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Active packaging employing cacao husk flour as antioxidant and antimicrobial in sodium caseinate edible film to extend vacuum-packed ham shelf-life

Empaque activo empleando harina de cáscara de cacao como antioxidante y antimicrobiano en películas comestibles de caseinato de sodio para prolongar la vida de anaquel de jamón envasado al vacío

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Abstract

The quality of processed meat products can be altered by lipid oxidation during storage. In the same manner, food safety determines no pathogenic microorganisms due to foodborne illness outbreaks. The objective of this work was to incorporate cacao husk flour into edible film formulation as an antioxidant and antimicrobial agent, to reduce oxidative rancidity and inhibit the growth of four pathogenic bacteria. Cacao husk flour was incorporated into a sodium caseinate edible film with glycerol as a plasticizer. Film thickness, soluble matter, soluble protein, water vapor permeability, mechanical properties, and surface topology by AFM were determined, and applied to ham slices inoculated with the pathogen bacteria and stored for 21 days to determine oxidative rancidity and the pathogens' specific growth rate. Edible film protein matrix was modified by cacao husk flour, resulting in a more permeable and stiffer structure, a rougher surface, and less soluble films. The application of these edible films in ham slices reduced oxidative rancidity and inhibited the growth of pathogen microorganisms. Sodium caseinate edible films with cacao husk flour are an alternative to improve shelf life, avoiding changes related to lipid oxidation and the presence of pathogenic microorganisms in foods.

Keywords: active packaging, antioxidants, oxidative stability, antimicrobial, meat products.

Resumen

El objetivo de este trabajo fue incorporar harina de cáscara de cacao en la formulación de películas comestibles como agente antioxidante y antimicrobiano, para reducir la rancidez oxidativa e inhibir el crecimiento de cuatro bacterias patógenas. Se incorporó harina de cáscara de cacao a una película comestible de caseinato de sodio con glicerol como plastificante. Se determinaron el espesor de la película, la materia soluble, la proteína soluble, la permeabilidad al vapor de agua, las propiedades mecánicas y la topología superficial mediante MFA, y se aplicaron a lonchas de jamón inoculadas con las bacterias patógenas y almacenadas durante 21 días para determinar la rancidez oxidativa y la tasa de crecimiento específica de los patógenos. La matriz proteica de la película comestible se modificó con harina de cáscara de cacao, lo que resultó en una estructura más permeable y rígida, una superficie más rugosa y películas menos solubles. Las películas comestibles de caseinato de sodio con harina de cascarilla de cacao son una alternativa para mejorar la vida útil, evitando cambios relacionados con la oxidación lipídica y la presencia de microorganismos patógenos en los alimentos.

Palabras clave: empaque activo, antioxidantes, estabilidad oxidativa, antimicrobiales, productos cárnicos.

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1 Introduction

Meat products imply the manipulation of raw material, meat or fish, in order to convert/preserve their nutritional value. Most processed meat products are safe, but most of the time during the storage period in the shelf life several changes can occur due to four main scenarios. According to Umaraw *et al*. (2020), the current trend in the development of edible films is to enhance their functionality by incorporating components with antimicrobial and antioxidant activities, thereby extending food stability during storage and inhibiting the growth of spoilage or pathogenic microorganisms, as well as retarding lipid oxidation. The edible films can be employed to incorporate bioactive substances into food surfaces for: controlling microbial population (no pathogens or spoilage microorganisms, improving beneficial bacteria proliferation, as probiotics); counteracting oxidative stress [delaying oxidation, neutralizing free radicals); after processing and packaging (coadjutant material as moisture barrier, regulating gas exchange and active carriers); and prevent certain deteriorative reactions and microbial spoilage (in high moisture content food surfaces). Recently, active packaging, as an emerging technology, has been used instead of the direct addition of active agents, specifically in the oxygen-sensitive food surfaces, to decrease the presence of reactive oxygen compounds that acts as oxidation initiators, as well as antimicrobial compounds, in order to extend food shelf life (Benbettaïeb *et al*., 2018; Shetty *et al*., 2026), most of the times irrespectively of the filmogenic biomaterial utilized to develop the film matrix. From the food engineering point of view, edible films are designed as a sustainable alternative to conventional plastics to increase shelf-life of foodstuffs. Manufactured with biopolymers as proteins and polysaccharides, present concomitantly poor mechanical and physical properties than plastics, so, the importance of edible films' physical and mechanical properties on edible film performance focused to solve food engineering problems incorporating them as active packaging systems in reusable containers.

The agro-industrial co-products, such as peels, husks, pomace, and/or pulp from fruits and vegetables' processing, can be dehydrated, ground, and sieved to obtain flours or powders that comprise both bioactive ingredients: polyphenolic compounds as antioxidants and antimicrobials, and fiber as prebiotics. There are few studies on the direct use of powders or flours from agro-industrial co-products in the development of active packaging, as compared to the obtention of extracts. For example, ultrasonicated freeze-dry dragon fruit peel powder was employed in starch-based films (Santos *et al*., 2014), pomegranate peel powder exhibited antioxidant properties and antibacterial activity against *S. aureus* in fish gelatin films (Hanani *et al*., 2018), beetroot powder enhanced antioxidant properties in pectin-based films (Sucheta *et al*., 2019), and mandarin peel powder enhanced antioxidant and antimicrobial activity in sodium alginate films (Yun *et al*., 2022). In this view, agro-industrial co-products have been employed to reinforce edible films. For example, Hernández-Carrillo *et al*. (2015) reported the use of orange and pomegranate as source of phenolic compounds in edible films. Also, Piñeros-Guerrero *et al*. (2009) employed rice husk in edible films.

Henceforward, the objective of this work was to use the cacao husk flour as a source of bioactive compounds, since its antioxidant and antimicrobial capacity was previously reported (Pacheco-Martínez *et al*., 2024), to elaborate an edible film as an active carrier to improve two of the main concerns in packed meat products, i. e., oxidative rancidity stability and pathogens presence.

2 Materials and methods

2.1 Edible film formulation

For the edible film, sodium caseinate (6%, w/v) and glycerol (2%, w/v) were dissolved in distilled water. The suspension was heated to 80 °C, cooled to room temperature, and poured into glass plates (20 cm × 20 cm). Cacao husk flour was elaborated as previously reported (Pacheco-Martínez *et al*., 2024), and, based on previous research, 0.5% (w/v) was added to the protein and glycerol solution. The film-forming solutions were set to dry for at least 24 h. Edible films from each treatment were carefully separated from the glass plates and stored in plastic bags until use.

2.1.1 Physical properties

The samples' thickness was determined with a Mitutoyo digital micrometer, taking four to six random measurements along the edible film.

The edible films' soluble matter and soluble protein was determined adapting the methodology proposed by Jangchud and Chinnan (1999). A 20 × 20 mm film sample was weighed before being submerged in 30 mL of distilled water for 24 h. The remaining sample was removed, dried at 70 °C overnight, and weighed to report the weight difference as the percentage of film solubility. From the soaking water, soluble protein (g/mL) was determined by the biuret method to calculate protein solubility as:

$$\text{Protein solubility} = \frac{\text{g of soluble protein in 30 mL}}{\text{initial weigh} \times \text{protein in film} \times \%\ \text{dry matter}} \times 100 \quad (1)$$

The water vapor permeability was determined by adapting the methodology reported by Kokoska *et al*. (2010). Edible films were fixed to the mouth of 100 mL glass jars, filled with distilled water (90 mL). Flasks were weighed every 30 min for at least 8 h, recording the weight change to calculate the moisture loss slope during the test ($\Delta m/\Delta t$, in g per s). The water vapor permeability was calculated as:

$$\text{WVP} = \frac{\Delta_m}{\Delta_t} \frac{T}{A\ \Delta_p} \quad (2)$$

Where $\Delta m/\Delta t$ is the gradient of weight lost during time, A is the mouth flask area, T is the film thickness (m), and Δp is the water vapor pressure difference between the two sides of the edible film (3,168.74 Pa at 25°C).

2.2 Mechanical properties

The puncture deformation test was carried out following Gontard *et al*. (1993) methodology. The film samples were fixed in an acrylic cell and plunged with a 3 mm aluminum probe at a speed of one mm/s in a Brookfield CT-3 (Brookfield Engineering Laboratories, Middleboro) until they broke down. The maximum force detected at break point was reported as the deformation force, and puncture deformation was calculated as the relationship between the film initial length (26.5 mm, the acrylic cell radius) and the distance traveled by the probe before break according to the Pythagoras's theorem (as the square root of initial lenght2+traveled distance2).

The tensile properties were determined according to the method reported by Gennadios *et al*. (1993). Film samples were cut 10 mm wide by 100 mm long and fixed to clamps in the same texturometer. Initial separation was 70 mm before samples were extended at 1 mm/s until breakdown. From the force-distance curves, the maximum charge detected per transversal section area was reported as the tensile strength, and the percentage of elongation as the rate of maximum distance before break and initial distance.

2.3 Surface topography

The edible films surface topography was made with atomic force microscopy, using a Nanosurf Naio AFM microscope (Nanosurf, Liestal) provided with silicon carbide tips under static force operating mode in contact mode. The average roughness (Ra) and the root mean square roughness (Rq) were determined with the Naio Control Software as the average of at least three measurements (Farajpour *et al*., 2020).

2.4 Edible film as active carrier

The edible film with cacao husk flour with previously demonstrated *in vitro* antioxidant capacity and antimicrobial properties (Pacheco-Martínez *et al*., 2024) was employed as active carrier in the packaging of a meat product, cooked ham. Ham ingredients (50% lean pork, 20% lard, 8% potato starch, 2% salt, 0.8% sodium phosphates, 0.3% curing salt, and 20% ice) were mixed in a cutter, and the mix was formed in one-kilo stainless steel molds and cooked at 80 °C for 60 min. After cooling, ham was cut into 1 cm thick slices, covered with the cacao husk edible film, and vacuum packaged to be stored at 4 °C for 28 days.

Oxidative rancidity was determined according to Nam and Ahn (2003). Samples were homogenized in an Oster blender (10 g with 30 mL of distilled water), transferring 1 mL to a test tube and mixed with 50 μL of butylated hydroxytoluene ethanolic solution (7.2%, v/v) and 2 mL of 20 mM of thiobarbituric acid in a 15% trichloroacetic acid (w/v). The mixture was vortexed and incubated at 90 °C for 15 min, cooled in an ice bath, vortexed again, and centrifuged at 3,000 × g for 15 min. Absorbance of the upper layer was measured at 531 nm against a distilled water blank. Malonaldehyde concentration was determined with the same procedure, extrapolating against a 1,1,3,3-Tetraethoxypropane standard curve. Moisture was determined in agreement with the AOAC 950.46 method (AOAC, 1996) by weight difference of the samples (20 g) after drying in a convection oven at 98±2 °C for 16 h.

The surface color of packaged ham samples was measured with a HunterLab ColorFlex colorimeter (Hunter Associates Laboratory, Reston). CIE-Lab parameters (L*, a* and b*) were determined by taking six random lectures on samples surface. Color chroma and hue angle were determined to correct the non-uniform spacing of the CIE diagram, considered a disadvantage by some in comparing color differences throughout the space, according to the reported by Savadkoohi *et al*. (2014), as follows:

$$\text{Chroma (C)} = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$\text{Hue angle (H)} = \tan^{-1} \frac{b^*}{a^*} \quad (4)$$

The color difference between cacao husk edible film and control film was determined as follows:

$$\text{Color difference } \Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (5)$$

To determine the antimicrobial effect of the polyphenols in cacao husk flour incorporated in edible films, four pathogenic bacteria were employed: *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Previously, bacteria were activated to inoculate a similar bacterial charge, making pertinent dilutions. The D.O.= 0.76 corresponded to 5.2 log CFU/mL for *E. coli*; D.O.= 0.80 corresponded to 5.8 log CFU/mL for *S. typhimurium*; D.O.= 1.23 corresponded to 6.1 log CFU/mL for *S. aureus*; and D.O.= 1.76 corresponded to 5.5 log CFU/mL for *L. monocytogenes*. Ham samples were immersed in each bacterial broth (ca. 5-6 log CFU/mL) for 30 min, drained under sterile conditions, and covered with the cacao husk edible film. A no-film ham sample was the control. The samples were vacuum-packed and stored at 4 °C for 21 days, sampling at 7, 14, and 21 days, to enumerate the respective microbial counts of each pathogen bacterium. Bacterial specific growth rate was determined by D.O. at 585 nm to calculate the specific growth rate (μ) according to:

$$\mu,\ (h^{-1}) = \frac{Ln\left[\frac{X}{X_0}\right]}{[t-t_0]} \qquad (6)$$

where X and X_0 are the CFU per mL sampling days (t) and initial time (t_0), respectively. The percentage of microbial inhibition was reported as the difference of the control sample μ minus the μ of the cacao husk edible film sample, considering the control sample μ as 100%.

2.5 Experimental design and data analysis

The effect of the cacao husk incorporation in the edible films' properties was determined by one-way ANOVA. The effect of edible film as active carrier on moisture and oxidative stability of packaged samples during storage was determined by two-way ANOVA. Data analysis, analysis of variance (ANOVA) was performed using the R Studio® platform (https://www.rstudio.com/), and the significant difference (P<0.05) between means was determined by Tukey's honestly significant difference in the same platform.

3 Results and discussion

3.1 Physical properties

The effect of cacao husk flour on edible films' physical and mechanical properties are listed in Table 1. Cacao husk samples resulted in significant (p<0.05) higher thickness values. The cacao husk edible films presented significant (p<0.05) lower values in both film soluble matter and soluble protein. The WVP of these samples was significant (p<0.05) higher than control samples.

In general, the incorporation of peels or husk powder or flour increases the solid content of edible films, resulting in higher film thickness (Silva *et al*., 2020). In the same manner, the decrease in film solubility is due to the presence of insoluble compounds (Chhatariya *et al*., 2022). Sucheta *et al*. (2019) reported that crystalline granules in beetroot flour decrease solubility in starch edible films. Additionally, the constituents of peels or husks bind water molecules (Silva *et al*., 2022, De Moraes Crizel *et al*., 2017) which affect the solubility of edible films. In addition, the lower film solubility is due to the crosslinking effect of phenolic compounds, since the presence of multiple crosslinking compounds decreases the water solubility of protein films (Hanani *et al*., 2018). Since the cacao husk employed in this research contains about 70% dietary fiber and 4 mg/g of total phenolic compounds (Pacheco-Martínez *et al*., 2024), the main compounds in fiber and the presence of phenolic compounds modified the film thickness and solubility. The film's thickness and solubility also resulted in different water vapor permeability. Peel powder incorporation in edible films increased the WVP, since the insoluble compounds in peel powder resulted in incomplete dissolution in protein film, reducing the strong bonds formation, allowing the water vapor passage through the film (Hanani *et al*., 2018).

Table 1. Physical properties and mechanical properties of the edible films formulated with and without cacao husk flour.

Parameter	Treatment	
	No cacao husk flour	With cacao husk flour
Thickness (mm)	0.232±0.001 b	0.291±0.003 a
Soluble matter (%)	37.50±2.14 a	34.22±2.5 b
Soluble protein (%)	3.94±0.52 a	3.67±0.05 b
Water vapor permeability (g/s m^2 Pa $\times 10^{-10}$)	1.283±0.01 b	1.306±0.02 a
Puncture strength (N)	8.63±0.73 b	10.95±0.51 a
Puncture deformation (%)	4.88±0.35 a	1.54±0.25 b
Tensile force (N/mm^2)	4.78±0.60 b	8.66 ± 0.60 a
Elongation (%)	49.53±0.05 a	20.19±1.50 b

a, b, c... Means with the same letter in the same column are not significant (p<0.05) different for treatment.

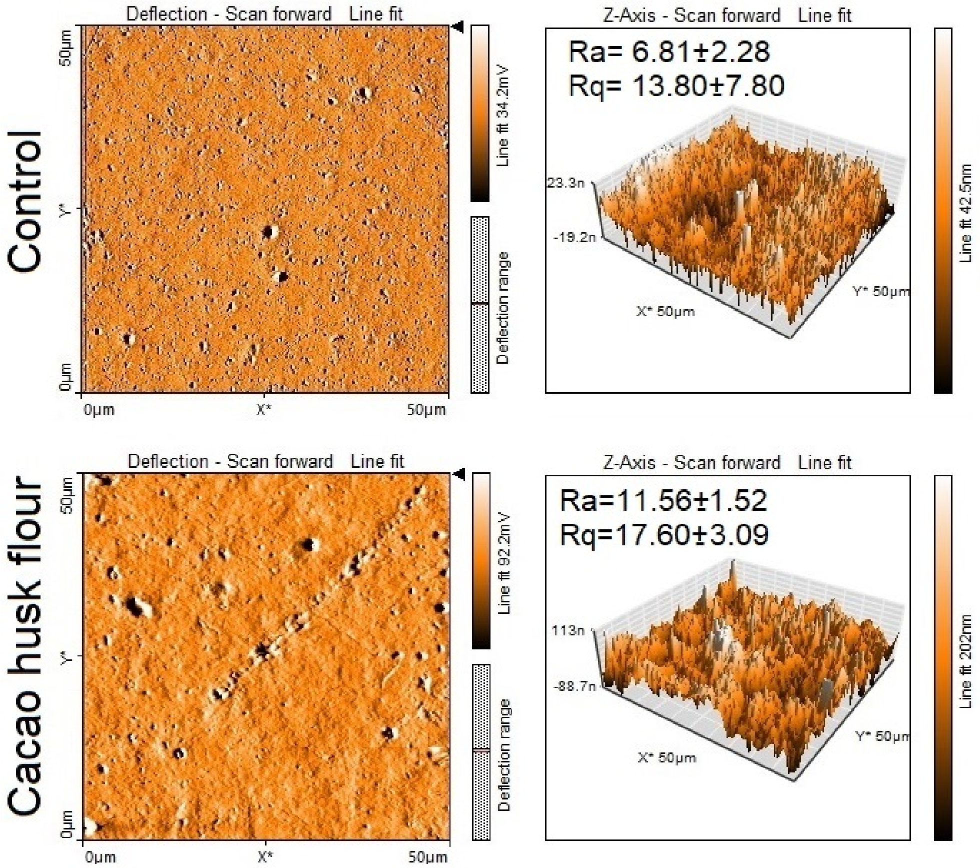

Fig. 1. Bi- and three-dimensional atomic force micrographs (right) of the different protein edible films (Ra: roughness average, Rq: roughness mean square).

3.2 *Mechanical properties*

In the mechanical properties, the incorporation of cacao husk flour into edible films' formulation resulted in significant (p<0.05) higher puncture force and tensile strength values (10.95±0.51 and 8.66±0.60 against 8.63±0.73 and 4.78±0.60, respectively), although decreased significant (p<0.05) the puncture deformation and percentage of elongation (1.54±0.25 and 4.88±0.35 versus 20.19±1.50 and 49.53±0.05, respectively) of the edible films, as compared to control (Table 1).

The changes in the mechanical properties of edible films were due to the presence of sugars, fiber and other compounds in cacao husk flour (Silva *et al*., 2020, Caetano *et al*., 2018). The cohesion of the filmogenic solution matrix was hindered by the insoluble peel or husk powder, modifying or disrupting the original matrix structure (Chhatariya *et al*., 2022, De Moraes Crizel *et al*., 2016). In peels and husks with considerable content of polyphenols, the side chains of complex polysaccharides, such as pectin, could interact with polyphenols' hydroxyl groups, improving film strength due to these intermolecular interactions like hydrogen bonds (Bermudéz-Oria *et al*., 2017, Kanatt *et al*., 2012, Chambi *et al*., 2011). In this view, brittle films, this is, poor elongation at break, resulted from structural gaps or discontinuities by the incorporation of peel or husk in edible films, since no soluble components disrupted the film matrix structure, acting as a filler entering in between the polymer chains and thus decreasing the mobility of the chain (Hanani *et al*., 2018, Chhatariya *et al*., 2022, Qin *et al*., 2013).

3.3 *Surface topography*

In the atomic force micrographs, the roughness average (Ra) values of cacao husk samples were higher than the control samples, as well as the root-mean-square roughness (Rq) values (Fig. 1). Since cell wall components from cacao husk disrupt protein film matrix, an uneven surface is expected. This means that cacao husk incorporation in edible films resulted in a rough surface in contrast with control edible film, with a more homogeneous surface. Particles in peel and husk interfered with film structure, provoking a less cohesive and fragile structure, and this lack of cohesion between the edible film matrix and the insoluble components provoked larger voids within the matrix, facilitating water diffusion (De Moraes Crizel *et al*., 2017). The formation of the edible film microstructure takes place during the production process, and during this time, several molecular interactions may take place, like hydrogen bonds between protein molecules and other substances, and/or with water molecules (Wigati *et al*., 2022). Subsequently, the presence of cementing materials around the cellulose fibers including pectin, hemicellulose and lignin resulted in an irregular surface (Li *et al*., 2014).

3.4 Edible film as active packaging

In the instrumental color, the application of the cacao husk edible film resulted in a significant (p<0.05) decrease in ham luminosity as compared to control samples. The luminosity of the samples significant (p<0.05) decreased with storage time. The hue angle resulted as well with significant (p<0.05) lower values in samples with cacao husk edible film. This color parameter significant (p<0.05) decreased with storage time. The saturation index of the samples with cacao husk edible film presented significant (p<0.05) lower values as compared to control samples. The saturation index significant (p<0.05) decreased with storage time. Finally, the color difference significant (p<0.05) increased with the storage time (Table 2).

The incorporation of cacao husk flour in edible film formulation resulted in darker coloration in samples under this treatment. This also affected the yellowness and redness (+a* and +b*, respectively, used to calculate ΔE) of the samples, resulting in a less hued and obscure coloration.

The edible film's color effect is an important feature, as consumer acceptance and visual appearance in direct application to the food surface are the primary considerations (Zhang *et al*., 2022). However, the color difference remained practically low (ΔE <1.5) during the first 8 days of storage, increasing to day 15 (ΔE <5.0), and ending in higher values (ΔE >7.0). Color differences in the range from 1.1–2.8 units and 2.8–5.6 units correspond to normal tolerances, and color differences >5.6 units could be

Table 2. Instrumental color of the samples packaged with the edible film with cacao husk and control (no edible film) during the storage period.

Storage time (days)	Control	Cacao husk edible film
Luminosity		
1	68.7±0.4 b, A	67.3±0.06 a, A
8	68.5±0.04 b, A	67.1±0.06 a, A
15	66.8±0.03 b, B	72.7±0.4 a, B
22	66.7±0.3 b, B	73.5±0.4 a, B
30	66.3±0.15 b, B	75.3±0.09 a, B
Chroma (C)		
1	13.62±0.22 b, A	13.85±0.21 a, A
8	13.80±0.06 b, A	13.74±0.22 a, A
15	14.30±0.04 b, B	12.16±0.10 a, B
22	14.65±0.22 b, B	11.99±0.20 a, B
30	14.92±0.11 b, B	11.45±0.03 a, B
Hue angle (H)		
1	1.163±0.20 b, A	1.178±0.50 a, A
8	1.168±0.10 b, A	1.198±0.20 a, A
15	1.176±0.20 b, a	1.191±0.20 a, A
22	1.171±0.10 b, B	1.257±0.15 a, B
30	1.164±0.09 b, B	1.323±0.16 a, B
Color difference		
1		1.43±0.18 A
8		1.45±0.19 A
15		4.27±0.37 A
22		7.39±0.12 C
30		8.52±0.10 C

a, b... Means with the same letter in the same row are not significant (p<0.05) different for treatment.

A, B, C... Means with the same letter in the same column are not significant (p<0.05) different for storage time.

Table 3. Oxidative rancidity of the samples packaged with the edible film with cacao husk and control (no edible film) during the storage period.

Storage time (days)	Oxidative rancidity (malonaldehyde mg/kg sample)	
	Cacao husk edible film	Control
1	2.9 ± 0.8 b, E	3.6 ± 0.7 a, E
8	3.4 ± 0.4 b, D	11.7 ±0.6 a, D
15	11.9 ± 0.4 b, C	29.9 ± 0.7 a, C
22	46.8 ± 0.5 b, B	55.2 ± 0.8 a, B
30	63.2 ± 0.8 b, A	78.7 ± 0.5 a, A

a, b... Means with the same letter in the same row are not significant (p<0.05) different for treatment.
A, B, C... Means with the same letter in the same column are not significant (p<0.05) different for storage time.

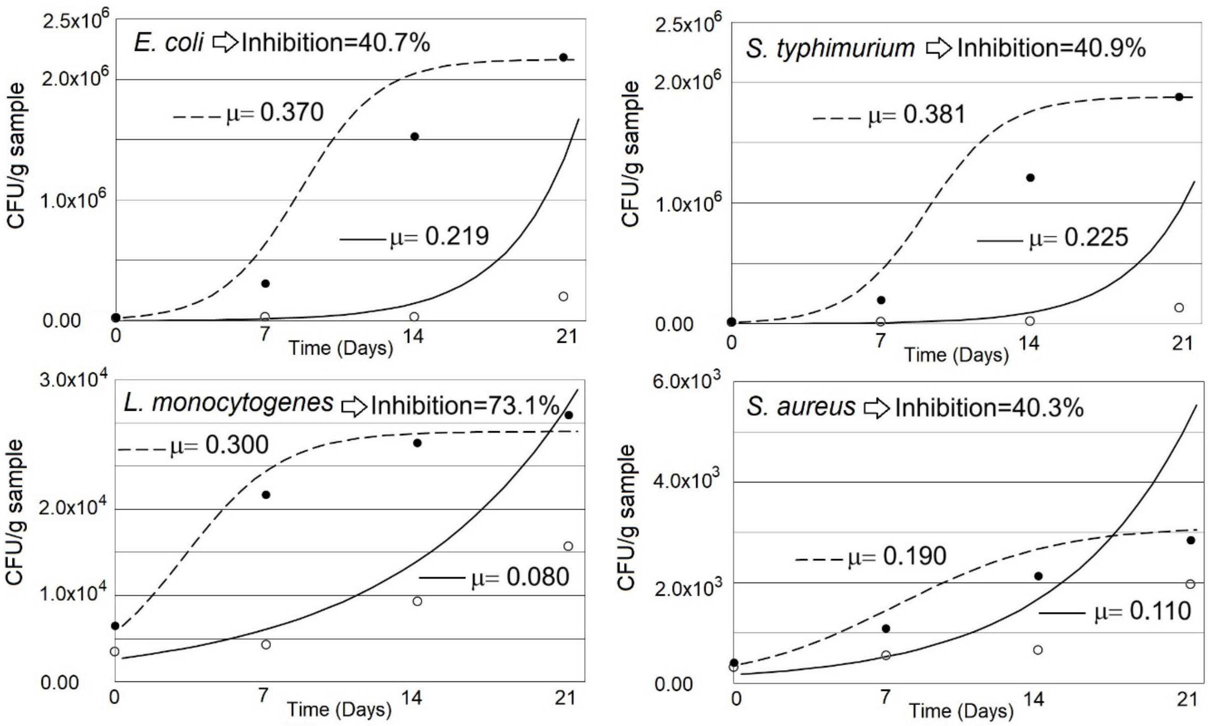

Fig. 2. Percentage of microbial inhibition of the cacao husk edible film on pathogen bacteria. The solid line is the adjusted model adjusted to calculate μ in cacao husk edible film samples (microbial count), and the dashed line is the adjusted model to calculate μ in control samples (microbial count).

easily distinguished (Meléndez-Martínez *et al*., 2004). Although there was an inherent color differences due to the edible film application, this is because their polymeric properties of most of the biopolymers employed do not result in a translucid material, depending on solubility conditions as well, the changes in edible film treated food surface must be considered to avoid consumer rejection or, since food color is the primary cue in the first sight food acceptation. Considering these results, the color difference observed in this research (ΔE= 1.43 to 4.27) can be considered negligible during the three weeks of storage.

During the period of storage of vacuum packaged samples, control samples presented significant (p<0.05) higher malonaldehyde values than the samples with cacao husk edible film. The oxidative rancidity significant (p<0.05) increased with storage time (Table 2). The moisture content was significant (p<0.05) lower in control samples, with a significant (p<0.05) decrease with storage time (Table 3).

The polyphenolic compounds and flavonoid content in cacao husk flour (4.12 and 1.30 mg/g, respectively), with relatively higher antioxidant properties, determined as TEAC (Trolox equivalent antioxidant capacity), DPPH (1,1-diphenyl-2-picrylhydrazyl assay), and linoleic acid peroxidation, as reported by Pacheco-Martínez *et al*. (2024), are the explanation for the oxidative rancidity inhibition during the period of storage. Edible film coating provides effective protection to meat products against lipid oxidation (Sánchez-González *et al*., 2011), since the antioxidant compounds included in the edible film as active packaging, mainly polyphenols, flavonoids, among others, and even lipase inhibitors (Bi *et al*., 2019). The lower solubility of the edible film allowed the gradual release of these compounds on the ham surface, acting as natural antioxidants, preventing lipids' oxidation. In the same way, the insoluble compounds present in cacao husk that decreased film solubility binding water molecules (Silva *et al*., 2020, Chhatariya *et al*., 2022, De Moraes Crizel *et al*., 2017) could be responsible for the high moisture detected in edible film treatments. Since samples were vacuum packed, the higher WVP in cacao husk edible films allowed to maintain the surface moisture of these samples.

In Fig. 2 the inhibitory effect of the cacao husk edible film on microbial growth can be observed by comparing the adjusted growth model for each treatment, with an acceptable correlation coefficient (R^2). The inhibitory percent for *E. coli* was 40.7%, with a μ= 0.219 for cacao husk samples (R^2=0.6162) and μ= 0.370 for control samples (R^2= 0.9030). For *S. typhimurium* the inhibitory percent was 40.9%, with a μ= 0.225 for cacao husk samples (R^2= 0.6075) and μ= 0.381 for control samples (R^2= 0.9106). The inhibitory percentage for *L. monocytogenes* was 73.1%, with μ= 0.080 for cacao husk samples (R^2= 0.9040) and μ= 0.300 for control samples (R^2= 0.7228). Finally, for *S. aureus* the inhibitory percent was 40.3%, with μ= 0.110 for cacao husk samples (R^2= 0.8387) and μ= 0.190 for control samples (R^2= 0.9571). These results are explained in based to the previous results of Pacheco-Martínez *et al*. (2024), who reported that the cacao husk ethanolic extracts demonstrated *in vitro* antimicrobial activity to inhibit pathogens, with an antimicrobial activity similar to chloramphenicol, where the main compounds detected in cacao husk flour ethanolic extracts were: 2.68 mg/g of condensed tannins, 0.03 mg/g of ferulic acid, and 0.07 mg/g of vanillic acid, bioactive compounds with demonstrated antimicrobial activity (El-Lateef *et al*., 2024; Kayaputri *et al*., 2020). Is important to concise that the implementation of edible films as active packaging could be more efficient than antimicrobial additives dispersed in the bulk of the foods, since they can be released gradually from edible film to the food surface (Falguera *et al*., 2011), and still if sometimes the color or flavor of natural active compounds is often a limitation of their use, the amount necessary is relatively low in the controlled release by edible film than the direct incorporation on the food surface or dispersed in the food bulk (Benbettaïeb *et al*., 2018). At the experimental conditions employed, the antimicrobial effect is related to *in vivo* results obtained with ethanolic extracts based on polyphenols content, since into the edible film scaffolding the antimicrobial effect of cacao husk flour remained considering water as the solvent during filmogenic process.

Conclusions

According to the results obtained under experimental conditions, cacao husk flour is a feasible alternative for formulating edible films as a vector to add bioactive compounds to the surface of processed meat products. The inclusion of cacao husk flour into the edible film protein matrix resulted in more permeable and stiffer films, since crystalline insoluble compounds in flour resulted in a rougher surface, besides being less soluble than control films. These features allowed the manipulation of edible film for their incorporation as active packaging on the ham slices' surface, where, although the inherent coloration due to cacao husk add in to edible film formulation, the oxidative rancidity was reduced, and inhibitory percent of pathogens was ca. 40% for *E. coli*, *S. aureus*, and *S. typhimurium*, and over 70% for *L. monocytogenes*. Thus, sodium caseinate edible films with cacao husk flour are an alternative to improve shelf life, avoiding changes related to lipid oxidation and the presence of pathogenic microorganisms. Even though protein or/and edible films cannot be employed as a physical and mechanical container to transport and preserve processed foods from factories to food shelves, edible films as active packaging are a viable alternative to incorporate natural antioxidants and antimicrobials in high moisture foods to improve shel-life, if a reusable container is employed.

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