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Research Article

Conservation of commercial quality and bioactive compounds of guava pieces by application of an alginate-acemannan coating

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Abstract

The objective of this study was to evaluate the effect of an edible coating based on alginate and acemannan on the commercial quality and concentration of bioactive compounds of guava pieces stored in refrigeration. Pieces of guava were coated with an alginate film enriched with acemannan (A-Ac), with samples without cover used as a control. The fruit pieces with and without the A-Ac coating were stored for 12 days at 6 ± 2 °C. Analyses of colour, firmness, ethylene production, ascorbic acid, total polyphenols, antioxidant potential, and polyphenol profile were performed at 0, 3, 6, 9, and 12 days of storage. In addition, a sensory analysis was carried out after nine days of refrigeration. The coated guava pieces retained firmness at 2.8 N, while in the uncoated pieces, firmness decreased to 1.3 N. Similarly, the coated pieces showed better colour retention (ΔE<9) and lower ethylene emission during storage. Also, the content of ascorbic acid, total polyphenols, antioxidant capacity, and the concentration of individually identified polyphenols were higher in the pieces of guava coated with the A-Ac film. In addition, coated guava pieces, stored for nine days in refrigeration, showed greater sensory acceptability compared to uncoated samples. Therefore, applying the A-Ac cover could be a good alternative for conserving sensory attributes and bioactive compounds of guava pieces stored at low temperatures.

Keywords: acemannan; edible coating; fresh-cut; guava; sodium alginate

Introduction

Guava is an exotic fruit highly appreciated for its creamy texture and characteristic aroma. World production of guava in 2020 was 54.7 million tons, with Mexico being the fourth largest producer (Tridge, 2020). This fruit contains high levels of vitamin C (~190 mg / 100 g) and is an essential source of pectin, dietary

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fiber, and minerals (Nora *et al.*, 2014; Yadav *et al.*, 2022). In addition, it contains bioactive compounds such as carotenoids, flavonoids, and polyphenols. These compounds are considered secondary metabolites of plants that possess antioxidant activity by capturing free radicals produced during oxidative stress (Peretto *et al.*, 2017). This property has been associated with significant health benefits for consumers, such as anti-inflammatory, anti-cancer, and hypoglycemic activity (Mascaraque *et al.*, 2014; Szymanowska *et al.*, 2015; Castro-Acosta *et al.*, 2016). Some bioactive compounds found in guava are gallic acid, chlorogenic acid, ellagic acid, catechin, and rutin (Santos *et al.*, 2017).

The consumption of minimally processed products has increased significantly due to changes in the lifestyle of consumers, who demand ready-to-eat products with quality, freshness, and easy acquisition (Qadri *et al.*, 2015). However, this processing accelerates the metabolism of vegetables and increases the rate of respiration, darkening, softening, and the production of unpleasant aromas and tastes. In addition, open-cut fruit are more susceptible to microbial contamination, which significantly reduces the shelf life of these products (Benítez *et al.*, 2015). These losses of minimally processed products can exceed $9 billion (Botondi *et al.*, 2021). Therefore, processing to increase their shelf life is necessary to preserve the quality of the products and decrease losses. One of the preservation methods to improve the shelf life of these products include sanitization and disinfection with hypochlorite, hydrogen peroxide, organic acids, and ozone. Yet, both of these have not been effective enough. In recent years, innovative packaging and edible films have been developed and used for this purpose (Jideani *et al.*, 2017; Mostafidi *et al.*, 2020).

Specifically, edible covers can be a promising alternative to preserve fruits and vegetables' physical, chemical, nutritional, and sensory properties. This thin layer provides a barrier to mass and gas transfer, resulting in lower respiration and dehydration rates and prevention of loss of compounds responsible for color (Maringgal *et al.*, 2020).Edible films can be made from polysaccharides, proteins, and lipids, among others (Maringgal *et al.*, 2020). Sodium alginate is a polysaccharide obtained from seaweed composed of β-D-manuronic acid and α-L-guluronic acid (Acevedo *et al.*, 2012). This compound is widely used to prepare edible coatings, providing an excellent barrier to lipids, gases, and water vapor (Blanco-Pascual *et al.*, 2014). In addition, sodium alginate coatings have been enriched with ascorbic acid, oregano oil, or thyme, among others, which improves the properties of the cover by preserving the color, inhibiting microbial activity, and increasing the antioxidant potential of the coated vegetables (Robles-Sánchez *et al.*, 2013; Sarengaowa *et al.*, 2019; Tabassum and Khan, 2020).

In the same way, aloe vera gel, AVG, has been investigated as a form of edible coating (Mendy *et al.*, 2019; Alkaabi *et al.*, 2022).The gel is obtained from the aloe vera leaves (*Aloe barbadensis* Miller) and has shown a tremendous therapeutic, pharmaceutical, and cosmetic importance. Aloe vera coatings can maintain color, decrease enzyme activity, and prevent the coated fruit's weight loss and firmness (Supapvanich *et al.*, 2016).In addition, it is well known that the consumption of aloe vera gel has health benefits (Martínez-Burgos *et al.*, 2022). These benefits are attributed to the presence of acemannan, which is considered to be the main functional component of aloe vera gel. Acemannan is a polysaccharide composed mainly of partially acetylated mannose, glucose, and galactose units (Minjares-Fuentes *et al.*, 2017). Acemannan has excellent activity in cell regeneration and modulation of the immune system (Kumar and Kumar, 2019). In addition, it allows the transport of other bioactive compounds through the intestinal epithelium, improving their absorption in the intestine (Chokboribal *et al.*, 2015). It has been reported to exhibit antioxidant and antimicrobial effects (Kumar and Kumar, 2019).

However, although AVG has been used as a coating material, there are some challenges regarding its application as an edible covering. AVG has low film-forming abilities, and in the research, it has been used in different amounts and concentrations (Maan *et al.*, 2021). Depending on the species of the plant and the conditions in which it is obtained, AVG contains biologically active compounds such as anthraquinones (aloin and emodin), which can cause toxicity and genotoxicity (Guo and Mei, 2016). These compounds are associated

with diarrhea, hypokalemia, *Pseudomelanosis coli*, renal failure, phototoxicity, and hypersensitivity reactions (Guo and Mei, 2016). In addition, the concentration of acemannan differs between crops due to factors such as the plant's variety, age, and environmental stress (Minjares-Fuentes *et al*., 2017). Then, the acemannan incorporation into the formulation of an edible coating could help in the standardization of filmmaking, improve the product's functional properties and eliminate the presence of possible toxic compounds.

Therefore, the objective of the present study is to evaluate the effect of the application of an edible coating based on sodium alginate and enriched with acemannan, on the physicochemical properties, production of ethylene, ascorbic acid, total polyphenols, antioxidant activity, bioactive compounds and acceptability of pieces of guava stored in refrigeration.

Materials and Methods

Materials

Sodium alginate, soy lecithin, and Tween 80 were supplied by Golden Bell (México, D.F.), carnauba wax from Multiceras® S.A. de C.V. (Nuevo León, México). Ascorbic acid, acetic acid, calcium chloride, sorbitol, glycerol, ethylene standard, phosphoric acid, monobasic sodium phosphate, Folin-Ciocalteu reagent, gallic acid standards, cinnamic acid, vanill, catechin gallate, rutin, ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), DPPH (1,1-difenil-2-picrilhidrazil), potassium persulfate, Trolox, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), water, and methanol HPLC grade were acquired from Sigma-Aldrich (Louis MI, USA). EDTA was acquired from ProductosQuímicos Monterrey, S.A. (Nuevo León, México) and metaphosphoric acid from Merck (México, D.F.). Glycerol, acetic acid, and hydrochloric acid came from J.T. Baker, (Phillipsburg, N.J. USA), sodium carbonate, 96% ethanol, and distilled water from Analytyka® S.A de CV (Nuevo León, México).

Plant material

The guava used in this study was collected in August-September in Calvillo (Durango, Mexico; 25° 32′ 10″ N/103° 31′ 28″ W). Fruits free of any physical damage and microbial contamination and at commercial maturity were selected and washed with 0.002% sodium hypochlorite solution.

Aloe vera (*Aloe barbadensis* Miller) leaves, used as a raw material, were supplied by the "Instituto Tecnológico Superior de la Región de los Llanos" (Guadalupe Victoria, Durango, Mexico), corresponding to 3-year-old plants.

Extraction of acemannan

Approximately 200 g of aloe vera gel was mixed with 1.4 L of ethanol and homogenized at 13,000 rpm for 2 min with ULTRA-TURRAX T18 (IKA® Works, Inc., Wilmington, USA). The mixture was heated to boiling for 5 min, homogenized for 1 min and filtered through GF/C grade glass microfiber to obtain alcohol-insoluble solids (AIS); the procedure was repeated twice. The AIRS were rinsed with acetone and dried at room temperature for 24 hours. Subsequently, 200 mg of AIRS were mixed with 300 mL of distilled water and homogenized for 1 min with Ultraturrax at 13,000 rpm; the mixture was stirred for 2 h at 300 rpm and centrifuged at 4500 rpm for 30 min. The supernatant was filtered on glass fiber, frozen and lyophilized for 72 h. The product obtained was characterized by gas chromatography and nuclear magnetic resonance to validate the presence of acemannan concentrate (Rodríguez-González *et al*., 2011).

Coating preparation

The sodium-acemannan alginate (A-Ac) coating was prepared as described by Reyes-Avalos *et al*. (2016)with modifications. First, approximately 2 g of sodium alginate was dissolved in 100 mL distilled water at 55-60 °C. Subsequently, 600 mg of sorbitol, 7 mL of glycerol, 0.7 mL of Tween 80, 0.5 mL of soy lecithin,

and 3 g of carnauba wax were added. The mixture was homogenized at 22,000 rpm for 8 min with an Ultraturrax. Then, the mixture was heated to 85±5 °C, homogenized at 22,000 rpm for 5 min, and cooled (25 °C). Subsequently, 10 mg of acemannan was added to the sodium alginate solution and mixed. The amount of acemannan incorporated depended on the concentration present in the fresh gel described previously (Minjares-Fuentes *et al.*, 2017)and the ease of handling the coating.

Characterization of A-Ac film

Opacity

The opacity of the film was finalized according to the methodology proposed by Kanatt and Makwana (2020). Opacity was calculated using the following formula:

Opacity = (600 nm)/t

Where t is the thickness of the film in mm (~0.08 mm). The results were expressed in $A \cdot mm^{-1}$.

Water vapor permeability (WVP) and mechanical properties

These properties were determined according to the methodologies proposed by Reyes-Avalos *et al.* (2016).

Coating application and storage

The guava fruits were manually cut into four parts and were randomly distributed in two batches. The first batch was taken as a control group (no coating application), and the second batch was coated with A-Ac film. The guava pieces were immersed in A-Ac emulsions for 30 seconds, and the excess was removed. Subsequently, the coating was gelled by immersion in a 2% calcium chloride solution and dried at room temperature. Guava pieces, coated and uncoated, were stored at 6 ± 2 °C and 95% RH for 12 days. Ethylene production, color, firmness, ascorbic acid concentration, total polyphenols (PT), antioxidant potential, and bioactive compound profile were evaluated at 0, 3, 6, 9, and 12 days of storage. Sensory analysis was carried out on samples with nine days of storage.

Ethylene production

The analysis was evaluated as described by Reyes-Avalos *et al.* (2016). Approximately 150 g of guava pieces were placed in a 1.2 L container for one h at 6 ± 2 °C, and subsequently, a 1 mL sample of the gases from the head space was injected into a gas chromatograph HP 6820 (Agilent Technology, CA, EUA). Ethylene analysis was performed on a Carboxen column (Supelco, PA, USA) of 30 m x 0.53 mm x 0.25 μm and a flame ionization detector; the injector was at 120 °C and the detector at 250 °C; the temperature of the furnace gradually rose from 35 °C to 120 °C. Chromatograph readings were recorded and analyzed with the software Agilent Cerity NDS (Agilent Technologies, EUA). They were compared to an ethylene standard calibration curve (R^2= 0.99). The results were expressed in μL of $C_2H_4 kg^{-1} h^{-1}$.

Color

A colorimeter was used Minolta CR-300, equipped with an included ocular component, C and 0° viewing angle geometry, used to obtain L* (luminosity), a* (greenness), and b* (yellowness). The color was measured in the pulp of 5 pieces of guava, twice in each of the pieces. The total color difference (ΔE*) was calculated using the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Firmness

A puncture method was used to evaluate the firmness in the pulp of guava pieces. The Texture Analyzer TA-XT plus (Stable Micro Systems, England), was fitted with a 2 mm cylindrical stainless-steel probe

penetrating to a depth of 8 mm at a speed of 1.5 mm s^{-1} and with a load cell of 30 kg. Firmness was measured in six pieces of guava per treatment at each storage time. All guava samples were tempered to 25 °C prior to measurement. Firmness was obtained from the average of six replications and reported in Newtons.

Ascorbic acid

Ascorbic acid extraction and analysis were performed as described by Oliveira *et al.* (2010)with modifications. Approximately 0.2 g of guava were homogenized with 12 mL of 3% metaphosphoric acid solution, 8% acetic acid, and EDTA 1 mM using T 18 digital ULTRA-TURRAX® (IKA® Works, Inc., Wilmington, USA) at 5,000 rpm for 1 min. Samples were centrifuged at 250 g for 30 min at 4 °C; the supernatant was recoated and graduated to 14 mL with HPLC water. The mixture was filtered with Millipore® 0.45 µM and injected into HPLC Agilent 1200 (Agilent Technology, Palo Alto, CA) equipped with a diode array detector, 5 µM (250 mm x 4.6 mm) Kintex C18 column and 20 µL loops. The temperature and flow rate were 25 °C and 0.5 mL/min, respectively. The mobile phase consisted of NaH_2PO_4 at 1 mM with EDTA at 1 mM at pH of 3.0. Reduced ascorbic acid was monitored at 245 nm, and the content was expressed as mg ascorbic acid/g of dry matter (dm). The calibration curve was performed with standard ascorbic acid solutions ($R^2 = 0.99$).

Analysis of polyphenolic compounds

Extraction

The phenolic compounds were extracted with the method proposed by Eim *et al.* (2013) with modifications. Approximately 0.5 g of freeze-dried guava were homogenized with 10 ml of methanol with ULTRA-TURRAX® (IKA® Works, Inc, Wilmington, USA) at 13,500 rpm for 1 min. The samples were stored in the dark and mechanically shaken for 16 h at 4 °C. Then, the samples were centrifuged (Centurion Scientific, UK) at 1520 g for 15 min and filtered. The supernatant was stored at 4 °C for further analysis. All extractions were performed in duplicate.

Total polyphenolic content

Total polyphenols were determined according to the Folin-Ciocalteu method, using 96-well microplates, as described by González-Centeno *et al.* (2012), with slight modifications. 95 uL of distilled water and 10 uL of extract were mixed in each well; subsequently, 5 uL of the Folin-Ciocalteu reagent was added. The mixture was incubated at 25 °C for 5 min, in the absence of light, and 80 uL of solution of Na_2CO_3 at 7.5% (w/v) was added. The sample was incubated for 30 min and measured absorbance at 765 nm in a Multiskan Spectrum spectrophotometer (Thermo Fisher Scientific, Vanda, Finland). A Gallic acid calibration curve was used to determine the PT content; the results were expressed as mg/g GAE dm. Analyses were performed in triplicate.

Evaluation of antioxidant capacity

The effect of the application of the edible film of A-Ac on the antioxidant capacity of guava pieces was measured. The 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays were used, as described by Reyes-Avalos et al. (2019). For both antioxidant assays, an automated microplate reader Multiskan Spectrum (Thermo Scientific, Waltham, MA USA) was used. The results were expressed as µM eq. Trolox /g dm. Analyses were performed in triplicate.

Identification and quantification of polyphenolic compounds

The phenolic compounds were extracted according to the method proposed by Eim *et al.* (2013)with modifications. 0.5 g of freeze-dried guava were homogenized with 10 mL of methanol and stirred mechanically for 16 h at 4 °C. The samples were centrifuged at 1520 g for 10 min and concentrated at 1 mL under vacuum conditions at 40 °C. The samples were filtered through a 0.45 µm PTFE filter and injected into an HPLC.

Chromatographic analysis was performed using an Agilent 1200 chromatograph (Agilent Technology, Palo Alto, CA) equipped with DAD, quaternary pump, and two LiChrospher C18 columns 5 µm (4 mm x 150 mm) (Phenomenex, Torrance, CA, USA) connected in series. The temperature, flow rate, and amount injected were 25 °C, 0.8 mL/min, and 20 µL, respectively. The mobile phase was composed of (A) 2% acetic acid solution and (B) Methanol; gradient composition was 5% (B) for 2 min; 25% (B) up to 10 min; 40, 50, 60, 70 and 80% (B) every 10 min. Phenolic compounds were analysed at three different wavelengths: 280 nm for catechins and benzoic acids, 316 nm for hydroxycinnamic acids, and 365 nm for flavonols. To identify and quantify the phenolic compounds, cinnamic and vanillic acid, catechin gallate, and rutin standards were used.

Sensory analysis

The sensory analysis of coated and uncoated guava pieces (control) was carried out following the methodology described by Reyes-Avalos *et al.* (2019). The level of pleasure was evaluated by appearance, colour, odour, firmness, flavour, and overall acceptability in 3 samples: one sample corresponding to 0 days of storage (fresh guava) and 2 samples (coated and uncoated) stored for 9 days 6 ± 2 °C. The samples were labelled with 3-digit number codes that were randomly provided to the panelists. The evaluation was conducted by 60 untrained judges and regular guava users. Drinking water was provided to the panelists to eliminate residual flavour between the samples. Each attribute was scored on a category scale labelled from "I really dislike it" to "I like it very much."

Statistical analysis

The results obtained were analysed using an analysis of variance. Whereas the difference between means was carried out using Fischer's minimum significant difference (SMD)($p \leq 0.05$). SAS version software was used 8 (SAS Institute Inc. 2005).

Results and Discussion

Characterization of the A-Ac film

Opacity and permeability to water vapor

The opacity of a film is a clue of how much light passes through the material, which might influence the product's colour, thus, the consumers' acceptance (Fakhouri *et al.*, 2015). The opacity of the A-Ac film was determined to be 10.43 ± 0.11A·mm^{-1}. This value is similar to that documented by Nazir and Wani (2022) in basil seed mucilage and sodium alginate films. However, the A-Ac film presented greater opacity than reported by Paula *et al.* (2015) (0.732-1.311) in films based on carrageenan and sodium alginate. The opacity of an edible film depends on the composition (polymer, plasticizer, lipids, etc.) and thickness (Hadi *et al.*, 2022). Coatings with a certain degree of opacity can reduce lipid oxidation and nutrient loss problems (Soukoulis *et al.*, 2014). Likewise, the A-Ac film had a water vapor permeability, WVP, of 1.51 ± 0.15 x 10^{-12} g m^{-1} h^{-1} Pa^{-1}, which is similar to the permeability reported by Reyes-Avalos *et al.* (2016) in coatings formed from alginate-chitosan. However, Bourbon *et al.* (2011) and Olivas and Barbosa-Cánovas (2008) obtained higher WVPs in films based on chitosan and alginate. Salama *et al.* (2021) showed that adding AVG to alginate-based edible film formulation improved the barrier properties, and WVP decreased. The application of edible films with low WVP values can elongate the shelf life of coated fruit and vegetables(Radev and Dimitrov, 2019).

Mechanical properties

The mechanical properties measured in the A-Ac film were the percentage of elongation, tensile force, and Young's modulus(Reyes-Avalos *et al.*, 2016). The percentage of elongation of the film A-Ac was 19.36 ± 2.10%, while the tensile strength and the modulus of Young were 0.07 ± 0.01 MPa and 0.94 ± 0.50 MPa,

respectively. Compared to an alginate-chitosan bilayer film, these values are significantly lower in tensile strength and Young's modulus, 0.96 and 6.55 Mpa, respectively, but higher in elongation percentage (16.6%) (Reyes-Avalos *et al.*, 2016).Similarly, alginate-AVG films showed much better mechanical strength than those reported here (Salama *et al.*, 2021).The differences may be due to other coating components, such as chitosan (a nanocomponent that typically increases mechanical strength) (Wang *et al.*, 2021) or essential oil that can act as a plasticizer(Song *et al.*, 2018). Regardless, as reported here, A-Ac films show adequate elongation and mechanical strength to maintain integrity during handling and storage.

Ethylene production

The ethylene results in coated and uncoated guava pieces stored at 6 ± 2 °C are shown in Figure 1. Ethylene release in coated guava pieces was lower than that of the control throughout the experiment. At the end of the storage time, they showed ~27% lower ethylene released than in the uncoated slices ($p \leq 0.05$). Guerreiro *et al.* (2017) y Ahmed *et al.* (2009)observed a reduction in ethylene production when applying alginate and AVG coatings in minimally processed fruits. The coating of A-Ac decreases the gaseous exchange of the fruit with the environment by a barrier effect. The lower production of ethylene has been correlated with lower ripening speed and susceptibility to fruit decomposition (Maqbool *et al.*, 2010).

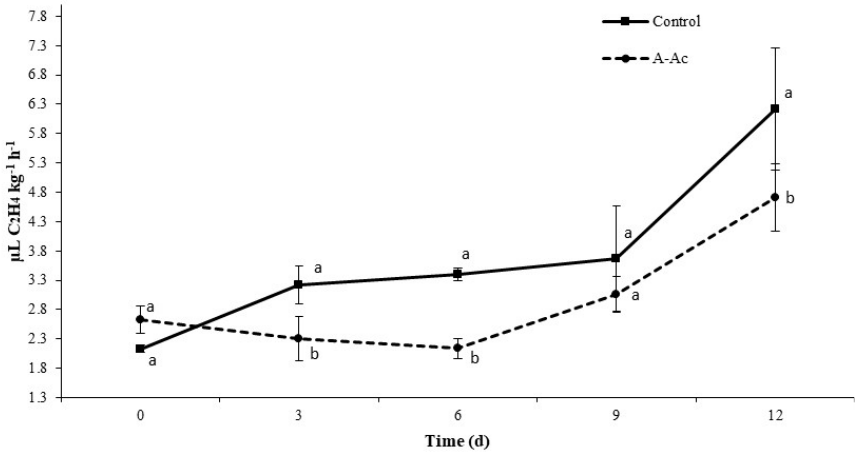

Figure 1. Ethylene production (µL kg^{-1} h^{-1}) of guava pieces coated with an A-Ac film and uncoated and stored for 12 days at 6 ± 2 °C

Different letters indicate a significant difference between means established by the MSD test ($p \leq 0.05$). Vertical bars indicate standard deviation (n = 4)

Colour changes

The colour of fruits is directly related to the perception of quality by consumers. Table 1 shows the values of L* (luminosity), a* (greenness), and ΔE (colour difference) in the pulp of cold-stored guava pieces. Here, L* decreased more in uncoated samples (~16.5%) compared to coated pieces (~9.4%) during storage. Likewise, the uncoated pieces of guava showed a more significant change in a* (~5.7) with respect to the coated samples (~1.6). These changes in the parameters mentioned suggest a more substantial darkening in the samples without the coating. As a result, coated guava pieces had lower ΔE (~9.1) than uncoated pieces (~23.9) during cold storage ($p \leq 0.05$). The results indicate that the use of the A-Ac coating helps in the retention of the colour of the guava piece pulp by acting as a physical barrier against dehydration, gas exchange, and microbial contamination, as well as by decreasing metabolism and enzymatic darkening(Figure 2) (Forato *et al.*, 2015).

These results coincide with reports in pineapple cubes(Azarakhsh *et al.*, 2014) and melon spheres(Koh *et al.*, 2017) coated with alginate, which retained the colour of the fruit for a longer time than the control.

Table 1. L*, a* and, ΔE* parameter values of coated (with an A–Ac film) and uncoated (control) guava pieces stored for 12 days at 6 ± 2 °C

	Day	Uncoated				Coated			
L*	0	74.50	±	0.83	^{aX}	73.48	±	0.41	^{aX}
	3	66.62	±	1.32	^{bX}	67.06	±	0.10	^{bX}
	6	64.71	±	0.34	^{bX}	65.86	±	0.68	^{bX}
	9	62.68	±	0.35	^{cX}	65.77	±	0.40	^{bY}
	12	62.24	±	0.41	^{cX}	67.17	±	0.45	^{bY}
a*	0	-1.55	±	0.17	^{dX}	0.11	±	0.44	^{bY}
	3	0.94	±	0.73	^{cX}	1.11	±	0.06	^{aX}
	6	2.22	±	0.45	^{bX}	1.32	±	0.07	^{aY}
	9	3.05	±	0.51	^{bX}	1.59	±	0.17	^{aY}
	12	4.16	±	0.07	^{aX}	1.80	±	0.29	^{aY}
ΔE*	0	0.00	±	0.00	^{eX}	0.00	±	0.00	^{bX}
	3	9.30	±	0.92	^{dX}	8.03	±	0.45	^{aX}
	6	13.81	±	2.11	^{cX}	9.50	±	0.95	^{aY}
	9	19.65	±	3.41	^{bX}	9.10	±	0.56	^{aY}
	12	23.90	±	2.17	^{aX}	9.06	±	0.26	^{aY}

Lowercase letters (a, b, c, d, e) indicate significant differences in storage days according to the LSD test (p≤0.05). Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test (p≤0.05) (± standard deviation; n = 4).

Figure 2. Pieces of guava (*Psidium guajava*) variety Media China after 12 days of storage at 6 ± 2°C, a: uncoated and b: A-Ac coated

Firmness

The texture of food is perhaps the main quality attribute judged by the consumer and is, therefore, extremely important in the general acceptance of the product. Generally, guava pieces coated with A-Ac film were more firm than uncoated samples (p≤0.5). The coated pieces of guava retained their texture during the cold storage period (~2.8 N). On the contrary, uncoated samples showed ~50% less firmness after 12 days of storage (~1.3 N) (Figure 3). Similar results were found in pineapple cubes coated with an alginate film, which delayed the fruit's softening (Azarakhsh *et al.*, 2014).Guava pieces coated with A-Ac film were able to retain the firmness of their tissues due to lower water loss (~38% water loss in coated fruit versus ~45% in uncoated samples) and delayed enzymatic activity related to fruit softening (Brasil *et al.*, 2012). In addition, since calcium

ions may penetrate into the fruit, it can be incorporated into the shell, interacting with the pectic acids of the fruit's cell wall, forming polymeric networks, and improving plant tissue's integrity (Oms-Oliu *et al.*, 2010; Verdini *et al.*, 2008). In the case of uncoated fruits, the highest ethylene production could accelerate the ripening process producing softer tissues (Hayama *et al.*, 2006).

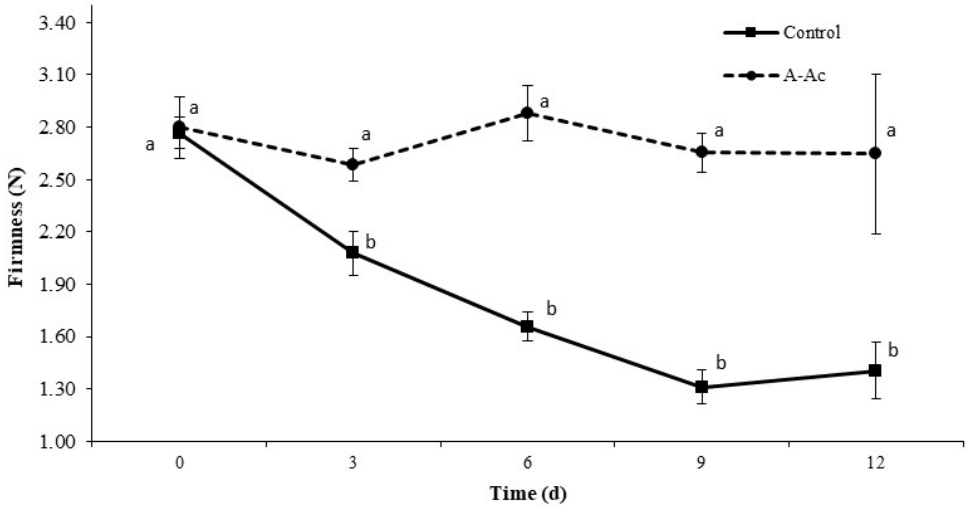

Figure 3. Changes in firmness (compressive force) of guava pieces coated with an A-Ac film and uncoated stored for 12 days at 6 ± 2 °C
Different letters indicate a significant difference between means established by the MSD test (p≤0.05). Vertical bars indicate standard deviation (n = 4)

The concentration of ascorbic acid

Typically, when an alginate coating is placed on cut fruit pieces, it creates a protective barrier that slows down oxidative processes. With time, the bio-compounds, such as ascorbic acid, that the presence of oxygen could influence are much higher in the coated samples (Ehteshami *et al.*, 2022; Montone *et al.*, 2022; Zhang *et al.*, 2016). The ascorbic acid content of coated and uncoated guava pieces, stored at 6 ± 2 °C, can be seen in Figure 4. The ascorbic acid concentration of guava samples decreased during storage (p≤0.05); however, uncoated guava pieces showed a significantly greater decrease (~64%) than coated guava pieces (~51 %) (p≤ 0.05). Chen *et al.* (2021) reported similar results when applying an edible coating of sodium alginate on freshly cut apples and retaining a higher amount of ascorbic acid than uncoated apple pieces. It is known that films made with sodium alginate can decrease the concentration of O_2 in fruit tissues (Reyes-Avalos *et al.*, 2019), which is one of the critical factors for the oxidation and loss of ascorbic acid. In addition, the A-Ac covers presented a certain degree of opacity, which decreased light penetration and hindered the degradation of this vitamin(Duncan and Chang, 2012).

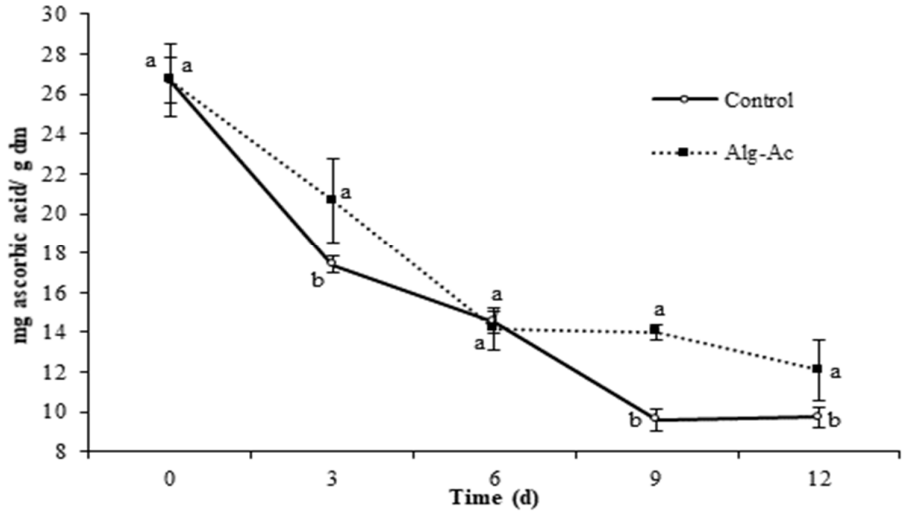

Figure 4. Ascorbic acid content (mg / g dm) of guava pieces coated with an A-Ac film and uncoated and stored for 12 days at 6 ± 2 °C.
Different letters indicate a significant difference between means established by the MSD test (p≤0.05). Vertical bars indicate standard deviation (n = 4)

Total polyphenols and antioxidant capacity

The effect of the A-Ac coating on total polyphenol content (TPC) and the antioxidant capacity (ABTS and FRAP) of guava pieces stored for 12 days at 6 ± 2 °C are listed in Table 2.

The uncoated guava pieces showed an increase and subsequent decrease of TPC regarding storage time (p≤0.05). The initial increase in TPC was probably caused by damage as a consequence of cutting, which increased respiratory rate and ethylene production, promoting the synthesis of polyphenols as a defense mechanism (Hu *et al.*, 2022; Li *et al.*, 2022). However, the prolonged storage of minimally processed fruit could promote the chemical and enzymatic degradation of phenolic compounds, decreasing the concentration of these compounds (Li *et al.*, 2022). Meanwhile, the TPC values in the coated fruits did not decrease during the period from 0 to 9 days of refrigerated storage (p≥0.05), showing higher TPC (~21.2 mg/g GAE) than uncoated fruits at day 9 (~17.0 mg/g GAE) (p≤0.05).

These results are supported by similar trends seen in other studies of the application of alginate covers in sweet cherry fruit (Díaz-Mula *et al.*, 2012) and whole guava (Nair *et al.*, 2018),concluding that the fruit coated with sodium alginate retained a higher amount of TPC. The greater presence of TPC, in the pieces of guava coated, could be due to lowered concentrations of oxygen inside the fruit, a consequence of the barrier effect to the gases of the film (Hambleton *et al.*, 2012; Reyes-Avalos *et al.*, 2019).

Likewise, the A-Ac layer significantly influenced the antioxidant capacity of guava pieces stored in refrigeration (p≤0.05). In general, it can be observed that the samples with the A-Ac coating presented a higher value of antioxidant capacity, determined by ABTS and FRAP, compared to the pieces of guava without a coating (p≤0.05). This property decreased in the samples without covers during refrigeration storage, while the guava pieces coated with the A-Ac layer showed no changes in the levels of the antioxidant capacity under the same storage conditions. Maximum antioxidant capacity values were found in the samples with the A-Ac coating (~162 and ~158 μM eq. Trolox /g dm for ABTS and FRAP, respectively), while the lowest values were presented by samples without cover (137 and 110 μM eq. Trolox /g dm for ABTS y FRAP, respectively). The results of lower antioxidant capacity in the uncoated fruit pieces are supported by various investigations of the application of edible coatings on fruit (Reyes-Avalos *et al.*, 2019; Wang and Gao, 2013). The higher antioxidant capacity in the coated fruit may be linked to the delay of the ripening processes observed in these pieces of guava

which could be attributed to reduction of the ripening rate as a consequence of decreasing of the ethylene production (see Figure 1).

Table 2. Total phenols (mg/g GAE dm), ABTS (µM eq. Trolox /g dm), and FRAP (µM eq. Trolox/ g dm) Antioxidant activity measured in methanolic extracts of coated guava pieces with an Alginate-acemannan coating and uncoated (control) guava pieces, stored for 12 days at 6 ± 2 °C

	Day	Uncoated					Coated			
Total Polyphenols	0	18.32	±	0.58	^{bcX}		20.70	±	0.73	^{aX}
	3	19.79	±	0.94	^{abX}		20.03	±	0.81	^{abX}
	6	20.89	±	0.68	^{aX}		19.10	±	0.88	^{abX}
	9	16.92	±	0.39	^{cX}		21.23	±	1.24	^{aY}
	12	17.49	±	0.93	^{bcX}		17.89	±	0.56	^{bX}
ABTS	0	155.61	±	3.19	^{aX}		152.88	±	7.97	^{aX}
	3	142.55	±	9.96	^{aX}		162.54	±	8.31	^{aX}
	6	149.29	±	4.35	^{aX}		157.95	±	8.14	^{aX}
	9	153.03	±	7.12	^{aX}		146.70	±	5.80	^{aX}
	12	137.24	±	7.95	^{aX}		160.24	±	11.43	^{aY}
FRAP	0	152.85	±	2.72	^{aX}		148.40	±	5.40	^{aX}
	3	143.99	±	11.03	^{aX}		158.57	±	12.43	^{aX}
	6	158.56	±	2.76	^{aX}		155.44	±	8.91	^{aX}
	9	143.04	±	2.76	^{aX}		146.91	±	4.62	^{aX}
	12	110.55	±	10.10	^{bX}		150.10	±	7.73	^{aY}

Lowercase letters (a, b, c) indicate significant differences in storage days according to the LSD test ($p \leq 0.05$). Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test ($p \leq 0.05$) (± standard deviation; n = 4).

Effect of A-Ac coating on polyphenolic compounds and flavonoids

In order to learn more about the different polyphenolic compounds contained in the guava pieces, methanolic extracts were analysed by HPLC-DAD study. The bioactive compounds identified and quantified in the pieces of guava not coated and coated with the A-Ac film were catechin gallate, rutin (quercetin-3-O-rutinoside), cinnamic acid, and vanillic acid. The consumption of these compounds has been linked to potential health benefits, such as antimutagenic, anti-inflammatory, antidiabetic, immune-stimulating, hepatoprotective activity, and preventing oxidative stress (Babu *et al.*, 2012; Nishimura *et al.*, 2016). Table 3 shows the influence of the A-Ac coat on the concentration of catechin gallate, rutin, cinnamic acid, and vanillic acid in guava pieces stored for 12 days at 6 ± 2 °C. Here, significant changes in the amount of these compounds can be observed by applying the A-Ac film and the passage of cold storage time ($p \leq 0.05$). Generally, guava pieces coated with the A-Ac film showed a higher concentration of these bioactive compounds than uncoated ones ($p \leq 0.05$). Specifically, samples containing the A-Ac coat had ~10%, ~22%, ~17%, and ~31% more catechin gallate, rutin, cinnamic acid, and vanillic acid, respectively, compared to uncoated guavas. Specifically, the concentration of the bioactive compounds presents in the pieces of guava with the A-Ac cover did not decrease during the period of refrigeration storage, while in the uncoated samples, they had decreases of ~30%, with the highest loss for vanillic acid at 12 days (~38% in comparison to the coated pieces). Similar results have been reported by Reyes-Avalos *et al.* (2019)when coating figs with a film of alginate-chitosan. They observed that bioactive compounds such as anthocyanins, rutin, and polyphenolic acids maintained their concentration for 15 days in refrigeration.

Likewise, Rößle *et al.* (2011) reported that the concentration of rutin in apple pieces was not affected after the application of an alginate film. These results support the idea that the A-Ac coatings act as a barrier to atmospheric gases, preventing the reaction of these bioactive compounds with oxygen and subsequent degradation. Also, applying polymeric coatings on fresh fruits can change the concentration of internal fruit gases, promoting a modified atmosphere and causing cellular metabolism changes that involve conserving these molecules (Reyes-Avalos *et al.*, 2019).

Table 3. Concentration of the phenols (μg/g dm) identified in methanolic extracts of coated guava pieces with the alginate-acemannan coating and uncoated (control) guava pieces, stored for 12 days at 6 ± 2 °C

	Day	Uncoated					Coated			
Catechin gallate	**0**	110.04	±	7.46	^{bcX}		103.18	±	10.81	^{bX}
	3	133.18	±	6.14	^{aX}		98.93	±	9.08	^{bY}
	6	103.70	±	16.75	^{cX}		163.96	±	14.62	^{aY}
	9	130.04	±	3.60	^{abX}		115.79	±	7.73	^{bX}
	12	97.05	±	36.23	^{cX}		161.46	±	9.38	^{aY}
Rutin	**0**	90.38	±	6.60	^{aX}		102.18	±	16.71	^{aX}
	3	72.30	±	3.86	^{abX}		118.67	±	8.20	^{aY}
	6	74.63	±	5.78	^{abX}		107.04	±	14.52	^{aY}
	9	79.28	±	3.72	^{abX}		70.88	±	8.88	^{bX}
	12	54.19	±	5.74	^{bX}		77.75	±	3.02	^{bX}
Cinnamic acid	**0**	2.42	±	0.10	^{aX}		2.28	±	0.08	^{abX}
	3	2.01	±	0.04	^{bX}		2.35	±	0.06	^{abY}
	6	1.58	±	0.22	^{cX}		2.37	±	0.18	^{aY}
	9	1.88	±	0.07	^{bcX}		2.05	±	0.20	^{abX}
	12	1.55	±	0.03	^{cX}		1.99	±	0.05	^{bY}
Vanillic acid	**0**	15.62	±	0.83	^{aX}		15.32	±	0.50	^{bX}
	3	11.18	±	0.52	^{bX}		15.38	±	0.56	^{bY}
	6	12.74	±	0.93	^{abX}		15.49	±	1.94	^{bX}
	9	10.70	±	1.41	^{bX}		13.77	±	0.76	^{bX}
	12	9.68	±	0.34	^{bX}		19.08	±	1.67	^{aY}

Lowercase letters (a, b, c) indicate significant differences in storage days according to the LSD test ($p \leq 0.05$). Uppercase letters (X, Y) indicate significant differences between coated and uncoated fruits according to the LSD test ($p \leq 0.05$) (± standard deviation; n = 4).

Sensory analysis

Figure 5 shows the results from the sensory attributes analysed in the coated and uncoated pieces of guava. The samples coated with the A-Ac film presented significantly higher scores in acceptability, appearance, colour, and firmness compared to the control samples ($p \leq 0.05$).

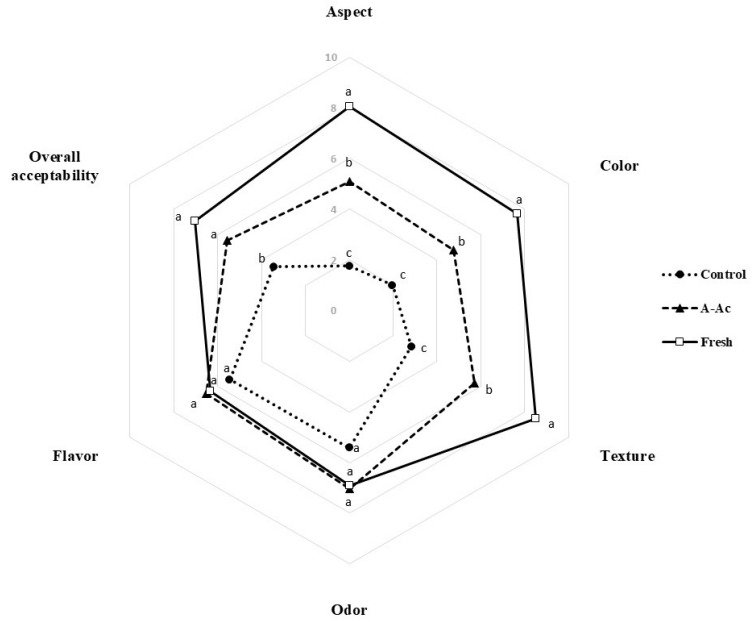

Figure 5. Comparison of the sensory quality of coated guava pieces with an A–Ac coating and uncoated (control) samples stored for 15 days at 6 ± 2 °C with fresh fruit
Means with the same letters are not significantly different according to the LSD test (p≤0.05)

Interestingly, the coated samples scored closer to the fresh guava pieces than the uncoated pieces. This difference is possibly due to the fact that the control samples showed more significant changes in colour, firmness, and weight loss compared to the coated ones. Similar results have been reported in watermelon cylinders coated with alginate and pectin (Sipahi *et al.*, 2013)and in figs coated with an alginate-chitosan film (Reyes-Avalos *et al.*, 2016). In these reports, it was observed that the coating did not affect the odour or flavour and preserved the appearance of the fruit longer.

Conclusions

The effect of the application of an alginate-acemannan (A-Ac) coating on ethylene production, colour, texture, total polyphenols, antioxidant potential, ascorbic acid, bioactive compounds, and sensory quality of guava pieces stored in refrigeration was analysed. The A-Ac coating decreased the release of ethylene and maintained the colour and texture of the guava pieces. Likewise, the use of the A-Ac cover improved the retention of ascorbic acid and bioactive compounds, particularly catechin gallate, rutin, cinnamic, and vanillic acid, which manifested itself in more substantial antioxidant potential and polyphenol content of guava pieces coated with A-Ac film. Additionally, the application of the A-Ac coating maintained, more efficiently, the sensory attributes of the fruit during storage.

Therefore, the application of the A-Ac cover can be an excellent alternative to preserve the sensory, nutritional, and functional properties of chopped guava pieces stored at low temperatures with the health benefits conferred by the consumption of acemannan, but without the possible technological and toxicological drawbacks of the use of aloe Vera gel.

Authors' Contributions

Conceptualization EALZ, RMF and JAMV; Data curation EALZ, RMF and JAMV; Formal analysis EALZ, RMF and JAMV; Funding acquisition JAMV; Investigation EALZ and JAMV; Methodology EALZ, ESC, SIBG; Project administration JAMV; Resources;JAM Software; EALZ and JAMV; Supervision JAMV, RMF; Validation ESC, JEM and SIBG; Visualization EALZ and JAMV; Writing - original draft EALZ; Writing - review and editing JAMV and JEM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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