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ORIGINAL ARTICLE

Effect of the addition of American cinnamon essential oil (*Ocotea quixos*) on the water vapor permeability of chitosan films

Efecto de la adición de aceite esencial de canela americana (*Ocotea quixos*) en la permeabilidad al vapor de agua de películas de quitosana

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Abstract The objective of this study was to develop chitosan films with Tween 80 and the essential oil of American cinnamon (*Ocotea quixos*) with good water vapor barrier properties. The thickness of the films (42-92 µm) was consistent with those reported for similar biomaterials. The moisture content ranged between 23 and 48%, showing no significant trend (p>0.05) about the concentrations of chitosan and essential oil, likely due to the low amounts of essential oil added (0.1; 0.3; 0.5% v/v). The water vapor permeability (WVP) values ranged from 0.349 to 0.802 g mm m^{-2} h^{-1} kPa^{-1}, with no relevant changes due to polymer concentration or the addition of essential oil. A cubic model explained 99.76% of the variability in WVP, with a confidence level of 95%. The optimal formulation was 1.5% (m/v) chitosan, 0.3% (v/v) Tween 80, and 0.5% (v/v) essential oil. The optimized film exhibited consistent properties in WVP, thickness, and moisture content with the other formulations, due to the standardization of the film production process. The addition of essential oil reduced the water solubility of the films.

Keywords chitosan films, American cinnamon essential oil, barrier properties, water vapor permeability.

Resumen El objetivo de este trabajo fue desarrollar películas de quitosana con Tween 80 y aceite esencial de canela americana (*Ocotea quixos*) con buenas propiedades de barrera al vapor de agua. Los espesores de las películas (42-92 µm) fueron consistentes con los reportados para biomateriales similares. El contenido de humedad osciló entre 23 y 48 %, sin mostrar una tendencia significativa (p>0,05) en función de las concentraciones de quitosana y aceite esencial, probablemente debido a las bajas cantidades de aceite esencial añadidas (0,1; 0,3; 0,5 % v/v). Las permeabilidades al vapor de agua (WVP) variaron entre 0,349 y 0,802 g mm m^{-2} h^{-1} kPa^{-1}, sin cambios relevantes por la concentración de polímero o la adición del aceite esencial. Un modelo cúbico explicó el 99,76 % de la variabilidad de la WVP con un nivel de confianza del 95 %. La formulación óptima fue de 1,5 % (m/v) de quitosana, 0,3 % (v/v) de Tween 80 y 0,5 % (v/v) de aceite esencial. La película optimizada mostró propiedades consistentes en WVP, espesor y contenido de humedad con las demás formulaciones, debido a la estandarización del proceso. La adición de aceite esencial redujo la solubilidad de las películas en agua.

Palabras clave películas de quitosana, aceite esencial de canela americana, propiedades de barrera, permeabilidad al vapor de agua.

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PUBLIS EDITORIAL

Introduction

In the past, consumers demanded more natural foods that were organoleptically and nutritionally similar to fresh products, while also being safe, hygienic, and having a long shelf life. Meeting these growing demands drove significant technological advancements in food packaging (Fadiji & Pathare, 2023).

Active packaging techniques emerged as one of the most interesting innovations in this context, allowing for favorable interactions between the packaging and the product to enhance quality and acceptability (Salgado et al., 2021). An example of active packaging for food includes edible films and coatings that can incorporate active substances into their formulations. Their use in the food industry generated considerable interest due to their potential to extend the shelf life of many food products (Priya et al., 2023). These films and coatings are made from biopolymers such as polysaccharides, proteins, and lipids, which are edible and biodegradable, making them non-toxic to the environment (Hashemi et al., 2023).

Among the polysaccharides used in the preparation of edible films and coatings is chitosan, the main derivative of chitin, which is obtained industrially through chemical or enzymatic deacetylation. The primary source of chitin comes from the exoskeletons of industrially processed crustaceans such as lobster, crab, and shrimp, contributing to the utilization of these waste products and reducing their environmental impact (Ngasotter et al., 2023).

Chitosan is noted for its excellent film-forming and mechanical properties, with no limitations regarding biocompatibility, biodegradability, and toxicity. Additionally, it is naturally abundant and renewable (de Sousa et al., 2020). The antimicrobial properties of chitosan solutions and films have been reported in several studies, demonstrating this biopolymer's ability to inhibit the growth of a wide variety of bacteria (Khubiev et al., 2023). Although its antioxidant properties are limited, research has focused on incorporating antioxidant and antimicrobial substances into chitosan films to enhance these characteristics.

Essential oils have been included in chitosan polymer matrices due to their effective antimicrobial and antioxidant effects (Casalini & Giacinti, 2023). As natural compounds, they are biodegradable, leaving no residues, and do not harm the environment (Ponnusamy & Mani, 2022). The essential oil from the leaves of Ishpink (*Ocotea quixos*) has a high capacity to inhibit the growth of strains such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus mutans*, and the yeast *Candida albicans* (Valarezo et al., 2021). These characteristics give the essential oil the ability to enhance the biological properties of chitosan films when incorporated as an active substance, without affecting the inherent characteristics of the film. Therefore, the following general objective was proposed: to evaluate the influence of the concentrations of polymer, Tween 80, and essential oil of American cinnamon (*O. quixos*) on the thickness and moisture content of chitosan films.

Materials and methods

The research was conducted in the laboratories of the Institute of Pharmacy and Food at the University of Havana, as part of the project on the extraction of chitosan and its salts from lobster chitin (*Panulirus argus*) for pharmaceutical and food applications.

The experimental design and analysis of water vapor permeability (WVP) of chitosan films with Tween 80 and essential oil of American cinnamon (*O. quixos*) were performed using Design Expert 8.0.6 software (Stat-Ease Inc., Minneapolis, USA). The film with the lowest WVP value was selected using a cubic response surface model for numerical optimization. The evaluated factors included the concentration of essential oil (A), chitosan (B), and Tween 80 (C), while WVP served as the response variable. The software defined 18 experimental combinations, including three replicates (Table 1).

Table 1. Experimental design matrix

Run	Essential oil (% v/v)	Chitosan (% m/v)	Tween 80 (% v/v)
1	0.5	2.0	0.5
2	0.1	1.5	0.1
3	0.5	2.0	0.3
4	0.5	1.5	0.1
5	0.5	1.5	0.5
6	0.1	2.0	0.1
7	0.1	2.0	0.3
8	0.1	1.5	0.5
9	0.3	2.0	0.1
10	0.3	2.0	0.5
11	0.5	2.0	0.1
12	0.3	1.5	0.3
13	0.3	1.5	0.5
14	0.3	2.0	0.5
15	0.3	1.5	0.3
16	0.1	1.5	0.1
17	0.1	2.0	0.5
18	0.3	2.0	0.3

Chitosan (221 kDa and 76% degree of deacetylation) was used, obtained through thermo-alkaline N-deacetylation of chitin from common lobster (*P. argus*) at the Production Plant for Natural and Synthetic Products of the Center for Research and Development of Medicines (Havana, Cuba) (de la Paz et al., 2012). Other materials used included the essential oil of American cinnamon (*O. quixos*) supplied by the Chankuap Foundation (Ecuador), Tween 80 (Acros Organics, Belgium), 90% lactic acid (Merck, Germany), and distilled water.

The film-forming solutions were prepared with chitosan at 1.5% and 2.0% (m/v) in a 1% lactic acid solution and agitated for 2 hours. Tween 80 was then added at various concentrations according to the experimental design, and the mixture was filtered to remove impurities. Next, the essential oil of cinnamon was incorporated, and the mixture was emulsified at 12000 min^{-1} for 5 minutes using an Ultra-Turrax homogenizer. The emulsions were allowed to rest for deaeration and poured into glass molds, drying at 40 °C for 24 hours.

The concentrations of chitosan were selected based on previous studies on the physical properties of the films (Casariego, 2009). The obtained films were stored in double-sealed Ziploc® bags within a desiccator, maintained at a controlled relative humidity of 21-22%.

To evaluate the physical properties of the chitosan films, thickness, moisture content, water solubility, and water vapor permeability (WVP) were measured. The thickness of the films was determined using a digital micrometer with an accuracy of ±1 µm. Moisture content was evaluated by weighing the samples before and after drying at 105 ºC for 24 hours, while water solubility was measured by immersing the films in distilled water at room temperature for 24 hours and calculating the percentage of dissolved material. WVP was determined following the standard gravimetric method using permeable capsules and measuring weight loss through the films at 25 ºC and relative humidity of 50%. The results obtained were statistically analyzed using Statistica software.

Results and discussion

Table 2 shows the thickness and moisture content values of chitosan films with the essential oil of American cinnamon (*O. quixos*). The thickness of the films ranged from 42 to 92 µm, which could be attributed to differences in moisture content, as protonated chitosan has a high affinity for water, increasing its hydrophilicity compared to its powdered form (Desai et al., 2023). During film formation, the interaction between chitosan and lactic acid increases water absorption, likely due to the loss of integrity in the molecular structure, which exposes more functional groups for water absorption (Yadav et al., 2023).

Table 2. Effect of the addition of essential oil of American cinnamon (*O. quixos*) on the thickness and moisture content of chitosan films

Run	Essential oil (% v/v)	Chitosan (% m/v)	Tween 80 (% v/v)	Thickness (µm)	Moisture (% m/m)
1	0.5	2.0	0.5	72 (2) abcd	32 (5) bcdefg
2	0.1	1.5	0.1	73 (9) abcd	31 (8) cdefgh
3	0.5	2.0	0.3	68 (6) bcd	38 (4) bc
4	0.5	1.5	0.1	92 (5) a	29 (4) defgh
5	0.5	1.5	0.5	68 (1) bcd	25 (4) gh
6	0.1	2.0	0.1	61 (9) bcde	25 (3) gh
7	0.1	2.0	0.3	57 (7) cde	28 (1) efgh
8	0.1	1.5	0.5	75 (1) abcd	27 (6) fgh
9	0.3	2.0	0.1	58 (2) bcde	23 (3) h
10	0.3	2.0	0.5	53 (7) de	32 (3) bcdefg
11	0.5	2.0	0.1	42 (5) e	35 (3) bcdef
12	0.3	1.5	0.3	65 (4) bcd	35 (1) bcdef
13	0.3	1.5	0.5	59 (1) bcde	36 (5) bcde
14	0.3	2.0	0.5	61 (8) bcde	37 (4) bcd
15	0.3	1.5	0.3	79 (2) ab	48 (3) a
16	0.1	1.5	0.1	77 (1) abc	36 (4) bcdef
17	0.1	2.0	0.5	58 (4) bcde	40 (1) b
18	0.3	2.0	0.3	78 (9) abc	32 (3) bcdefg

Mean (Standard deviation).

Different letters indicate significant differences ($p \leq 0.05$).

The thickness values obtained in this study are generally lower than those reported by Peng et al. (2013), who reported thicknesses ranging from 72 to 131 µm for 2% (m/v) chitosan films with green and black tea extracts, acetic acid, and glycerol. Conversely, Moradi et al. (2012) reported thickness values ranging from 70 to 80 µm for 2% chitosan films in acetic acid with essential oil of *Zataria multiflora* Boiss and grape seed extract, using glycerol as a plasticizer. Under similar conditions but with the addition of Tween 80, Ojagh et al. (2010) obtained values between 95 and 107 µm.

In general, the thickness values obtained in this research fall within the range reported in the literature. However, the thickness of the films can vary depending on factors such as temperature and humidity, which are difficult to control, leading many authors not to report this parameter in studies of biodegradable films.

Table 2 shows that the moisture content of the films varied between 23 and 48% (m/m), with no significant trend ($p>0.05$) observed concerning the concentrations of chitosan and essential oil, which could be attributed to the low concentrations of essential oil used (0.1, 0.3, and 0.5% v/v). Ojagh et al. (2010) reported a decrease in moisture content with increasing concentrations of essential oils (0.4 to 2%) in 1, 2, and 3% chitosan films in acetic acid. However, Bonilla et al. (2013) indicated that the addition of hydrophobic compounds to 1% chitosan films did not significantly affect ($p>0.05$) moisture content, likely due to the low solid content. In contrast to the results of this study, Peng et al. (2013) observed a decrease in moisture content in 2% chitosan films with glycerol and green and black tea extracts (0.5%), reducing from 28 to 19% and 23%, respectively. Wang et al. (2013) reported similar results with the addition of tea polyphenols (10-40%), with values ranging from 42 to 25%.

A key function of films is to prevent the transfer of moisture between the food and its environment (Azevedo et al., 2022). Water vapor permeability (WVP) develops in three phases: absorption of water from the area of higher relative humidity, diffusion through the film, and desorption in the area of lower relative humidity (Turan et al., 2021). The WVP values for chitosan films with essential oil of American cinnamon were calculated from the WVTR results (with R^2 between 0.9989 and 0.9999) and are presented in Table 3. Neither the concentration of chitosan nor the addition of essential oil significantly modified the WVP, with values ranging from 0.349 to 0.802 g mm m^{-2} h^{-1} kPa^{-1}. Bonilla et al. (2013) and Pranoto et al. (2005) also found that the addition of basil, thyme extracts, and garlic essential oil did not notably affect this property.

Table 3. Effect of the addition of essential oil of American cinnamon (*O. quixos*) on the water vapor permeability of chitosan films

Run	Essential oil (% v/v)	Chitosan (% m/v)	Tween 80 (% v/v)	R^2	WVTR (g/h m^2)	WVP (g mm/m^2 h kPa)
1	0.5	2.0	0.5	0.9989	37.1 (0.6)	0.566 (0.009) cdef
2	0.1	1.5	0.1	0.9994	36.2 (3.1)	0.505 (0.03) cdefg
3	0.5	2.0	0.3	0.9989	35.4 (0.7)	0.479 (0.06) efg
4	0.5	1.5	0.1	0.9993	33.5 (1.9)	0.588 (0.1) bcde
5	0.5	1.5	0.5	0.9997	33.8 (0.7)	0.469 (0.003) efgh
6	0.1	2.0	0.1	0.9997	34.4 (2.9)	0.456 (0.07) fghi
7	0.1	2.0	0.3	0.9996	33.7 (1.0)	0.602 (0.05) bcd
8	0.1	1.5	0.5	0.9999	32.5 (0.5)	0.608 (0.03) bc
9	0.3	2.0	0.1	0.9996	34.9 (1.8)	0.697 (0.03) b
10	0.3	2.0	0.5	0.9999	33.4 (1.1)	0.526 (0.07) cdefg
11	0.5	2.0	0.1	0.9998	36.7 (0.3)	0.486 (0.1) defg
12	0.3	1.5	0.3	0.9997	31.8 (0.6)	0.363 (0.04) hi
13	0.3	1.5	0.5	0.9996	31.8 (0.5)	0.544 (0.05) cdef
14	0.3	2.0	0.5	0.9997	32.5 (1.8)	0.546 (0.02) cdef
15	0.3	1.5	0.3	0.9996	34.6 (3.8)	0.349 (0.03) i
16	0.1	1.5	0.1	0.9991	32.3 (1.1)	0.525 (0.008) cdefg
17	0.1	2.0	0.5	0.9989	32.7 (0.8)	0.802 (0.01) a
18	0.3	2.0	0.3	0.9992	31.7 (1.2)	0.414 (0.1) ghi

WVRT: water vapor transmission rate; WVP: water vapor permeability.
Mean (Standard deviation).
Different letters indicate significant differences ($p \leq 0.05$).

Several researchers (Peng et al., 2013; Wang et al., 2013; Siripatrawan and Harte, 2010) have demonstrated that the addition of polyphenols reduces WVP. In the study by Peng et al. (2013), a decrease of up to 62.1% compared to the control was observed when using a 2% extract. Siripatrawan & Harte (2010) attribute this decrease to the interaction between polyphenols and the chitosan structure.

Although the general patterns observed in this study and previous ones do not coincide, the WVP values with essential oil concentrations and extracts similar to those in our research are comparable. The differences could be related to the fact that all these films were made with 2% chitosan in an acetic acid solution and glycerol as a plasticizer. Acetic acid has been shown to produce films with lower WVP compared to lactic acid, while the addition of a plasticizer tends to increase WVP (Eslami et al., 2023). Furthermore, factors such as the type and amount of plasticizer and solvent, as well as the molecular weight and degree of deacetylation of chitosan, may also influence permeability (Wang et al., 2013). The influence of active compounds on reducing WVP was reported by Ojagh et al. (2010) when evaluating the permeability of films with cinnamon essential oil, findings that coincide with those of Pastor et al. (2013) on the incorporation of resveratrol. On the other hand, Bonilla et al. (2013) found that citric acid and α-tocopherol had no significant impact ($p>0.05$) on WVP values.

The results of the analysis of variance for regression and the estimated coefficients related to the water vapor permeability of chitosan films. The best fit was achieved with a cubic model, which was significant at a 95.0% confidence level, indicating a significant relationship between the factors and the dependent variable of the model. The R^2 statistic showed that the fitted model explains 99.76% of the variability in WVP. The cubic term of the essential oil concentration and the linear interaction of the three factors did not significantly affect the WVP of the films.

Figure 1 illustrates the influence of these factors on the WVP of chitosan films with Tween 80 and the essential oil of American cinnamon. It is observed that the addition of Tween 80 at 0.3% (v/v) improved the water vapor barrier properties of chitosan films with essential oil of American cinnamon (*O. quixos*), likely due to the hydrogen bonds established between the polar groups of chitosan and the polar groups of Tween 80, thereby reducing the number of polar groups available to interact with water molecules (Bide et al., 2021).

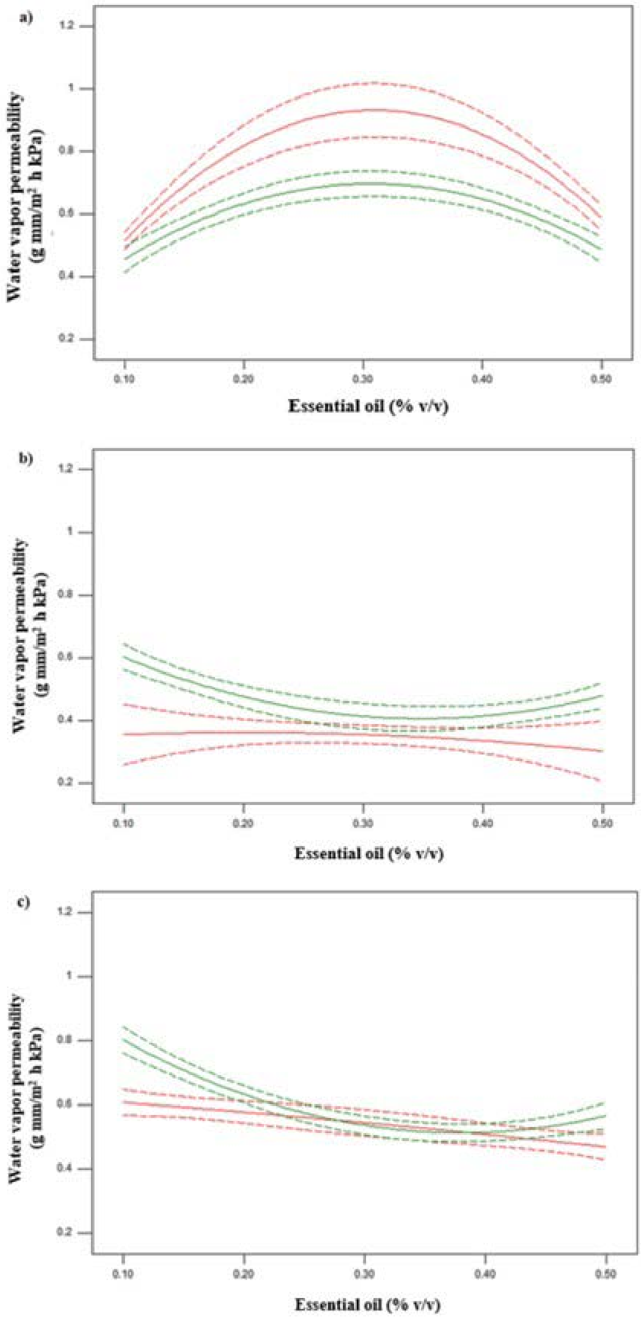

Figure 1. Water vapor permeability of chitosan films with essential oil of American cinnamon (*O. quixos*) and Tween 80 at concentrations of a) 0.1; b) 0.3; and c) 0.5% (v/v). --- Chitosan 1.5% --- Chitosan 2% (m/v).

The mechanism predicting water transport through hydrophilic films, such as those made of chitosan, is complex due to the non-linear nature of the absorption isotherms and the fact that diffusivity varies with water content (Souza et al., 2009). Additionally, the water vapor flow through these films behaves non-linearly about the partial vapor pressure gradient. If the films are cationic and highly hydrophilic, they may interact with the polymer matrix, which could increase the WVP.

Villalobos et al. (2006) found that the WVP of hydroxypropyl methylcellulose films with a mixture of Span 60 and sucrose esters, which had HLB values between 4.7 and 8.0, was lower than that of control films; moreover, WVP decreased with low ratios of hydrocolloid to surfactant. Rodríguez et al. (2006) reported that the WVP of starch films was not significantly affected by the addition of Tween 20 and lecithin at concentrations of 0.5 to 5%. On the other hand, Chen et al. (2009) observed that the WVP of starch and decolorized hsian-tsao gum films with surfactants (sucrose esters S-0770 with HLB = 7, S-1170 with HLB = 11, and S-1570 with HLB = 15) was significantly lower compared to control films, and this decrease was correlated with the increase in HLB values of the surfactants, indicating that the effect of the surfactant on WVP depends on its type and concentration, as well as the properties of the film-forming material.

The verification of the normality assumption was conducted through an analysis of variance, analyzing the normal probability of the residuals. The values of the internally studentized residuals fit a straight line, indicating a normal distribution of errors and confirming the normality hypothesis. For the numerical optimization of the formulation of chitosan films with essential oil of American cinnamon, the ranges of the independent variables (concentrations of chitosan, Tween 80, and essential oil) were used as constraints to achieve the lowest WVP values in the films (Table 4).

Table 4. Constraints for the optimization of the formulation of chitosan films with the essential oil of American cinnamon (*Ocotea quixos*)

Parameter	Lower limit	Upper limit	Criterion
Essential oil (% v/v)	0.1	0.5	In the range
Chitosan (% m/v)	1.5	2.0	In the range
Tween 80 (% v/v)	0.1	0.5	In the range
WVP (g mm/m^2 h kPa)	0.349196	0.8023	Minimize

The program suggested 25 optimized solutions for the formulation of chitosan films with the essential oil of American cinnamon based on the previous constraints. Solution 5 was selected, as it had the lowest WVP and high statistical convenience, corresponding to the lowest percentage of chitosan and the highest of essential oil.

Table 5 shows the results of the evaluated properties of the film obtained from the optimized formulation. The WVP of the optimized film was higher than the value estimated from the numerical optimization of the film formulation, although the differences are practically insignificant.

Table 5. Properties of the optimized chitosan film with Tween 80 and the essential oil of American cinnamon (n = 3)

Parameter	Mean (Standard deviation)
Water vapor permeability (g mm/m^2 h kPa)	0.50 (0.02)
Thickness (µm)	64 (1)
Solubility (% m/m)	34 (2)
Moisture (% m/v)	35 (4)

WVP: Water vapor permeability.

The incorporation of essential oil of American cinnamon into chitosan films resulted in a decrease in their water solubility. Rodríguez (2015) reported solubility values of 49 and 47% for chitosan films at 1.5 and 2%, respectively, with thicknesses similar to those in this study. Ojagh et al. (2010) documented a water solubility of 23.2% for chitosan films that were 95 ± 2.5 µm thick at 2% (w/v) in a 1% (v/v) acetic acid solution, which was lower than the results obtained in this work, supporting the hypothesis that greater thickness results in lower water solubility. Wang et al. (2013) reported a solubility of 23.5% for films at 4% (w/v) in 2% (v/v) acetic acid, showing a similar trend, although without significant differences. Despite the concentration of essential oil used to reduce solubility, Ojagh et al. (2010) observed a significant reduction in this indicator starting at 1.5% of the essential oil of cinnamon. On the other hand, Peng et al. (2013) reported an increase in solubility upon adding tea extracts.

Conclusions

Chitosan films with the essential oil of American cinnamon (*O. quixos*) exhibited thicknesses consistent with those reported for this type of biomaterial. The moisture content in the films did not show a significant trend concerning the concentrations of chitosan and essential oil, which could be attributed to the low amounts of essential oil used in the formulation. Additionally, water vapor permeability was not affected by either polymer concentration or the addition of essential oil. A cubic model was found to be the most suitable for explaining the variability in water vapor permeability, resulting significantly in a 95.0% confidence level. The optimal formulation for the films was identified and related to permeability properties. The permeability, thickness, and moisture content characteristics of the optimized film were consistent with those of the other analyzed films, indicating standardization in the laboratory-scale production process. Furthermore, the incorporation of the essential oil of American cinnamon reduced the solubility of the films in water.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Inalvis Escalante, Flor Marina Fon-Fay and Jorge A. Pino: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, validation, visualization, drafting the original manuscript and writing, review, and editing.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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