* sp.) [21]. Based on these findings, the present study aimed to develop and characterize a bioactive film composed of a chitosan-gelatin mixture with guava leaf extract. This film is intended as a bio-based, bioactive packaging material to extend the shelf life of meat.

2. Materials and methods

2.1. Plant materials and extracts

The guava (*Psidium guajava* L.) variety Calvillo was collected from the Mexican city of Uruapan (19°25′16″N, 102°3′47″W). During summertime, the arboreal leaves of Guava were harvested from trees of different ages in the countryside. Only intact leaves were sampled.

Plant extracts were made according to Archundia et al. [2019] Hydroalcoholic plant extracts were made. Hydroalcoholic extractions were produced with 1 g of guava leaves per 8 mL of ethanol-water mixture (50/50 vol/vol). Ethanol was sourced from Fermont (Monterrey, Mexico). Leaves were ground, stored (72 h, room temperature) in amber flasks (50 mL screw-capped glass bottles), then placed in a water bath (39 °C, 30 min), followed by filtration using Whatman N45 filter paper. Before using it, the extract was stored in the amber flasks at 4 °C.

2.2. Development of gelatin–chitosan biopolymeric films without extract and with guava extract

The following reagents were used in this study: Bloom 290 Type A gelatin from beef and pork skin and hide (Coloidales Duché, Mexico City, Mexico), chitosan from shrimp exoskeleton (Sigma-Aldrich, USA), purified glycerol (99.0 %; Sigma-Aldrich, USA), Tween 80™ (Sigma-Aldrich, USA), and acetic acid (99 %, analytic grade; Fermont, Monterrey, Mexico).

For the formulation of the polymeric base, a factorial experimental design of 3 × 3 random mixtures is used: glycerol (0, 5, 10 mL), chitosan (0, 2 and 4 g), and gelatin (0, 8 and 16 g), evaluating the formation of biofilms by the casting method and obtaining the best results with the combination of chitosan (2 g), gelatin (8 g), and glycerol (5 mL), which were used to make the biofilms as described below.

Two types of films were prepared: a gelatin-chitosan film without extract (BF) and a gelatin-chitosan film with hydroalcoholic guava leaf extract (BFGE), using the "casting" method with some modifications [22]. To prepare the chitosan solution, 2 g of chitosan whereas dissolved in 100 mL of distilled water, 2 mL of acetic acid were dissolved in 100 mL of distilled water, and 2 mL of acetic acid was added to create an acidified medium (pH 3.5). The solution was heated to 75 °C and combined with 8 g of gelatin, followed by stirring for 10 min at a temperature of 75 °C. Subsequently, the temperature was reduced to 30 °C, and 5 mL of glycerol and 0.6 mL of Tween 80 were added. The mixture stirred for an additional 10 min at 30 °C.

Half of the prepared mixture was then activated with 4 mL of hydroalcoholic guava leaf extract (50/50 v/v). Both mixtures (with and without extract) were degassed under vacuum for 1 h at 30 °C. Approximately 20 mL of each mixture was poured into 10 × 15 cm^2 tempered glass molds. The films were dried in a forced-air chamber for 24 h at 30 °C (Figs. 1 and 2.)

Fig. 1. Biopolymeric films without extract (BF). Size 10 cm × 15 cm.

Fig. 2. Biopolymeric film with guava extract (BFGE). Size 10 cm × 15 cm.

2.3. Characterization biopolymers films gelatin–chitosan biopolymeric films without extract and with guava extract

2.3.1. Thickness of the biofilms

Biofilm thickness was measured using a digital hand micrometer (Mitutoyo IP 65, Model 293-348-30 Mituyoto Corp., Kawasaki-shi, Japan) with a 0. 5–1 μm precision. For this analysis, three replicates of measurements from 10 randomly placed locations on the film were taken [23].

2.3.2. Mechanical properties

The tensile strength (TS), elongation at break (EAB), and Young's modulus (E) were measured using a texture analyzer TA.XT2 plus (Stable Micro Systems, United Kingdom) with a load of 49.3 N, cells equipped with traction grips (model A/TG). Grip separation was established at 30 mm, and the cross-speed was 2 mm/s. TS and E were evaluated for 10 assay samples (20 mm × 150 mm strip) for each film [24].

2.3.3. Optical properties

2.3.3.1. Color (L, a*, and b*).* Color measurement was carried out using a Chromameter CR-400 colorimeter (Konica Minolta, Osaka, Japan), which uses a D65 illuminator and a diffuse illumination system and 0° angle of vision deploying the CIELAB color system, which is based on lightness (L^*, *lightness*), redness (a^*, red-green), and yellowness (b^*, yellow-blue). The total difference in color (ΔE) was calculated using the following equation [25]:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^{*2}) + (\Delta b^{*2})} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* stand for the differences between the parameters corresponding to each sample color and the white paper used as the background to take measurements, which presented the following values: $L^* = 89.99$, $a^* = -0.23$, $b^* = -8.94$. For this analysis, measurements were taken from 10 randomly placed spots on the film for each replicate [25].

2.3.3.2. Transparency. Transparency was calculated using spectrophotometry. Absorbance was measured at 600 nm using a UV–Vis spectrophotometer (model G10s UV–Vis, Genesys, Madison, USA) and the following equation [26]:

$$T = \frac{A_{600}}{M} \quad (2)$$

Where A_{600} stands for the absorbance at 600 nm and M for the thickness of the film (mm). Higher T numbers, therefore, describe films with lower transparency [25]. For this analysis, 10 randomly placed measurements were made for each replicate [27].

2.3.4. The barrier properties

2.3.4.1. Permeability to water vapor. Water vapor permeability (WVP) was determined using the gravimetric method. A total of 5 mL of distilled water was placed into an aluminum dish (6 cm in diameter), which was then sealed with the biofilm. The dish was maintained at a constant temperature of 25 °C, assuming a relative humidity (RH) of 100 % inside and approximately 0 % outside the dish. The ambient humidity was not directly measured; however, the experiment was conducted in a chamber containing a desiccant, ensuring nearly zero relative humidity on the outer side of the film [27].

The weight loss of the dish was measured hourly for 8 h, and the results were expressed in units of $g \cdot mm \cdot Pa^{-1}\ h^{-1}\ m^{-2}$. The WVP was calculated using the following equation:

$$WVP = \frac{GL}{At\Delta p} \quad (3)$$

- G represents the weight loss of the dish (g) during the test,
- L is the film thickness (mm),
- A is the total test area (m^2),
- t is the test duration (s)
- Δp is the pressure difference between the inside and outside of the dish (Pa).

2.3.4.2. Oxygen permeability. An M8001 oxygen permeability analyzer was used (Systech Illinois, Thame, United Kingdom) at 60 % relative humidity and 23 °C. A 5-cm^2 sample of each film was placed in each cell of the test. The samples had previously been purged with nitrogen and humidity-balanced before exposure to an oxygen flow of 10 mL/min [28,29].

2.3.5. Scanning electron microscopy

A scanning electron microscope was used (Model 6510LV, JEOL, Japan) to obtain surface images from 4 mm^2 pieces of film at a 13 mm working distance using secondary electron signals [30,31].

2.3.6. Thermogravimetric analysis and differential scanning colorimetric analysis

For thermogravimetric analysis (TGA) and differential scanning colorimetry (DSC), an SDT Q600 analyzer (TA instruments, USA) was used with a 25 °C–600 °C temperature ramp at 10 °C/min, using nitrogen as the purge gas [26,32].

2.4. Shelf life of bovine meat covered gelatin–chitosan biopolymeric films without extract and with guava extract

Microbiological analysis and color analysis were performed using a 4 × 5 bi-factorial experimental design. Treatment was considered the first factor: meat without film (Meat-Control); meat with commercial plastic film [Ziploc®] (Meat-CPF)) meat with biopolymeric film (Meat-BF); and meat with biopolymeric film and guava leaf extract (Meat-BFGE). The second factor was shelf life: 0, 3, 6, 9, 12, or 15- days (Fig. 3).

2.4.1. Microbiological analysis

To determine shelf life, 100 g of meat samples (*Longissimus dorsi*) were obtained from the municipal slaughterhouse of Toluca, Mexico State. The samples were placed in sterilized 10 × 15 × 5 cm^3 aluminum trays, which were covered as per the experimental design described.

A destructive method was used, where samples of meat were ground using an immersion blender (model MXC/250, Oster), and filtered with sterile gauze. Relevant dilutions were performed as described in NOM-110-SSA1-1994 [33].

2.4.1.1. Aerobic mesophilic and psychrophilic bacteria. To evaluate the total count of aerobic mesophilic (AM) and psychrophilic (PS) bacteria, a counting plating technique was used. All dilutions were inoculated in triplicate on standard counting agar. Plates were incubated at 35 °C ± 2 °C for 48 ± 1 h for AM bacteria and at 4 °C for 5–10 days for PS bacteria [34].

2.4.1.2. Total coliforms. All samples were seeded in duplicate on violet and red bile agar. Plates were incubated at 35 °C ± 2 °C for 24 ± 1 h [35] before visual colony counting.

2.4.1.3. Fecal coliforms. The French Association norm was used for the total count of fecal coliform (FC), seeding in triplicate on violet, red bile agar at 45 °C ± 2 °C for 48 ± 1 h [36].

2.5. Statistic analysis

All analyses were performed in triplicate, and the data were analyzed using the statistical program SAS (SAS Institute, USA)- [37]. Statistically significant results obtained for the different samples were assessed via the analysis of variance (ANOVA) with a 95 % significance level ($P \leq$

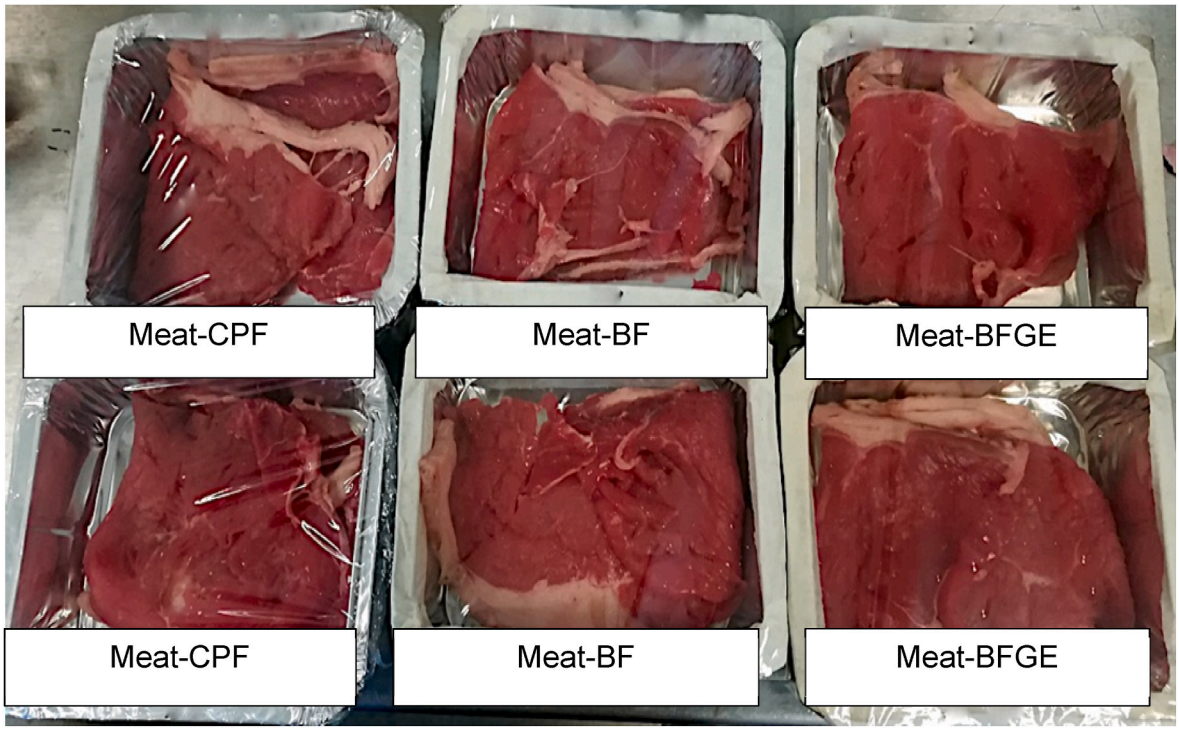

Fig. 3. Shelf-life treatment (commercial plastic film (Meat-CPF), chitosan-gelatin biopolymers film (Meat-BF), chitosan-gelatin biopolymers film with guava leaf extract (Meat-BFGE)).

0.05) and a multiple comparison test (Tukey).

3. Results and discussion

3.1. Thickness and mechanical properties (tensile strength, Young's modulus

The thickness of both films was measured to be less than 0.1 mm, with no statistically significant differences observed between BF and BFGE ($P < 0.05$) (Table 1). These measurements align with the specifications for plastic films as defined by the ASTM D88-02 standard [38]. The tensile strength (TS) test revealed values ranging from 24.74 to 27.12 MPa, with a significant reduction in TS observed in BFGE films compared to BF ($P < 0.05$). This reduction can be attributed to the distribution of extracted micelles within the material, which disrupts the interactions between gelatin and chitosan molecules in the film matrix. This disruption weakens the structural network, resulting in fewer stable bonds and causing the films to fracture under lower applied forces [39]. Despite this, the tensile strength values obtained in this study were higher than those reported by Wang et al. [40], which ranged from 3.10 to 24.88 MPa in films made from unmixed proteins or carbohydrates. However, the values were lower than those reported by Wang et al. [26] for starch–gelatin films (54.53–63.01 MPa), and those found in biopolymer mixtures containing cellulose, chitosan, and polyvinyl alcohol (27.75–78.48 MPa), as well as in chitosan–gelatin and chitosan–gelatin blend films (38.73–76.79 MPa) [41,42]. Regarding elongation at break (EAB) and Young's modulus, both BF and BFGE films exhibited elongation values close to 47 %, with no significant differences. Comparable elongation values (approximately 49 %) were observed in previous studies that analyzed chitosan–gelatin polymeric biofilms (50/50 w/w) [43]. Variations in these properties can be influenced by factors such as the source and composition of the chitosan, as well as the preparation and storage conditions of the biofilms.

3.2. Optical properties

For packaging, optical appeal is a critical property. The film displays the contained product and should be homogeneous and transparent [44].

3.2.1. Color measurements

The results of the color (L^*, a^*, b^*, and ΔE) and transparency tests are shown in Table 2. In both, a significant difference ($P < 0.05$) was observed between BF and BFGE. Color measurements of BF were closer to the white standard paper alone ($L^* = 93.68$, $a^* = 1.93$, $b^* = -1.87$) than BFGE. BF ($L^* = 90.7$) was more luminous than BFGE ($L^* = 78.8$). By contrast, a^* and b^* values for BF were lower than those for BFGE, indicating that BFGE films have slightly increased red and blue tones in the CIELAB color space. This result quantifies the slight grayish-brown color appearance in Figs. 1 and 2, which is likely due to plant pigments in the extract.

Regarding transparency, BF demonstrated higher transparency values compared to BFGE. While transparent bio-based films are generally desirable for highlighting the favorable visual qualities of food products, this increased transparency can influence the color stability of meat on the shelf. Myoglobin, the pigment responsible for the red color of meat, degrades when exposed to light. However, studies suggest that active biofilms with plant-derived pigments, such as BFGE, may counteract this effect. Although these pigments reduce transparency, they provide antioxidant properties that help minimize the degradation and oxidation of meat, thereby preserving its quality during storage [45].

Table 1
Results of mechanical tests and thickness of biopolymeric films without extract vs. film with guava extract.

Film	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
BF	0.08 ± 0.10^a	27.12 ± 0.68^a	47.37 ± 3.86^a
BFGE	0.09 ± 0.01^a	24.74 ± 0.68^b	47.20 ± 2.70^a
SE	0.001	0.574	0.687
P value	0.0537	0.0263	0.9473

The values are averages ± standard deviations. Values with different letters (a, b) in the same column show significant differences between biopolymeric films ($P < 0.05$), Legend: film without extract (BF), film with guava extract (BFGE), Standard error (SE), Value of significance (P value).

3.3. Permeability properties

3.3.1. Water vapor permeability

The driving force for moisture transfer between a packaged food and its surroundings is the vapor pressure difference between the inner and outer sides of the biofilm. In the case of hydrophilic films, WVP was dependent not only on the relative humidity difference but also on the absolute humidity [46]. Concerning the values in this study for WVP, no significant difference ($P < 0.05$) between BF and BFGE (Table 3) was found. Another study reported values of 0.932–1.884 g mm Pa^{-1} h^{-1} m^{-2} in mammal- and fish-derived gelatin of that work [47], a higher permeability to water vapor than our films (8.95 a 9.29×10^{-8} g mm Pa^{-1} h^{-1} m^{-2}) was found, for comparable thickness. By contrast, films of chitosan combined with tapioca starch have a much lower WVP ($2.8 \pm 0.3 \times 10^{-10}$ g mm Pa^{-1} h^{-1} m^{-2}) [48]. Chitosan with higher molecular weight formed a stronger barrier to water vapor ($0.62–1.27 \times 10^{-10}$ g mm Pa^{-1} h^{-1} m^{-2}) [49]. This could be explained by the stronger water barrier properties of chitosan compared to gelatin. Because our biofilms were prepared using gelatin and chitosan, it is reasonable that their vapor-barrier qualities yielded intermediate values between gelatin- and chitosan-based films. For the BFGE, there was no improvement in the vapor-barrier properties, in contrast to reports where film permeability decreased with the addition of plant essential oils [50,51].

3.3.2. Oxygen permeability

There was no significant difference between BF and BFGE ($P < 0.05$) in the permeability value of oxygen (Table 3). The range of values was $9.7–9.10 \times 10^{-18}$ m^3 m^2 $s^1 \cdot Pa^1$. These are higher barrier values compared with other studies, e.g. Figueroa-López et al. [28], which reported values of $13.8 \pm 1.7 \times 10^{-15}$ m^3 m^2 $s^1 \cdot Pa^1$ in a gelatin film. This effect may be due to an improvement of the barrier properties when gelatin and chitosan are combined compared with when they are used separately.

3.4. Morphology of biopolymeric films

Scanning Electron Microscopy (SEM) was applied to visualize the cross-section of different biopolymeric films of BF and BFGE (Figs. 4 and 5). Smooth surfaces were observed. They appeared uniform and homogenous, except for some particles of chitosan, which did not impact on the properties of the film. The BFGE (Fig. 5) showed dark spots, which were identified as micelles. These structures are due to hydrophobic compounds from plants. These can be bioproducts, migrating from plant extracts. Similar results were obtained from films made from gelatin, chitosan, and boric acid [52]. Another study looked into the chitosan-gelatin nanocomposite films [53]. The presence of particles in the cross-section of the film can confirm component distribution inside the biopolymeric film.

3.5. Thermogravimetric analysis and differential scanning calorimetric analysis

The results of the thermogravimetric analysis (TGA) are presented in Fig. 6 (BF) and Fig. 7 (BFGE), illustrating four distinct temperature-related mass loss events in both polymeric films.

First stage: The first thermal event occurred at approximately 100 °C,

Table 2
Results of physical values of biopolymeric films without extract vs. film with guava extract.

Film	Color parameters				Transparency
	*L**	*a**	*b**	*ΔE**	
BF	90.7 ± 0.2[b]	0.38 ± 0.3[b]	7.53 ± 1.4[a]	11.2 ± 3.[1a]	0.79 ± 0.1[a]
BFGE	78.8 ± 1.8[a]	2.40 ± 0.3[a]	13.81 ± 1.5[b]	19.1 ± 3.7[b]	1.18 ± 0.1[b]
SE	0.76	0.18	0.87	1.99	0.12
P value	0.0004	0.0004	0.0069	0.0497	0.0775

Values are averages ± standard deviations. Values with different letters (a, b) within the same column show significant differences between biopolymeric films ($P < 0.05$), luminosity (L^*), red (a^*), yellow (b^*), difference in color ΔE*, film without extract (BF), film with guava extract (BFGE), Standard error (SE), value of significance (P value).

Table 3
Results of oxygen and water vapor permeability values of biopolymeric films without extract vs. film with guava extract.

Film	Water vapor permeability ($g \cdot mm \cdot Pa^{-1} \cdot h^{-1}\ m^{-2}$)	Oxygen permeability ($m^3 \cdot m^2 \cdot s^1 \cdot Pa^1$)
BF	$9.29 \pm 1.09 \times 10^{-18a}$	$9.7 \pm 3.5 \times 10^{-18a}$
BFGE	$8.95 \pm 1.06 \times 10^{-18a}$	$9.10 \pm 0.97 \times 10^{-18a}$
SE	5.19×10^{-9}	1.07×10^{-18}
P value	0.6587	0.6996

Values are averages ± standard deviations. Values with different letters (a, b) within the same column show significant difference differences between biopolymeric films ($P < 0.05$), film without extract (BF), film with guava extract (BFGE), Standard error (SE), level of significance (P value). The same column shows significant differences ($P < 0.05$) between films without extract (BF), between films without extract (BF), and films with guava extract (BFGE).

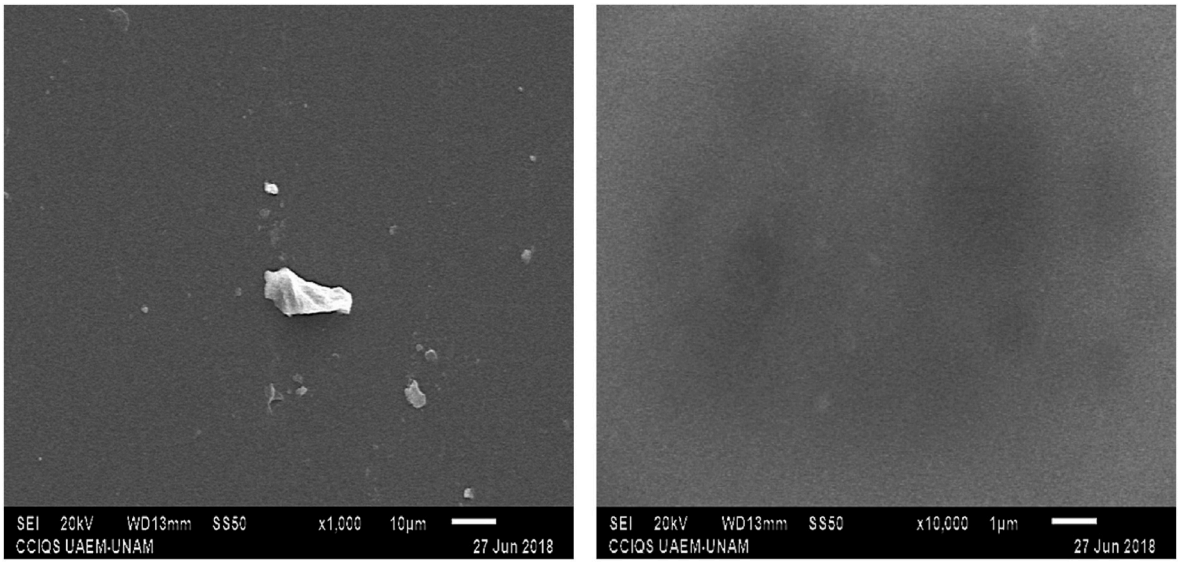

Fig. 4. (A) Micrography biopolymeric film without extract (BF) to 10 µm. Fig. 4 (B) Micrography biopolymeric film without extract (BF) to 10 µm.

with a weight loss of 9.58 % BF and 8.11 % BFGE. This mass loss is attributed to water evaporation. Second stage: The second mass loss was observed between 230 and 250 °C, with weight reductions of 35.1–34.10 % for BF and BFGE, respectively. This stage corresponds to the degradation of low molecular weight proteins in gelatin and the loss of glycerol, a plasticizer with a boiling point of 290 °C. Third stage: The third thermal event occurred at approximately 400 °C, with weight losses of 28.80 % for BF and 31.15 % for BFGE. This stage is likely associated with the thermal degradation of carbohydrates in the polymer, specifically chitosan. Final stage: In the final stage, between 500 and 510 °C, both films exhibited a weight loss of approximately 25 %. This loss is attributed to the degradation of high molecular weight proteins and carbohydrates and the non-volatile components of Tween 80™. Tween 80™ is a polysorbate surfactant and emulsifier consisting of polyethoxylated sorbitan and oleic acid and contributes to the degradation profile at this stage. However, BFGE did not show normal behavior because there is no differentiation in the fusion phase (Fig. 7). As noted above, it is suggested that the addition of hydroalcoholic extract makes films less thermostable, probably because hydrophobic compounds obstruct the interactions between molecules in the gelatin-chitosan network of the film. Some studies have demonstrated how structural modifications affect thermal behavior. For instance, when chitosan and gelatin interact, crosslinking enhances thermal stability due to stronger intermolecular bonds. However, introducing hydrophobic components may disrupt these interactions, reducing stability by limiting hydrogen bonding and polymer cohesion [54,55].

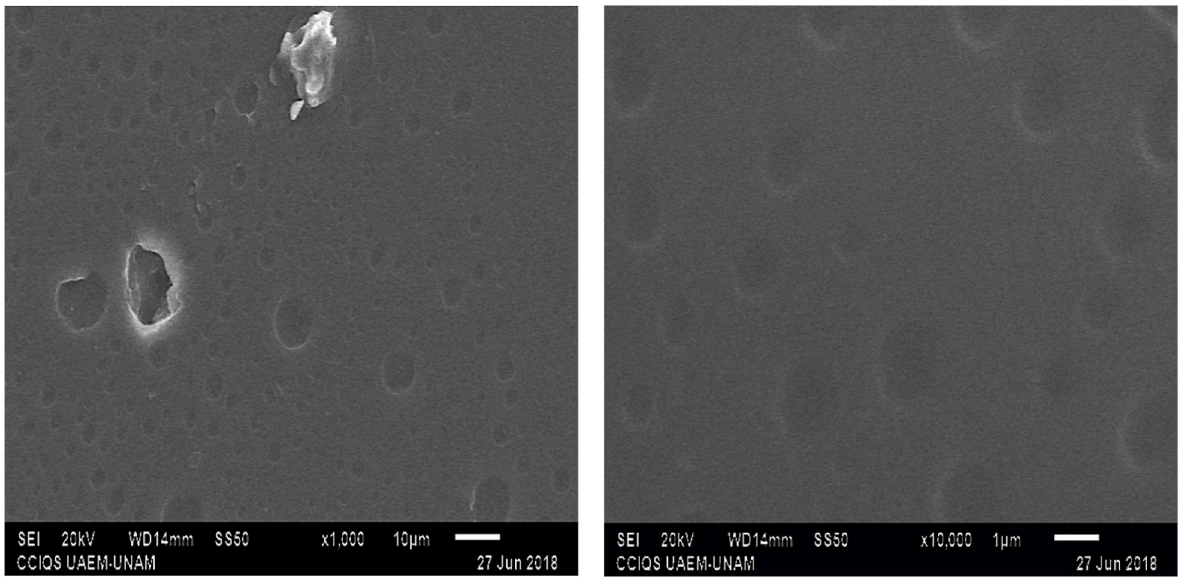

Fig. 5. (A) Micrography film with guava extract (BFGE) 10 µm. Fig. 5 (B) Micrography film with guava extract (BFGE) to 1 µm.

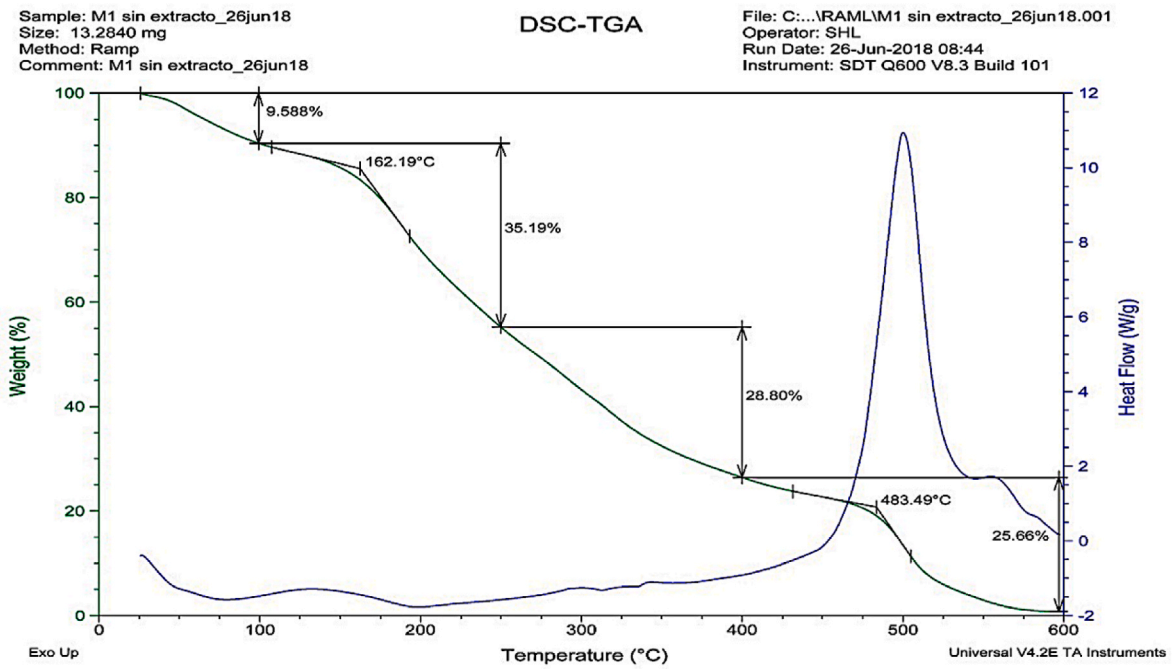

Fig. 6. DSC-TGA for the biopolymeric film without extract (BF).

3.6. Shelf life of bovine meat covered by gelatin–chitosan biopolymeric films with and without extract of guava

3.6.1. Microbiological analysis

3.6.1.1. Aerobic mesophilic bacteria. There were no significant differences ($P < 0.05$) among Meat-Control, Meat-CPF, Meat-BF, and Meat-BFGE treatments on day 0 in terms of AM (4.06–4.25 CFU/mL), CT (3.74–3.90 CFU/mL), or PS (2.65–3.01 CFU/mL) microorganisms, indicating that the microbial load was equivalent at the start of the experiment (Table 4). By day 9, the Meat-Control sample, which lacked a protective biofilm, exhibited cold-induced damage that negatively impacted its physicochemical properties, marking the end of its shelf life. For the remaining treatments (Meat-CPF, Meat-BF, and Meat-BFGE), the useful shelf life extended to day 15. The treatments displayed similar trends on days 3 and 6, with significant differences ($P < 0.05$) observed among groups. Quantitative microbial load results (CFU/mL) are detailed in Table 4. This effect is attributed to the antimicrobial and antifungal properties of chitosan [14]. Notably, Meat-BFGE demonstrated even stronger microbial inhibition due to the inclusion of hydroalcoholic guava leaf extract. On day 9, Meat-BFGE had the lowest microbial count, while Meat-Control exhibited the highest proliferation. At this stage, Meat-CPF and Meat-BF did not differ significantly. By day 12, a significant difference ($P < 0.05$) persisted among

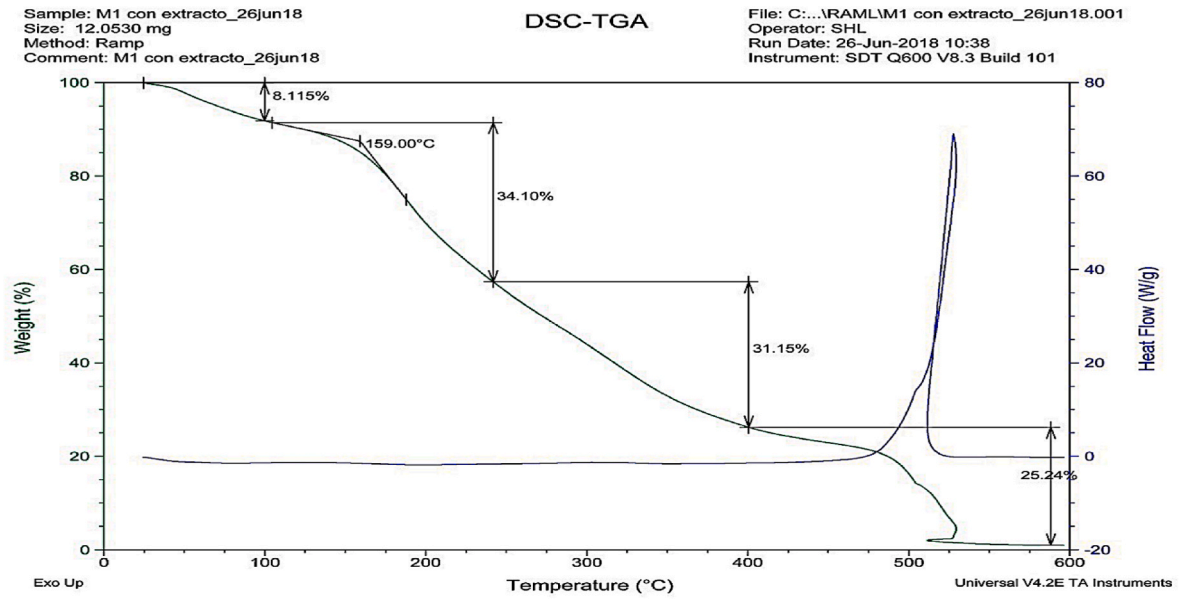

Fig. 7. DSC-TGA for the biopolymeric film with guava leaf extract (BFGE).

Table 4
Results of microbiological analysis over the shelf life of meat covered with polymeric commercial film vs. biopolymeric films without extract vs. film with guava extract.

Treatments					
	Meat-Control	Meat-CPF	Meat-BF	Meat-BFGE	*P* value
Aerobic mesophilic (log^{10} UFC/ml)					
0	4.25 ± 0.3^{aA}	$4.13 \pm 0.1b^{aA}$	4.17 ± 0.1^{aB}	4.06 ± 0.5^{aB}	0.5125
3	4.62 ± 0.05^{cB}	4.51 ± 0.04^{cAB}	3.59 ± 0.07^{bA}	3.07 ± 0.3^{aA}	0.0001
6	4.64 ± 0.16^{cB}	4.58 ± 0.02^{cB}	4.36 ± 0.13^{bB}	3.87 ± 0.1^{aB}	0.0000
9	5.47 ± 0.24^{cC}	5.20 ± 0.20^{abB}	5.34 ± 0.04^{bC}	5.08 ± 0.12^{aC}	0.0030
12	–	5.58 ± 0.02^{cD}	5.40 ± 0.03^{bC}	5.08 ± 0.04^{aC}	0.0000
15	–	5.31 ± 0.05^{aC}	5.19 ± 0.21^{aC}	5.04 ± 0.27^{aC}	0.1702
P value	0.0001	0.0001	0.0001	0.0001	
Total Coliforms (log^{10} UFC/ml)					
0	3.57 ± 0.13^{aA}	3.90 ± 0.17^{aA}	3.74 ± 0.20^{abB}	3.87 ± 0.15^{aB}	0.5036
3	4.44 ± 0.03^{cB}	3.75 ± 0.21^{bA}	2.73 ± 0.30^{aA}	2.35 ± 0.62^{aA}	0.0004
6	4.84 ± 0.06^{cC}	4.56 ± 0.06^{bB}	4.54 ± 0.05^{bC}	3.6 ± 0.06^{aB}	0.0000
9	5.38 ± 12^{cD}	4.75 ± 0.06^{bB}	4.80 ± 0.02^{bCD}	4.34 ± 0.14^{aBC}	0.0002
12	–	4.69 ± 0.05^{bB}	4.84 ± 0.15^{bCD}	4.67 ± 0.03^{aC}	0.0028
15	–	5.33 ± 0.02^{bC}	5.02 ± 0.04^{abD}	4.77 ± 0.07^{aC}	0.0000
P value	0.0001	0.0001	0.0001	0.0001	
Psychrophiles (log^{10} UFC/ml)					
0	3.01 ± 0.7^{aA}	2.65 ± 0.52^{aA}	2.65 ± 0.52^{aA}	2.69 ± 0.32^{aA}	0.8192
3	5.30 ± 0.17^{bB}	5.57 ± 0.38^{cB}	5.42 ± 0.03^{bcB}	4.90 ± 0.23^{aB}	0.0297
6	$5.59 \pm 0.06a^{BC}$	5.88 ± 0.02^{bB}	5.9 ± 0.02^{bC}	5.69 ± 0.09^{aAB}	0.0005
9	5.97 ± 0.12^{aABC}	6.63 ± 0.10^{cCD}	6.5 ± 0.12^{cC}	6.3 ± 0.02^{bCD}	0.0004
12	–	7.17 ± 0.02^{bCD}	7.3 ± 0.09^{bD}	6.9 ± 0.01^{aDE}	0.0000
15	–	7.82 ± 0.15^{bD}	8.1 ± 0.18^{bE}	7.39 ± 0.01^{aE}	0.0000
P value	0.0001	0.0001	0.0001	0.0001	

Lowercase letters (a, b, c, d) show significant differences in the growth of microorganisms between treatments for the day (among rows). Upper case letters (A, B, C, D, E) show significant differences in the growth of microorganisms during the shelf life: 0, 3, 6, 9, 12, 15 (among columns). Colony forming units (UFC), meat with no film (Meat-Control), commercial plastic film (Meat-CPF), chitosan-gelatin biopolymers film (Meat-BF), chitosan-gelatin biopolymers film with guava leaf extract (Meat-BFGE), level of significance (*P value*). Spoiled meat (−).

treatments, with Meat-BFGE continuing to exhibit the lowest CFU/mL and Meat-Control the highest. However, by day 15, no significant differences ($P < 0.05$) were detected among treatments samples' sensorial and physicochemical properties had deteriorated to levels unsuitable for human consumption, defining the end of their respective shelf life.

3.6.1.2. Total and fecal coliforms. Results of the FC results are not shown because no FC was found during the 15-day shelf life. For TC, the results on day 3 exhibited similar behavior to AM (Table 4). There was a significant difference ($P < 0.05$), where Meat-BFGE had the strongest antimicrobial effect over the 15 days of useful shelf life. It is important to note that Meat-BF and Meat-BFGE, in AM and TC variables, showed a significant effect ($P < 0.05$) regarding quantification per day. Interestingly, on day 3, some CFU values were lower than those on day 0, which is the initial load of the product. This suggests the antimicrobial effects of chitosan in the composition of the films and given that the treatment with guava leaf extract had even lower bacterial proliferation, an additional impact of the guava leaf extract can be inferred. This effect lasted for a limited period: at the end of 15 days, the results were similar among treatments. However, this is beneficial, considering that meat is a

perishable food and its life in supermarkets is limited. Thus, the film with guava leaf extract can maintain the product because of its bioactive antimicrobial properties, these results are consistent with those reported in another study [23].

3.6.1.3. Psychrophilic bacteria. Results were similar to other microorganisms regarding the 15-day shelf life, as well as Meat-BFGE showed the least proliferation compared with Meat-Control, Meat-PCF, and Meat-BF, corroborating the antimicrobial and antifungal effect of guava leaf extract (Table 4).

3.6.2. Physical analysis

3.6.2.1. Color (L, a*, and b*).* The results for the color variables *L**, a*, and *b** are presented in Table 5. At the start of the experiment day 0, no significant differences were observed among the treatments. Regarding the *L** values, significant differences ($P < 0.05$) were detected on days 3, 6, 9, 12, and 15 of shelf life, forming two distinct groups: Meat-Control and Meat-PCF, which were darker, compared to Meat-BF and Meat-BFGE. A similar trend was observed for the *a** values, where Meat-BF and Meat-BFGE exhibited less pigment degradation (myoglobin) throughout the shelf life, compared to Meat-Control and Meat-PCF. Notably, Meat-BFGE demonstrated more stable red coloration due to reduced pigment degradation. For *b** values, no significant differences were noted among treatments from days 0–9 (Table 5). However, on days 12 and 15, significant differences emerged. Meat-BFGE exhibited the highest *b** values, indicating a shift toward yellow tones. Importantly, a coloration shift toward green would indicate spoilage. These findings highlight the positive effect of the active biofilm in preserving meat color, allowing it to retain its original appearance for a longer duration. The results of this research align with findings from studies evaluating the use of essential oils, such as thyme and clove, in meat preservation. The results of this research are aligned with findings from a study that evaluated the use of essential oils, such as thyme and clove, in meat preservation. This study concludes that such oils, rich in secondary metabolites with strong antioxidant properties, effectively reduce lipid and protein oxidation. This mechanism helps to maintain the sensory and physicochemical quality of meat during storage, prolonging shelf life and preserving color and texture [56].

4. Conclusions

The packaging industry, spanning food and non-food applications, remains primarily dominated by fossil, non-degradable plastics. According to the waste management hierarchy, prioritizing reduction, reuse, and recycling are essential. However, contemporary meat packaging often employs multilayer films, which, combined with contamination issues, significantly limit the recyclability of such packaging materials. Furthermore, the low economic value of plastics and the prevalence of "on-the-go" packaging designs, improper waste disposal habits, and insufficient infrastructure lead to substantial amounts of plastic waste entering natural ecosystems. Due to their non-degradable properties, these materials persist in the environment, breaking down into micro- and nanoplastics, posing severe ecological and health challenges. Thus, exploring alternative biodegradable packaging solutions is imperative to address these environmental concerns. This study focused on developing and characterizing gelatin–chitosan films, both with and without guava leaf extract, to evaluate their potential as sustainable packaging materials. The films exhibited desirable mechanical properties, surface hydrophobicity, color stability, and effective barrier functions against oxygen and water vapor. Notably, adding guava leaf extract did not significantly alter key physical attributes such as thickness, elongation at break, water vapor permeability, or oxygen permeability. Similarly, thermal properties, analyzed through thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), remained comparable between films with and without the extract. Morphological analyses further confirmed structural consistency, with no adverse changes detected in the film matrix.

Nevertheless, incorporating guava leaf extract led to a measurable reduction in tensile strength (TS). Scanning electron microscopy (SEM) revealed the presence and homogeneous distribution of micelles derived from the extract, highlighting its uniform integration within the

Table 5
Results of color analysis over the shelf life of meat covered with polymeric commercial film vs. biopolymeric films without extract vs. film with guava extract.

Treatments					
	Meat-Control	Meat-CPF	Meat-BF	Meat-BFGE	*P* value
Lightness (*L**)					
0	40.22 ± 1.95^{aD}	39.53 ± 0.64^{aE}	40.13 ± 0.60^{aC}	38.64 ± 0.62^{aD}	0.3457
3	34.11 ± 0.59^{aC}	32.23 ± 1.49^{aD}	39.58 ± 1.45^{bBC}	36.28 ± 1.29^{bD}	0.0028
6	26.53 ± 0.74^{aB}	27.91 ± 0.22^{bC}	35.54 ± 2.41^{dB}	31.26 ± 0.73^{cC}	0.0003
9	22.70 ± 0.78^{aA}	25.83 ± 0.88^{bB}	29.44 ± 0.57^{cA}	31.20 ± 0.56^{cBC}	0.0000
12	–	24.72 ± 0.4^{aB}	29.07 ± 0.60^{bA}	30.04 ± 0.68^{bB}	0.0000
15	–	22.89 ± 0.34^{aA}	28.84 ± 0.40^{bA}	27.77 ± 1.01^{bA}	0.0000
P value	0.0001	0.0001	0.0001	0.0318	
Redness (*a**)					
0	26.07 ± 2.47^{aC}	23.50 ± 0.51^{aF}	24.46 ± 0.7^{abF}	25.7 ± 0.53^{bcD}	0.4508
3	20.98 ± 2.26^{aB}	19.27 ± 1.01^{aE}	21.61 ± 0.24^{aE}	21.11 ± 1.66^{aC}	0.0525
6	13.46 ± 1.12^{aA}	15.51 ± 1.17^{abD}	16.89 ± 0.63^{bD}	20.02 ± 0.8^{cC}	0.0001
9	12.58 ± 1.65^{aA}	12.27 ± 0.75^{aC}	15.26 ± 0.54^{bC}	16.86 ± 0.81^{cB}	0.0134
12	–	6.01 ± 1.64^{aB}	14.13 ± 0.07^{bB}	14.85 ± 0.08^{cA}	0.0001
15	–	3.36 ± 0.47^{aA}	8.90 ± 1.37^{bA}	14.05 ± 0.7^{cA}	0.0000
P value	0.0001	0.0001	0.0003	0.0001	
Yellowness (*b**)					
0	$20.20 \pm 0.17^{*C}$	19.21 ± 1.51^{aE}	17.52 ± 0.33^{aE}	19.42 ± 2.34^{aC}	0.2045
3	19.93 ± 2.64^{aB}	17.28 ± 1.16^{aE}	16.51 ± 1.75^{aE}	18.15 ± 0.24^{aC}	0.1502
6	$12.58 \pm 2.03^{*A}$	15.04 ± 0.60^{aD}	11.86 ± 1.11^{aC}	13.65 ± 1.47^{aB}	0.1012
9	11.37 ± 1.36^{aA}	11.8 ± 0.67^{aC}	11.50 ± 0.07^{aC}	11.63 ± 2.21^{aAB}	0.9280
12	–	9.89 ± 0.64^{aB}	9.22 ± 0.63^{aB}	10.8 ± 0.4^{cA}	0.0318
15	–	7.53 ± 0.49^{aA}	7.78 ± 1.1^{aA}	9.98 ± 0.69^{bA}	0.0303
P value	0.0001	0.0001	0.0001	0.0001	

Lowercase letters (a, b, c, d) show substantial differences in color analysis between treatments for the day (among rows). Upper case letters (A, B, C, D, E) show significant differences in color analysis during the shelf life: 0, 3, 6, 9, 12, 15 (among columns). Colony forming units (UFC), meat with no film (Meat-Control), commercial plastic film (Meat-CPF), chitosan-gelatin biopolymers film (Meat-BF), chitosan-gelatin biopolymers film with guava leaf extract (Meat-BFGE), level of significance (*P value*). Spoiled meat (–).

biopolymer matrix. Importantly, guava leaf extract demonstrated bioactive benefits, particularly in extending the shelf life of bovine meat. This was evidenced by decreased microbial proliferation and enhanced color retention, contributing to preserving the meat's physicochemical qualities over extended periods compared to untreated packaging.

Gelatin–chitosan biopolymer films represent a promising, eco-friendly alternative to traditional packaging materials for meat and other perishable goods. These films have potential applications that extend beyond current practices, including integration into established manufacturing processes like extrusion for cast or blown films and thermoplastics for heat-sealable designs. Research into biodegradable packaging solutions, such as foamed trays, is an additional opportunity for innovation.

Further exploration of bioactive plant-based compounds can expand the development of active packaging and edible films that enhance food preservation and quality. These advances must align with sustainable design principles, minimizing environmental impact while meeting industrial feasibility and consumer demand. Adopting such technologies could drive significant progress toward sustainable food packaging solutions.

CRediT authorship contribution statement

Enrique D. Archundia Velarde: Writing – original draft, Validation, Formal analysis, Conceptualization; María D. Mariezcurrena Berasain, Raúl A. Morales Luckie: Dora I. Medina Medina: Gisela Velázquez Garduño: Investigation and data validation: Maximilian Lackner: Writing, review and editing; Abdelfattah Z.M. Salem: Supervision, Funding acquisition, data validation, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101555.

Data availability

Data will be made available on request.

References

[1] N.R.W. Geiker, H. C Bertram, H. Mejborn, L.O. Dragsted, L. Kristensen, J. R. Carrascal, S. Bügel, A. Astrup, Meat and human health—current knowledge and research gaps, Foods 10 (7) (2021) 1556, https://doi.org/10.3390/foods10071556.

[2] V.H. de Vargas, L.D. F Marczak, S.H. Flôres, et al., Advanced technologies applied to enhance properties and structure of films and coatings: a review, Food Bioprocess Technol. 15 (2022) 1224–1247, https://doi.org/10.1007/s11947-022-02768-6.

[3] P.N.T. Pilapitiya, A.S. Ratnayake, The world of plastic waste: a review, Cleaner Materials 11 (2024) 100–220, https://doi.org/10.1016/j.clema.2024.100220.

[4] S.A. Siddiqui, N.A. Bahmid, S.H.M. Salman, A. Nawaz, N. Walayat, G. K. Shekhawat, A.A. Nagdalian, Migration of microplastics from plastic packaging into foods and its potential threats on human health, Adv. Food Nutr. Res. 103 (1) (2022) 313–359, https://doi.org/10.1016/bs.afnr.2022.07.002.

[5] M. Lackner, M. Anindya, K. Martin, What are "bioplastics"? Defining renewability, biosynthesis, biodegradability, and biocompatibility, Polymers 15 (24) (2023) 4695, https://doi.org/10.3390/polym15244695.

[6] P. Gupta, B. Toksha, M. Rahaman, A review on biodegradable packaging films from vegetative and food waste, Chem. Rec. 22 (7) (2022) e202100326, https://doi.org/10.1002/tcr.202100326.

[7] M. Ramos, A. Valdés, A. Beltrán, M.C. Garrigós, Gelatin-based films and coatings for food packaging applications, Coatings 6 (1) (2016) 41, https://doi.org/10.3390/coatings6040041.

[8] P. Cazón, G. Velazquez, J.A. Ramírez, M. Vázquez, Polysaccharide-based films and coatings for food packaging, Food Hydrocolloids 68 (1) (2016) 136–148, https://doi.org/10.1016/j.foodhyd.2016.09.009.

[9] D.Z. Šuput, V.L. Lazić, S.Z. Popović, N.M. Hromiš, Edible films and coatings – sources, properties and application, Food Feed Res 42 (1) (2015) 11–22, https://doi.org/10.5937/FFR1501011S.

[10] N. Kaur, C. Somasundram, Z. Razali, A.I. Mourad, F. Hamed, Z.F. R Ahmed, Aloe vera/chitosan-based edible film with enhanced antioxidant, antimicrobial, thermal, and barrier properties for sustainable food preservation, Polymers 16 (2) (2024 Jan 15) 242, https://doi.org/10.3390/polym16020242. PMID: 38257041; PMCID: PMC10821446.

[11] Q. Wang, M. Li, P. Wang, K. Wang, L. Fang, F. Ren, G. Lu, X. Lu, The interaction of chitosan and BMP-2 tuned by deacetylation degree and pH value, J. Biomed. Mater. Res., Part A 107 (1) (2019) 769–779, https://doi.org/10.1002/jbm.a.36593.

[12] P. Sahariah, M. Másson, Antimicrobial chitosan and chitosan derivatives: a review of the structure-activity relationship, Biomacromolecules 18 (1) (2017) 3846–3868, https://doi.org/10.1021/acs.biomac.7b01058.

[13] D. Yan, Y. Li, Y. Liu, N. Li, X. Zhang, C. Yan, Antimicrobial properties of chitosan and chitosan derivatives in the treatment of enteric infections, Molecules 26 (23) (2021) 7136, https://doi.org/10.3390/molecules26237136.

[14] A. Yarahmadi, B. Dousti, M. Karami-Khorramabadi, H. Afkhami, Materials based on biodegradable polymers chitosan/gelatin: a review of potential applications, Front. Bioeng. Biotechnol. 12 (1) (2024) 1397668, https://doi.org/10.3389/fbioe.2024.1397668.

[15] T. Zhang, J. Xu, Y. Zhang, X. Wang, J.M. Lorenzo, J. Zhong, Gelatins as emulsifiers for oil-in-water emulsions: extraction, chemical composition, molecular structure, and molecular modification, Trends Food Sci. Technol. 106 (1) (2020) 113–131, https://doi.org/10.1016/j.tifs.2020.10.005.

[16] Y. Lu, Q. Luo, Y. Chu, N. Tao, S. Deng, L. Wang, L. Li, Application of gelatin in food packaging: a review, Polymers 14 (3) (2022) 436, https://doi.org/10.3390/polym14030436.

[17] Z.A. Nur Hanani, Y.H. Roos, J.P. Kerry, Use and application of gelatin as potential biodegradable packaging materials for food products, Int. J. Biol. Macromol. 71 (1) (2012) 94–102, https://doi.org/10.1016/j.ijbiomac.2014.04.027.

[18] H. Wang, F. Ding, L. Ma, Y. Zhang, Edible films from chitosan-gelatin: physical properties and food packaging application, Food Biosci. 40 (1) (2021) 100871, https://doi.org/10.1016/j.fbio.2020.100871.

[19] D. Šuput, S. Popović, N. Hromiš, S. Bulut, V. Lazić, Biopolymer films properties change affected by essential oils addition promene karakteristika biopolimernih filmova uzrokovane dodatkom esencijalnih ulja, JFST 23 (2) (2019) 61, https://doi.org/10.5937/jpea1902061S.

[20] S. Khan, A.A. Abdo, Y. Shu, Z. Zhang, T. Liang, The extraction and impact of essential oils on bioactive films and food preservation, with emphasis on antioxidant and antibacterial activities—a review, Foods 12 (22) (2023) 4169, https://doi.org/10.3390/foods12224169.

[21] V.E. D Archundia, D.L. M Pinzón, A.Z. M Salem, P.G.G. Mendoza, M.D. B. Mariezcurrena, Antioxidant and antimicrobial capacity of three agroindustrial residues as animal feeds, Agrofor. Syst. 94 (1) (2019) 1393–1402, https://doi.org/10.1007/s10457-018-00343-7.

[22] D. Sánchez, L.J.C. Contreras-Esquive, G.V. Nevárez-Moorillón, C.N. Aguilar, Caracterización de películas comestibles a base de extractos pécticos y aceite esencial de limón Mexicano, Int. J. Food Prop. 13 (1) (2015) 17–25, https://doi.org/10.1080/19476337.2014.904929.

[23] Y.A. Arfat, J. Ahmed, N. Hiremath, R. Auras, A. Joseph, Thermo-mechanical, rheological, structural and antimicrobial properties of bionanocomposite films based on fish skin gelatin and silver-copper nanoparticles, Food Hydrocolloids 39 (1) (2014) 58–67, https://doi.org/10.1016/j.foodhyd.2016.08.009.

[24] ASTM, Standard test methods for tensile properties of thin plastic sheeting designation: d 882 – 02, Annu. Book ASTM (Am. Soc. Test. Mater.) Stand. (2002) 1–10, https://doi.org/10.1520/E0096.

[25] M. Ahmad, N.M. Hani, N.P. Nirmal, F.F. Fazial, N.F. Mohtar, S.R. Romli, Optical and thermo-mechanical properties of composite films based on fish gelatin/rice flour fabricated by casting technique, Prog. Org. Coating 84 (1) (2015) 115–127, https://doi.org/10.1016/j.porgcoat.2015.02.016.

[26] W. Wang, K. Wang, J. Xiao, Y. Liu, Y. Zhao, A. Liu, Performance of high amylose starch-composited gelatin films influenced by gelatinization and concentration, Int. J. Biol. Macromol. 94 (1) (2016) 258–265, https://doi.org/10.1016/j.ijbiomac.2016.10.014.

[27] ASTM, Standard Test Methods for Water Vapor Transmission of Materials, 2005. E 96/E 96M – 05 1–8.

[28] K.J. Figueroa-Lopez, J.L. Castro-Mayorga, M.M. Andrade-Mahecha, L. Cabedo, J. M. Lagaron, Antibacterial and barrier properties of gelatin coated by electrospun polycaprolactone ultrathin fibers containing black pepper oleoresin of interest in active food biopackaging applications, Nanomaterials 8 (1) (2018) 1–13, https://doi.org/10.3390/nano8040199.

[29] K.J. Figueroa-Lopez, M.M. Andrade-Mahecha, O.L. Torres-Vargas, Development of antimicrobial biocomposite films to preserve the quality of bread, Molecules 23 (1) (2018) 1–18, https://doi.org/10.3390/molecules23010212.

[30] K.J. Figueroa-López, A.J. García-Salcedo, O. Torres-Vargas, O.L.T. H Ariza-calderón, Efecto de PVA y pimienta negra en las propiedades estructurales de películas de gelatina Effect of PVA and black pepper in the structural properties of gelatin films, Agron. Colomb. 34 (1) (2016) 156–160, https://doi.org/10.15446/agron.colomb.v34n1supl.58220.

[31] M.M. Andrade-Mahecha, D.R. Tapia-Blácido, F.C. Menegalli, Development and optimization of biodegradable film based on achira flour, Carbohydr. Polym. 8 (2012) 449–458, https://doi.org/10.1016/j.carbpol.2011.12.024.
[32] L.F. Wang, J.W. Rhim, Preparation and application of agar/alginate/collagen ternary blend functional food packaging films, Int. J. Biol. Macromol. 80 (2015) 460–468, https://doi.org/10.1016/j.ijbiomac.2015.07.007.
[33] NOM-110-SSA1-1994, Norma Oficial Mexicana, Bienes y servicios, Preparación y Dilución de Muestras de Alimentos para su Análisis Microbiológico, Diario Oficial de la Federación México DF, México, 1994.
[34] NOM-092-SSA1-1994, Norma Oficial Mexicana, Bienes y Servicios. Método para la cuenta de bacterias aerobias en placa, NOM-092-SSA1-1994 SSA, Diario Oficial de la Federación, México, DF, 1994, p. 8.
[35] NOM-113-SSA1-1994, Norma Oficial Mexicana, Bienes y Servicios, Método para la cuenta de microorganismos coliformes totales en placa, Diario Oficial de la Federación, México DF, México, 1994.
[36] AFNOR, Microbiology of food and animal feedings stuffs. Enumeration of Thermotolerant Coliforms by Colony-Count Technique at 44°C, Routine Method, Association Française de Normalisation, Paris, France, 1996.
[37] SAS Institute, SAS user's guide: statistics. Ver 9.0, SAS Institute, Cary, NC, USA, 2002, p. 956.
[38] ASTM, Standard test methods for tensile properties of thin plastic sheeting designation: d 882 – 02, Annu. Book ASTM (Am. Soc. Test. Mater.) Stand. (2002) 1–10, https://doi.org/10.1520/E0096.
[39] K.K. Sadasivuni, P. Saha, J. Adhikari, K. Deshmukh, M.B. Ahamed, J.J. Cabibihan, Recent advances in mechanical properties of biopolymer composites: a review, Polym. Compos. 41 (1) (2020) 32–59, https://doi.org/10.1002/pc.25356.
[40] L. Wang, L. Liu, J. Holmes, J.F. Kerry, J.P. Kerry, Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films, Int. J. Food Sci. Technol. 42 (2007) 1128–1138, https://doi.org/10.1111/j.1365-2621.2006.01440.x.
[41] P. Cazón, M. Vázquez, G. Velazquez, Novel composite films based on cellulose reinforced with chitosan and polyvinyl alcohol: effect on mechanical properties and water vapour permeability, Polym. Test. 69 (2018) 536–544, https://doi.org/10.1016/j.polymertesting.2018.06.016.
[42] N. Benbettaïeb, M. Kurek, S. Bornaz, F. Debeaufort, Barrier, structural and mechanical properties of bovine gelatin–chitosan blend films related to biopolymer interactions, J. Sci. Food Agric. 94 (12) (2014) 2409–2419, https://doi.org/10.1002/jsfa.6570.
[43] S.F. Hosseini, M. Rezaei, M. Zandi, F.F. Ghavi, Preparation and functional properties of fish gelatin–chitosan blend edible films, Food Chem. 136 (3–4) (2013) 1490–1495, https://doi.org/10.1016/j.foodchem.2012.09.081.
[44] J.H. Han, Innovations in Food Packaging, Elsevier, 2005.
[45] C.E. Figueroa-Enríquez, F. Rodríguez-Félix, S. Ruiz-Cruz, D.D. Castro-Enriquez, H. Gonzalez-Rios, J.Á. Perez-Alvarez, I.Y. López-Peña, Application of active packaging films for extending the shelf life of red meats: a review, Processes 12 (10) (2024) 1–25, https://doi.org/10.3390/pr12102115.
[46] M.A. Bertuzzi, E.C. Vidaurre, M. Armada, J.C. Gottifredi, Water vapor permeability of edible starch-based films, J. Food Eng. 80 (3) (2007) 972–978, https://doi.org/10.1016/j.ijbiomac.2024.130165.
[47] M. Jiménez, M. Domínguez, Películas comestibles formuladas con polisacáridos: propiedades y aplicaciones, TSIA 6 (2012) 110–121.
[48] M.B. Vásconez, S.K. Flores, C.A. Campos, J. Alvarado, L.N. Gerschenson, Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings, Food Res. Int. 42 (2009) 762–769, https://doi.org/10.1016/j.foodres.2009.02.026.
[49] J.M. Moura, B.S. Rodrigues D, A. Moura, G.M. Dotto, L.A. Pinto, Preparation of chitosan with different characteristics and its application for biofilms production, J. Polym. Environ. 2 (2015) 470–477, https://doi.org/10.1007/s10924-015-0730-y.
[50] J.F. Martucci, L.B. Gende, L.M. Neira, R.A. Ruseckaite, Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films, Ind. Crop. Prod. 71 (2015) 205–213, https://doi.org/10.1016/j.indcrop.2015.03.079.
[51] F.M. Pelissari, M.V.E. Grossmann, F. Yamashita, E.A.G. Pined, Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil, J. Agric. Food Chem. 57 (2009) 7499–7504, https://doi.org/10.1021/jf9002363.
[52] S. Ahmed, S. Ikram, Chitosan and gelatin based biodegradable packaging films with UV-light protection, J. Photochem. Photobiol., B 163 (2016) 115–124.
[53] M. Rezaee, G. Askari, Z.E. Djomeh, M. Salami, Effect of organic additives on physiochemical properties and anti-oxidant release from chitosan-gelatin composite films to fatty food simulant, Int. J. Biol. Macromol. 114 (2018) 844–850, https://doi.org/10.1016/j.ijbiomac.2018.03.122.
[54] S.A. Mir, B.N. Dar, A.A. Wani, M.A. Shah, Effect of plant extracts on the techno-functional properties of biodegradable packaging films, Trends Food Sci. Technol. 80 (2018) 141–154, https://doi.org/10.1016/j.tifs.2018.08.004.
[55] Y.A. Shah, S. Bhatia, A. Al-Harrasi, F. Oz, M.H. Khan, S. Roy, A. Pratap-Singh, Thermal properties of biopolymer films: insights for sustainable food packaging applications, Food Eng. Rev. 1 (2024) 1–16, https://doi.org/10.1007/s12393-024-09380-8.
[56] X. Chen, S. Shang, F. Yan, H. Jiang, G. Zhao, S. Tian, Y. Dang, Antioxidant activities of essential oils and their major components in scavenging free radicals, inhibiting lipid oxidation and reducing cellular oxidative stress, Molecules 28 (11) (2023) 45–59, https://doi.org/10.3390/molecules28114559.