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Development of Chitosan-Based Active Films with Medicinal Plant Extracts for Potential Food Packaging Applications

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Abstract: In this study, 2% chitosan (Ch) (*w*/*v*) was enriched with 1% *Lippia javanica*, *Syzygium cordatum*, and *Ximenia caffra* extract to form Ch+L, Ch+S, and Ch+X, respectively. The control film was the chitosan (Ch) film without plant extracts. The composite films were assessed for their antifungal ability using the agar diffusion method against economically relevant plant pathogens, *Botrytis cinerea*, and *Penicillium expansum*. These chitosan films were further evaluated using an X-ray diffractometer and scanning electron microscope, and their physical and mechanical properties were also assessed. The medicinal plants in the chitosan matrix had the highest inhibition zone (10 mm) against *P. expansum*, while the chitosan-only films had the lowest inhibition zone (3.3 mm). Notably, Ch+S and Ch+X films had the highest inhibition zone (10 mm) against *B. cinerea*, while chitosan-only films did not avert the spread of *B. cinerea*. Ch+L films had the highest film thickness (0.189 mm), density (1.62 g·cm^3), swelling degree (48.6%), and water solubility (32.8%). Films with other plant extracts had moderate properties, while chitosan without plant extract had the least film thickness (0.128 mm), density (1.08 g·cm^3), swelling degree (31.9%), and water solubility (18.9%). X-ray diffraction images revealed that the chitosan films fused with plant extracts altered the extent of crystallinity of the films because they ranged between 14,710.43 for chitosan-only films and 26,288.31 a.u. for Ch+S films. Enriching the chitosan-based films with the investigated medicinal plant extracts resulted in different favorable properties and could make good candidates for food preservation and packaging if optimized.

Keywords: food preservation; functional compounds; oxidation; green packaging; edible film

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1. Introduction

In the evolving food packaging landscape, there is a critical shift towards sustainable and functional materials. This shift is driven by increasing consumer awareness and regulatory pressures regarding food safety and environmental sustainability [1]. Recent concerns about food safety have led to an interest in the development of green and biodegradable functional packaging materials to increase the storage period of food [1–3]. Enhanced food safety is achieved by incorporating active agent composites, such as antioxidants and antimicrobial agents, into packing materials [1,2,4]. These additions provide the packaging with functional properties not originally present, thus enhancing food quality, safety, and shelf-life [5,6].

Chitosan, a popular edible coating known for its non-toxicity and biodegradability, is known for its film-forming ability, making it an ideal biopolymer for producing edible films [7,8]. However, despite these advantages, chitosan films often exhibit limitations such as poor physical, mechanical, antioxidant, and antimicrobial properties [3,7]. These shortcomings hinder their broader application in the horticultural fresh produce and food processing industries [3,7]. Enhancing chitosan films by incorporating functional

compounds from natural sources is, therefore, a critical area of research to overcome these limitations and fully harness the potential of chitosan in food protection.

Integrating plant extracts and edible film polymer matrices has been considered a promising strategy to enhance their functional activity, including improving antioxidant and antimicrobial properties [6,9]. Plant extracts, particularly those documented in folk medicine or indigenous knowledge systems (IKS), are considered safe and offer a viable alternative to synthetic agents [10–12]. The preference for natural plant-based products over chemicals in food applications is growing, driven by consumer demand for natural products that offer added benefits to consumers and the environment [4,13]. Studies on maqui berry (*Aristotelia chilensis*) extract [14], Shirazi thyme (*Zataria multiflora*) oil, and grape seed extract [4] have proved that integrating plant extracts into chitosan films significantly improved the film characteristics, including antibacterial activity, total phenolics, and antioxidant capacity. Enriching chitosan films with tea extracts improved the physicochemical characteristics of the films [2], while carboxymethyl chitosan fused with waterborne polyurethane–gelatin hydrolysate improved the film's tensile strength and elongation at the break [15]. Liquid-mass spectrophotometer analysis of *B. pilosa* identified 20 metabolites that were influential in enhancing the physical and mechanical characteristics, radical scavenging activity, and antifungal activity of chitosan composite films against *Penicillium expansum* and *Botrytis cinerea* [16].

Based on a compiled literature review on folk plants used in IKS to maintain food quality, medicinal plants such as *Lippia javanica* [17], *Syzygium cordatum* [18], and *Ximenia caffra* [19] compounds are promising choices. *L. javanica* (Burm.f.) of the Verbenaceae family has been used in IKS as a herbal tea, enhances food flavor, and is also used for food preservation among local communities [17,20,21]. *S. cordatum* Hochst. ex-Krauss (family Myrtaceae), commonly known as water berry, is a valuable plant species indigenously used as herbal medicine, and its fruits are a source of food. The bark and leaves of this medicinal plant are used in food preservation, and it has many other useful pharmacological properties [18,22]. *X. caffra's* leaves and root extract have been described to have anti-gonococcal and antimicrobial possessions [21,23,24], substantiating its use as food essences and enhancing the shelf-life of food in the IKS [25,26]. A study by Nxumalo et al. [27] reported that *L. javanica*, *S. cordatum*, and *X. caffra* have different metabolite compositions that influence their variations in antioxidant and antibacterial activity. Therefore, the IKS of these medicinal plants for food preservation indicates that they have the potential to inhibit reactive oxygen species in food, thereby increasing its edibility.

Given the recognized limitations of chitosan films' mechanical strength, barrier properties, and bioactivity, this research explored the potential of enhancing chitosan films by integrating selected medicinal plant extracts rooted in indigenous knowledge systems. The primary objective was to study the physicochemical properties, mechanical strength, barrier qualities, and antimicrobial efficacy of chitosan films fused with *L. javanica*, *S. cordatum*, and *X. caffra* compounds. Additionally, this study aimed to investigate the release kinetics of the extracts within the chitosan matrix. The overall goal was to establish the suitability of the enriched chitosan films as innovative biocomposite materials for food preservation applications in the food industry, potentially overcoming the current limitations of standard chitosan films.

2. Materials and Methods

2.1. Obtaining and Preserving Plant Material

Leaves of the three medicinal plants were obtained in Eswatini, Mafutseni (26°24′21.9″ S, 31°35′05.3″ E) at the Eswatini Institute for Research in Traditional Medicine, Medicinal and Indigenous Food Plants (EIRMIP) farm. An Agronomist and Research Fellow from EIRMIP identified these medicinal plants, and they were deported as KN1001 (*Lippia javanica*), KN1002 (*Syzygium cordatum*), and KN1003 (*Ximenia caffra*) [28]. To preserve the leaves, they were dried at 50 °C for 72 h, reduced into a fine powder, and stored in zip-lock bags until further use.

2.2. Medicinal Plant Extraction and Development of Chitosan Films

The medicinal plants were extracted following a method by Ramesh et al. [29], and Hassan et al. [30], with slight variations. In a 250 mL beaker, 100 mL of 70% ethanol was combined with 20 g powdered leaves for 30 min, sonicated for 1 h at 20 °C, and the extract was filtered through Whatman filter paper no. 1. The filtrate was then concentrated under reduced pressure at 45 °C using a rotary evaporator (Rota-vapor R-200, BUCHI Laboratory Equipment, Flawil, Switzerland), air-dried using fans and refrigerated at 4 °C and 90 ± 5% relative humidity (RH) in sterile vials.

Chitosan films (Sigma Aldrich, St. Louis, MO, USA) were set as outlined by Siripatrawan and Vitchayakitti [6] and adopted with slight modification. In short, chitosan (2% w/v) was mixed with acetic acid (1% v/v), glycerol (1% v/v), canola oil (1% v/v), and Tween-20 (1% v/v) under constant magnetic stirring for 1 h. The dried plant extracts were reconstituted in 70% aqueous ethanol to facilitate complete dissolution while ensuring the final concentration in the coating solution reached 1% w/v. This standardized extract solution was carefully mixed into the chitosan solution to create fused edible films. To ensure a homogenous distribution of the plant extracts within the film, the combination was then homogenized at 3000 rpm for 10 min and sonicated at 40 °C for 1 h at high frequency. To develop the composite films, specific formulations were created as follows: chitosan with 1% *L. javanica* (Ch+L), chitosan with 1% *S. cordatum* (Ch+S), and chitosan with 1% *X. caffra* (Ch+X). The control film was prepared using chitosan without any addition of plant extract (Ch). Subsequently, 30 mL of each chitosan solution was evenly spread on 90 × 15 mm Petri dishes. The developed chitosan films were then prepared in an oven at 50 °C and 100% RH for 72 h, followed by further conditioning at 20 ± 5 °C and 65 ± 5% RH for 48 h to achieve the desired consistency and properties.

2.3. Experimental Layout

The experiment was laid out in a completely randomized design. The composite film treatments were chitosan with *L. javanica* (Ch+L), chitosan with *S. cordatum* (Ch+S), and chitosan with *X. caffra* (Ch+X) and chitosan (Ch) without plant extract was the control, and each treatment was replicated three times.

2.4. Characterization of Films

2.4.1. X-ray Diffraction

Using copper Ka radioactivity (k = 1.543 Å) sifted by nickel, an X-ray diffractometer X'Pert Pro Panalytical (Almelo, The Netherlands), adjusted between 2θ = 5° and 80° with a step size 2θ = 0.017° (step time of 2000 s) at 45 kV and 35 mA was used to analyze the cut circular film (32 mm^2).

2.4.2. Film Morphological Characteristics

The film (10 × 10 mm) morphological characteristics were viewed under a scanning electron microscopy (SU8010, Hitachi, Japan) set at 10 kV.

2.4.3. Film Thickness and Density

A digital micrometer (Mitutoyo, Mitutoyo Corporation, Sakado, Japan) was used to analyze the developed film thickness at three separate positions per film per treatment (n = 3). As shown in Equation (1), the film weight (f_w) and volume (v) were used to calculate the film density (F_d).

$$F_d = \frac{f_w}{v} \quad (1)$$

2.4.4. Film Water Vapor Transmission Rate

A method by Nxumalo and Fawole [16] was used to study the water vapor transmission rate (WVTR) of the developed films. Distilled water (30 mL) was poured into aluminum permeability cups with an internal diameter of 32 mm. The film samples (n = 3)

were then set on top of the aluminum cups, tightened with clamps, and their initial weight was obtained (m_i). The samples were enclosed in airtight plastic containers together with 25 g saturated chloride salt in 90 × 15 mm Petri dishes. The weight of the aluminum cups was observed every 1 h for the first 10 h and thereafter, after 24 h (d) to obtain the final weight (m_f) and Equation (2) was used to calculate the $WVTR$ of the films.

$$WVTR = \frac{m_{f-m_i}}{d \times s} = \text{gm}^{-2}\,24^{-1}\,\text{h} \quad (2)$$

where s = effective area of the film

2.4.5. Water Content

The water content (W_c) properties of the chitosan films were completed as outlined by Riaz et al. [3] and Nxumalo and Fawole [16]. Briefly, in triplicates, 3 × 3 cm were initially weighed ($w1$) and thereafter, oven-dried at 105 °C for 48 h to obtain ($w2$). The final per cent water content was calculated using Equation (3).

$$W_c\ (\%) = \frac{w1 - w2}{w1} \times 100 \quad (3)$$

2.4.6. Film Solubility and Swelling Degree

A method as described by Nxumalo and Fawole [16] was used to evaluate the chitosan film strips' (3 × 3 cm) solubility and swelling degree. To study the solubility of the films, they were first oven-dried at 105 °C for 24 h to obtain their initial dry mass ($M1$). For 24 h at room temperature, the films were then dipped in 75 mL of distilled water and oven-dried to obtain their final dry mass ($M2$). The final per cent film solubility was determined using Equation (4).

$$Film\ solubility\ (\%) = \frac{M1 - M2}{M1} \times 100 \quad (4)$$

To obtain the percent film swelling degree (%), they were first weighed to obtain their initial weight ($M1$) and then placed in 40 mL distilled water for 24 h at 20 ± 5 °C and 65 ± 5% RH. For 1 min, the films were superficially dried using filter papers and then weighed to obtain the final weight ($M2$). The values obtained were then used to calculate the film swelling degree, as shown in Equation (5).

$$Film\ swelling\ degree(\%) = \frac{M2 - M1}{M1} \times 100 \quad (5)$$

2.4.7. Film Color Attributes and Glossiness

The film color attributes were characterized by the Hunter Lab scale (CIE Lab scale) values of L^*, a^*, and b^* at three different points of each film. The film's total color difference (ΔE), white index (WI), and yellow index (YI) were then calculated using Equations (6), (7) and (8), respectively.

$$\Delta E = \left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)^{0.5} \quad (6)$$

where $\Delta L = L_{\text{standard}} - L_{\text{sample}}$; $\Delta a = a_{\text{standard}} - a_{\text{sample}}$; and $\Delta b = b_{\text{standard}} - b_{\text{sample}}$

$$WI = 100 - \sqrt{\left(100 - L^*\right)^2 + a^{*2} + b^{*2}} \quad (7)$$

$$YI = \frac{142.86 \times b^*}{L^*} \quad (8)$$

A gloss meter set (Multi-Gloss 268, Minolta, Germany) calibrated at 20, 60, and 80° angles was used to determine the glossiness of the developed films. The gloss values were obtained from three different positions of each film.

2.4.8. Transmittance and Opacity

A procedure by Riaz et al. [3] and Nxumalo and Fawole [16] was used to evaluate the transmittance and opacity of the developed films. Film sizes (1 × 4 cm) were cut from each film, and a UV-vis spectrophotometer (Mapada, Shangai, China) was adjusted to 600 nm to determine their transmittance and opacity. The instrument was first calibrated using distilled water as a blank; the mode of the instrument was changed to determine percentage transparency. As shown in Equation (9), the film opacity (O) was determined by the ratio of the absorbance value at 600 nm and the film length (mm).

$$O = \frac{Abs600}{L} \tag{9}$$

2.4.9. Mechanical Characteristics

A texture analyzer (Agrosta texture analyzer, Calib, France) adjusted to initial grip separation and the detector speed was set at 5 cm and 100 mm/min, respectively, was used to determine the film's tensile strength (TS) and percentage elongation at break (%E) as described by Ferreira et al. [31] and Nxumalo and Fawole [16], with minor modifications. The ratio of the maximum load ($F_{\max}$) and the initial cross-sectional area (ϕ) of the film was used to calculate the TS of the developed films, as presented in Equation (10).

$$TS = \frac{F_{\max}}{\phi} \tag{10}$$

The per cent elongation at break (%E) of the chitosan films was obtained by dividing the film extension (Δl) and the initial film length (l_0), as presented in Equation (11).

$$\%E = \frac{\Delta l}{l_0} \times 100 \tag{11}$$

2.5. Release Kinetics of Phenolic Content and Antioxidant Capacity

The release kinetics of the developed films were conducted as outlined by Lian et al. [32] and Nxumalo and Fawole [16], with slight changes. Briefly, chitosan film strips (3 × 3 cm) were submerged in 50 mL of 50% ethanol and centrifuged at 1000 rpm for 10 min. At room temperature, the release solution was drawn at 10 min intervals up to 120 min, and a UV-vis spectrophotometer at different absorbance wavelengths measured the released phenolic content and antioxidant capacity of the developed films.

2.5.1. Total Phenolic Content

A methodology by Lian et al. [32] and Prior et al. [33] that uses the Folin–Ciocalteu reagent (Sigma-Aldrich, St Louis, MO, USA) and gallic acid as a standard was used to determine the released total phenolics content (TPC) of the chitosan films. In the dark, extracted 450 µL of 50% ethanol extract was mixed with the Folin–C reagent (500 µL). The solution was left to react for 2 min, and thereafter, 2% sodium carbonate (2% w/v) was added, vortexed for 30 s, and stored in a dark chamber for 2 h to complete the reaction process. The absorbance of the solution was then determined at 760 nm.

2.5.2. Radical Scavenging Activity

A procedure outlined by Siripatrawan and Vitchayakitti [6] and Lian et al. [32] was used to evaluate the radical scavenging activity (RSA) of 2,2-diphenyl-1-picryl-hidrazil (DPPH) and Trolox was used as a standard. A solution containing 15 µL of 50% ethanol was mixed with 0.1 mM of 735 µL methanolic DPPH. This was then kept in a dark room for 30 min, and the absorbance of the mixture was measured at 517 nm.

2.5.3. Ferric Reducing Antioxidant Power

A ferric-reducing antioxidant power (FRAP) solution consisting of 300 mM acetate buffer (50 mL), 2,4,6-tripyridyl-s-triazine (TPTZ) (5 mL), and 20 mM $FeCl_3$ (5 mL) was prepared as according to Genskowsky et al. [14] and Trolox was used as a standard. Then, 2850 µL of the FRAP solution was mixed with 150 µL of 50% ethanolic extract and stored in a dark chamber for 30 min. Absorbance was then determined at 593 nm.

2.6. Antifungal Activity Assay

Inoculates of *Penicillium expansum* (STE-U 7865) and *Botrytis cinerea* (STE-U 7866), postharvest pathogens of fruits [12–14], were sourced from the culture collection at Stellenbosch University, South Africa, to evaluate the antifungal properties of the developed chitosan films. Film discs (6 mm diameter) were put on nutrient agar (Merck, Darmstadt, Germany) plates, inoculated with indicator fungal strains, and then constantly nurtured at 37 °C for four days [6,16,34]. The diameter of the inhibitory zone adjacent film discs and the contact area of film discs with agar surface were measured. In instances where no distinct inhibitory zone was observed around a film disc, it was classified as demonstrating no inhibitory activity. To ensure the reproducibility and reliability of the results, each test was conducted in triplicate.

2.7. Statistical Analyses

Data was statistically analyzed using GenStat Statistical Software (GenStat, 18.2 edition, VSN International, Hemel Hempstead, UK) and subjected to a one-way analysis of variance. Where significant differences ($p < 0.05$) were detected, the mean separation ($n = 3$) was conducted using Duncan's multiple range test. XLSTAT software version 2020.4.1.1020 (Addinsoft, Paris, France) was used for Principal component analysis (PCA) of the obtained data.

3. Results and Discussion

3.1. Characterization of Films

3.1.1. X-ray Diffraction

According to Podgorbunskikh et al. [35], X-ray diffraction patterns of chitosan samples must not necessarily alter their crystalline structure. However, findings obtained from this experiment revealed that incorporating the medicinal plant extracts into chitosan affected the degree of crystallinity in the films (Figure 1), indicating that the amorphous state of chitosan was altered. The reflection around $2\theta = 10^\circ$ (peak I) is associated with the hydrated crystalline structure of chitosan films, which contain bound water even when extensively dried, as noted by Souza et al. [36]. Ch+S films showed the highest crystallinity (26,288.31 a.u.), while chitosan-only films exhibited the lowest crystallinity (14,710.43 a.u.). Peak II (reflection around $2\theta = 20^\circ$), which corresponds to the major peak of crystallinity, was observed in all films, with Ch+L films having the highest crystallinity (21,157.48 a.u.) and chitosan-only films exhibiting the lowest crystallinity (17,109.56 a.u.). Rubilar et al. [37] also observed that carvacrol and grape seed extract in a chitosan medium resulted in film reflections at $2\theta = 10^\circ$ and $2\theta = 20^\circ$, while Nxumalo and Fawole [16] also reported that the chitosan films loaded with *Bidens pilosa* had crystallinity increased with a rise in the plant extract concentration. Modifications in oxygen permeability of the developed chitosan-based films affect the extent of crystallinity of films [36].

3.1.2. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to study the structural arrangement of the developed chitosan films (Figure 2). The results revealed that incorporating different plant extracts into the chitosan compound altered their microstructure. The SEM results indicated a compact and uniform smooth microstructure comprising few cracks and openings for chitosan-only films (Figure 2A). The treated chitosan films had increased roughness, reduced pores, and white spots, and the distribution of the applied plant extracts into the

chitosan environment differed (Figure 2B–D). Similar microstructures were observed by Riaz et al. [3], whereby apple peel polyphenols were fused into chitosan films. Peng and Li [38] also reported that adding essential oils from lemon, thyme, and cinnamon increased the coarseness of the cross-section chitosan films. The increased roughness of the films might be due to the formation of insoluble polyphenol particles within the polymer matrix during film drying [39]. Furthermore, different microstructural changes in the chitosan films were also observed between the different medicinal plant extracts in the chitosan matrix. Ch+L films (Figure 2B) had patchy openings with no uniform distribution and a few white spots. Ch+S films (Figure 2C) had a uniform darker structure with a rough appearance accompanied by visible pores that were very small compared to chitosan-only films. The visible pores were well distributed throughout the entire film. Ch+X films (Figure 2D) had a solid appearance with white spots and no obvious visible pores. The different observed SEM images might result from the compounds found in the different plant extracts that can influence the distribution of these compounds in the chitosan matrix.

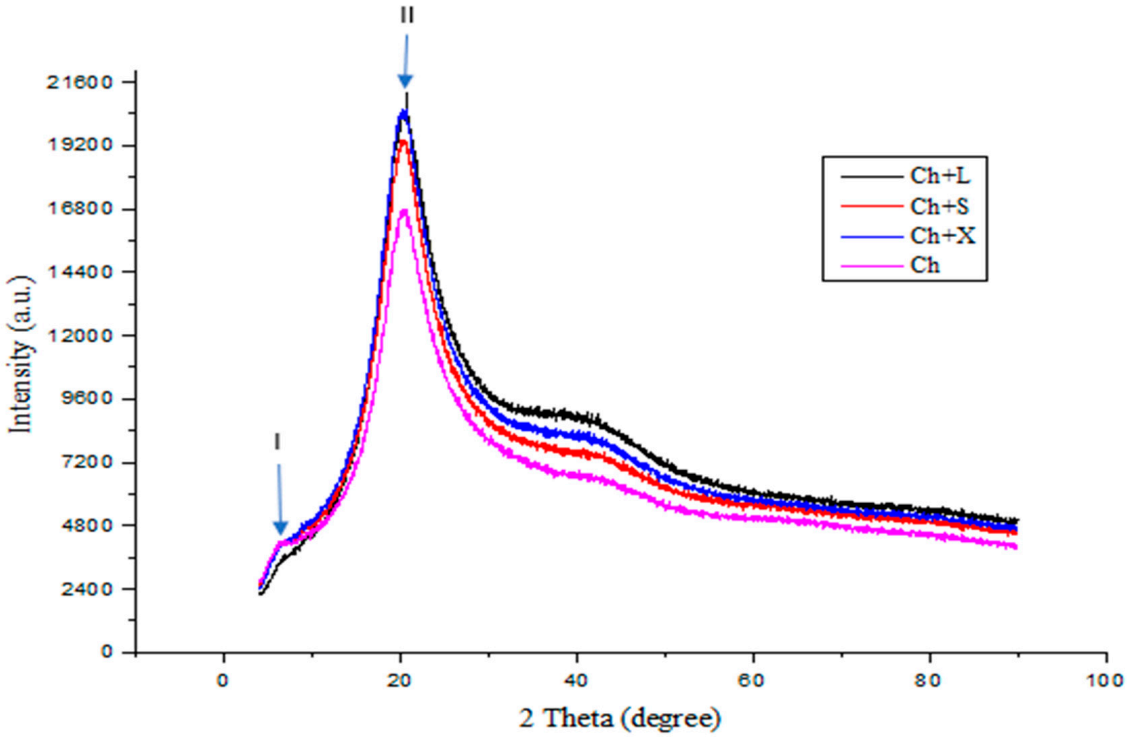

Figure 1. *X*-ray diffraction patterns of chitosan-based films enriched with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Ch; Chitosan. Peak I, reflection around 2θ = 10°; Peak II, reflection around 2θ = 20°.

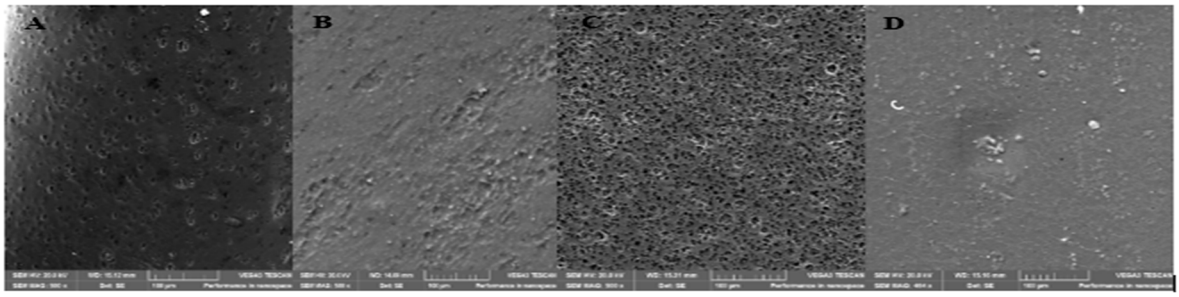

Figure 2. Scanning electron microscopy micrographs (SEM MAG: 100×) of chitosan functionalized with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Key: (**A**–**D**) are linked to the cross-sectional morphology of chitosan only, Ch+L, Ch+S, and Ch+X, respectively.

3.1.3. Film Thickness and Density

Fusing the different plant extracts into the chitosan medium significantly ($p < 0.05$) increased the thickness and density of the developed films (Figure 3A and Figure 3B, respectively). Ch+L films exhibited thicker films that had higher density (0.189 mm and 1.62 g·cm^{-3}, respectively), followed by Ch+X (0.183 mm, 1.59 g·cm^{-3}, respectively), Ch+S (0.168 mm and 1.39 g·cm^{-3}, respectively) and the lowest film thickness and density were observed in chitosan-only films (0.128 mm and 1.08 g·cm^{-3}, respectively). Similarly, Riaz et al. [3] observed an increase in chitosan film thickness and density after incorporating apple peel polyphenols, while Nxumalo and Fawole [16] reported that chitosan films had their density and thickness increased with an increase in *B. pilosa* extract concentration. According to Zhang et al. [40], fusing the plant extracts into the chitosan medium increases the interactions between the applied plant extracts and chitosan, causing a stronger bond in the developed films. Thus, it can be hypothesized that the space between the chitosan molecules was decreased, making the film structure more compact and, in this way, increasing the film thickness and density.

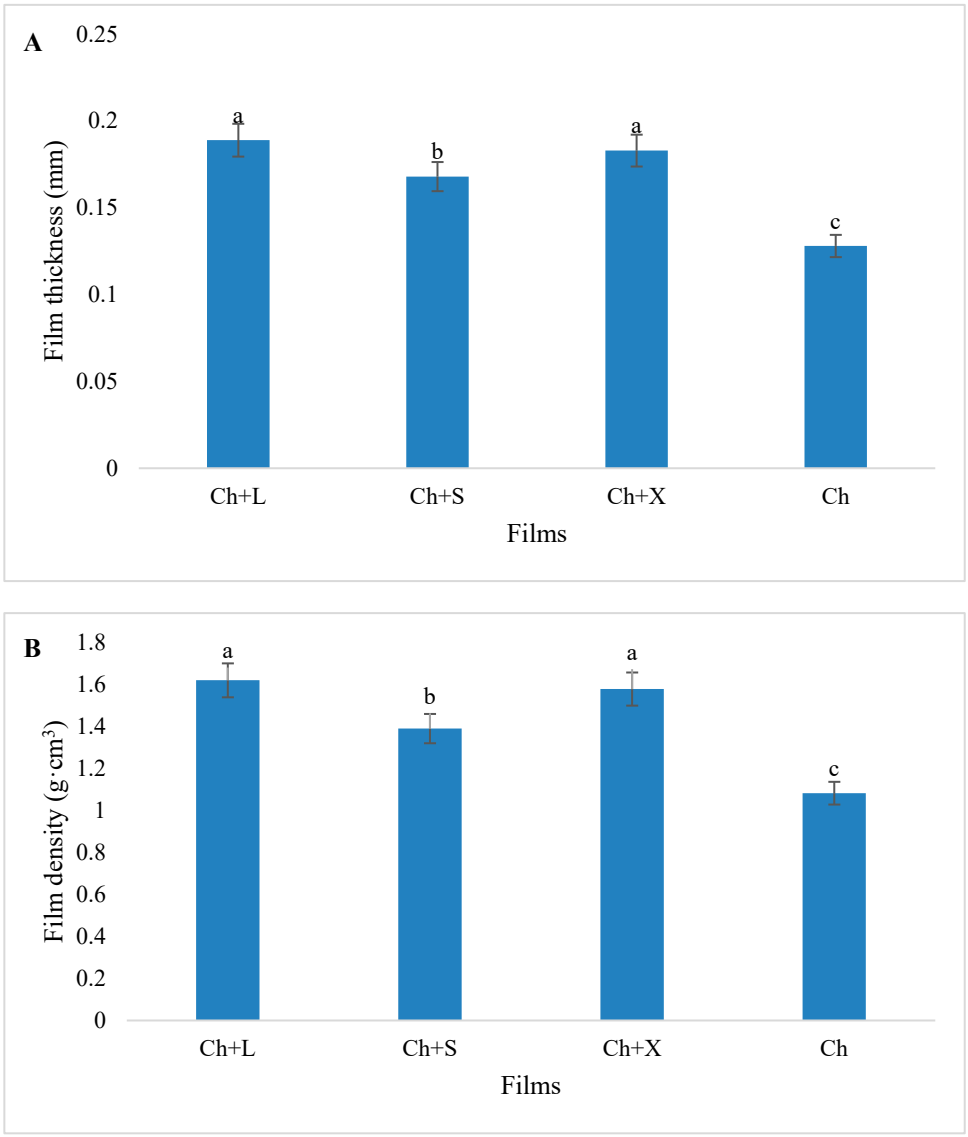

Figure 3. (**A**) Film thickness and (**B**) film density degree influenced by chitosan enriched with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Ch: chitosan only. Different letters indicate a statistical difference ($p < 0.05$). Error bars denote the standard error of the mean.

3.1.4. Film Water Vapor Transmission Rate and Water Content

Moradi et al. [4] defined the water vapor transmission rate (WVTR) as the amount of water vapor that can be diffused in a unit area at a given time, influenced by the barrier properties of the film and the changes in the amount of water vapor on both sides of the film. According to Bourtoom and Chinnan [41], the significance of a film is to prevent water exchange between the food and the nearby surroundings; thus, the film's WVTR should be very low. Generally, the WVTR significantly ($p < 0.05$) decreased after fusing plant extracts into the chitosan medium (Table 1). Ch+X films had the lowest WVTR (9.9 g m^{-1} 24 h^{-1}), followed by Ch+L (10.2 g m^{-1} 24 h^{-1}), Ch+S (13.1 g m^{-1} 24 h^{-1}) and the highest WVTR (17.2 g m^{-1} 24 h^{-1}) was observed in the chitosan-only films (Table 1). Similarly, Nxumalo and Fawole observed that interaction between chitosan and *B. pilosa* extract decreased the WVTR in chitosan films, resulting in control films having the highest WVTR. Wu et al. [42] reported that the decrease in WVTR of the films enriched with the different medicinal plant extracts might be caused by the interface between chitosan and the applied plant extracts, which reduced the disposal of hydrophilic groups in chitosan and reduced their interactions with water. Siripatrawan and Vitchayakiti [6] also observed that fusing propolis into the chitosan compound significantly lowered the WVTR of the films; however, it did not change with an increase in concentration. Thus, incorporating *X. caffra* into the chitosan matrix (Ch+X) decreased the distribution of water vapor through the chitosan-based film better than the other applied medicinal plant extracts, and therefore, this might result in better retardation of food deterioration when used as a packaging material.

Table 1. Water vapor transmission rate (WVTR) and water content of chitosan-based films enriched with different medicinal plant extracts.

Films	WVTR (g m^{-2} 24 h^{-1})	Water Content (%)
Ch+L	10.2 ± 1.45 ^{c}	27.8 ± 0.77 ^{b}
Ch+S	13.1 ± 1.73 ^{b}	26.2 ± 0.72 ^{b}
Ch+X	9.9 ± 1.76 ^{c}	26.3 ± 0.61 ^{b}
Ch	17.15 ± 2.54 ^{a}	33.3 ± 0.57 ^{a}

Water vapor transmission rate (WVTR) and water content of chitosan fused with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Ch: chitosan only. Different letters in the same column across treatments for each parameter are statistically different ($p < 0.05$) according to Duncan's multiple range test. Data presented as mean ± standard error (SE).

With regards to the moisture content of the developed chitosan films, the incorporation of the investigated medicinal plant extracts decreased the water content of the films, with no significant difference ($p > 0.05$) observed among the functionalized films (Table 1). It can be hypothesized that enriching the chitosan-based films with the investigated medicinal plant extracts improved the capacity of chitosan to bind with water molecules and enhanced its hydrophilicity, lowering their water content [2]. Fusing the medicinal plant extracts into the chitosan medium improved the capability of the chitosan to attach to water molecules and thus boosted its hydrophilic characteristics [16].

3.1.5. Film Swelling Degree and Water Solubility

Waterproof properties of films are affected by their rise in swelling degree and water solubility capacity [43]. Chitosan-based films fused with the investigated plant extracts significantly ($p < 0.05$) enhanced the film swelling degree and water solubility (Figure 4A and B, respectively). Ch+L films had a higher increase in swelling degree and water solubility (48.6% and 32.8%, respectively), followed by Ch+S (47.3% and 31.9%, respectively), Ch+X (44.8% and 29.9%, respectively) and the lowest film swelling degree and water solubility (31.9% and 18.9%, respectively) were observed in the chitosan-only films. Liu et al. [44] stated that the increased water solubility and swelling degree of the enriched films could be due to their improved hydrophilicity. Thus, fusing the plant extracts into the chitosan compound increased the hydrophilicity of the films. Similarly,

Moradi et al. [4] reported that chitosan-based film swelling degree and water solubility increased after incorporating Shirazi thyme oil and grape seed extract. It can, therefore, be assumed that fusing the chitosan films with the investigated medicinal plants could potentially be used to wrap food with high moisture.

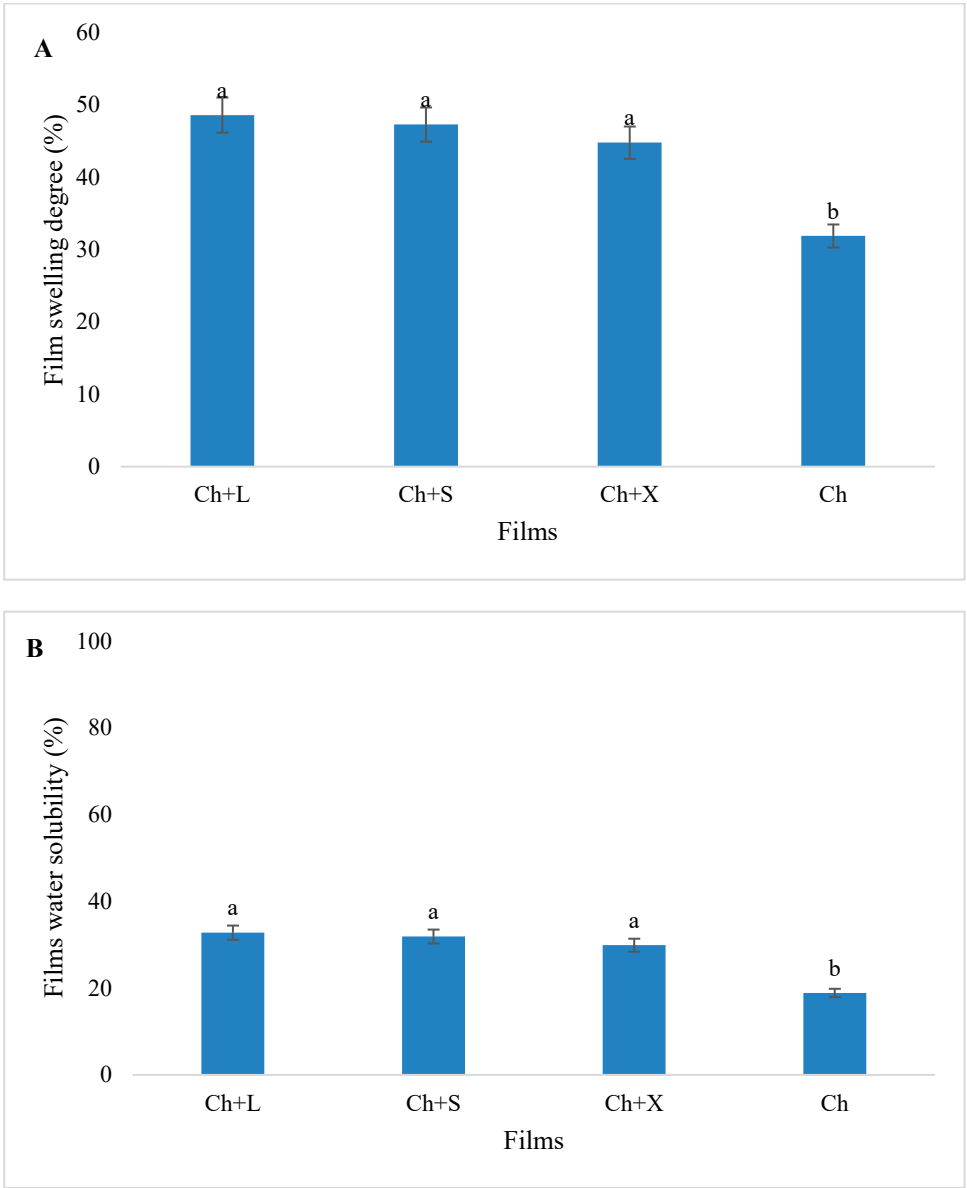

Figure 4. (**A**) Film swelling degree and (**B**) water solubility of chitosan films fused with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Ch: chitosan only. Different letters indicate a statistical difference ($p < 0.05$). Error bars denote the standard error of the mean.

3.1.6. Mechanical Properties of the Chitosan Films

Figure 5A,B illustrate the impact of the medicinal plant extracts on the mechanical properties of chitosan-based films. Bourtoom and Chinnan [41] describe tensile strength as the extreme strength that a film can withstand during a tension test, while elongation at break is the film's stretchability to break under tensile stretch and is expressed as a percentage. Fusing the different medicinal plant extracts into the chitosan medium significantly ($p < 0.05$) decreased the tensile strength and increased the elongation per cent at break of the chitosan-based films. Therefore, the highest tensile (23.4 MPa) was observed

in chitosan-only films, followed by Ch+L films (17.7 MPa), Ch+X films (14.30 MPa), and the lowest tensile strength (14.10 MPa) was observed in Ch+S films (Figure 5A). On the other hand, Ch+L films had the highest elongation per cent at break (41.9%), followed by Ch+S films (35.3%), Ch+X films (29.2%), and the lowest elongation per cent at break (25.4%) was obtained in chitosan-only films (Figure 5B). This suggests that the flexibility of chitosan-based films can be attributed to enriching them with investigated medicinal plant extracts. Riaz et al. [3] reported that incorporating apple peel polyphenols increased the elongation percentage and tensile strength of chitosan-based films. According to Bodini et al. [45], this observation suggests that the plant extracts and chitosan might have formed a cross-linking outcome that reduced the chitosan polymer's free volume and molecular mobility, thus causing an increase in elongation per cent at break and a decrease in tensile strength.

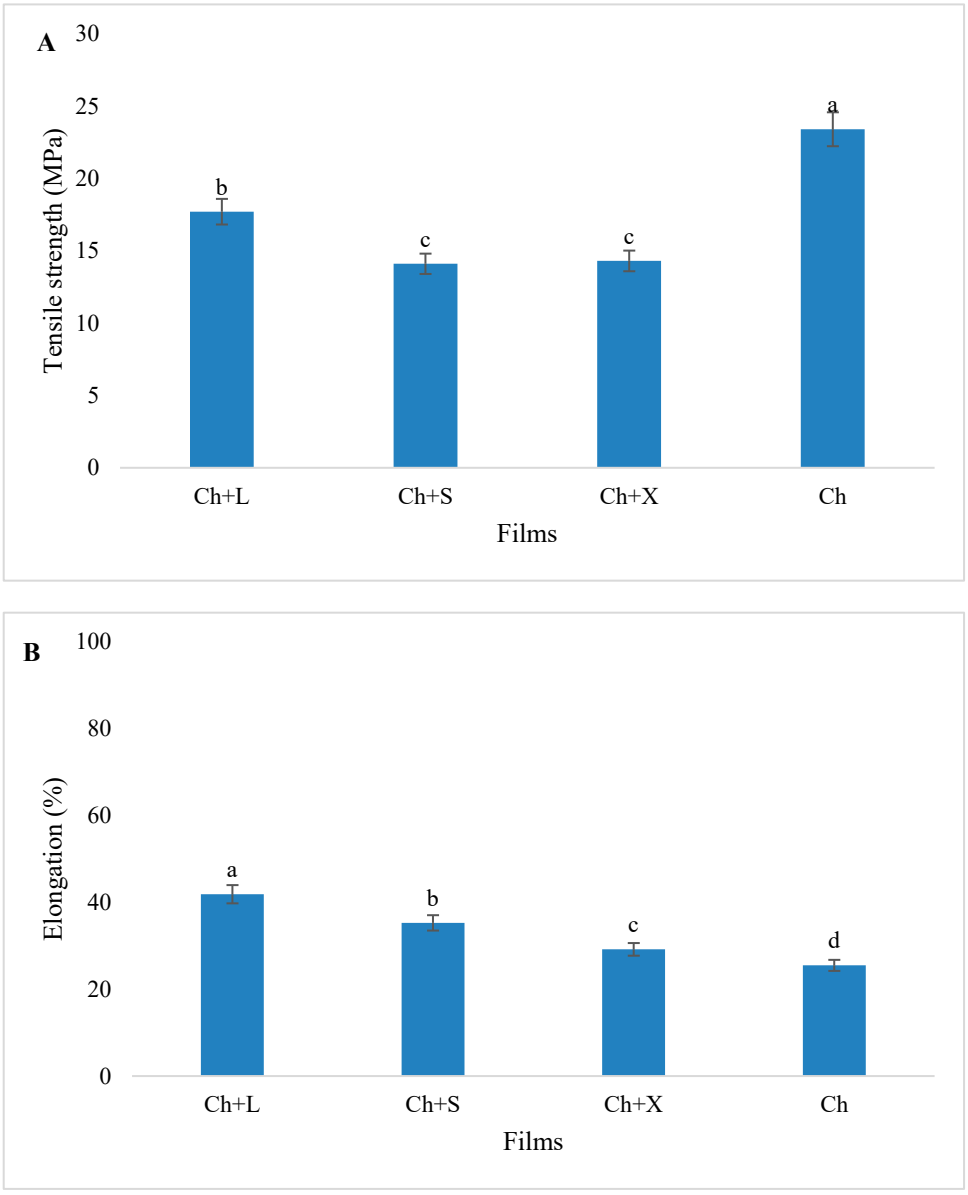

Figure 5. (**A**) Tensile strength and (**B**) elongation of chitosan films functionalized with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Ch: chitosan only. Different letters indicate a statistical difference ($p < 0.05$). Error bars denote the standard error of the mean.

3.1.7. Film Color Attributes

Color and opacity influence consumer acceptance of edible films, especially when packaging bright-colored foods [3]. The physical color appearance of the chitosan films is presented in Figure 6. Chitosan-based films enriched with different medicinal plant extracts significantly ($p < 0.05$) altered the film's color attributes (Table 2). The b^* (yellowness) of the films improved after incorporating the investigated medicinal plant extracts. Notably, Ch+X films had a significantly ($p < 0.05$) higher b^* (15.1), and chitosan-only films had the lowest (4.8). This changed the total color difference (TCD) in the films, with Ch+X film having a significantly ($p < 0.05$) higher TCD (11.5) and Ch+L film having the lowest TCD (8.01). The yellow index (*YI*) and white index (*WI*) were also significantly ($p < 0.05$) affected by the incorporated plant extracts.

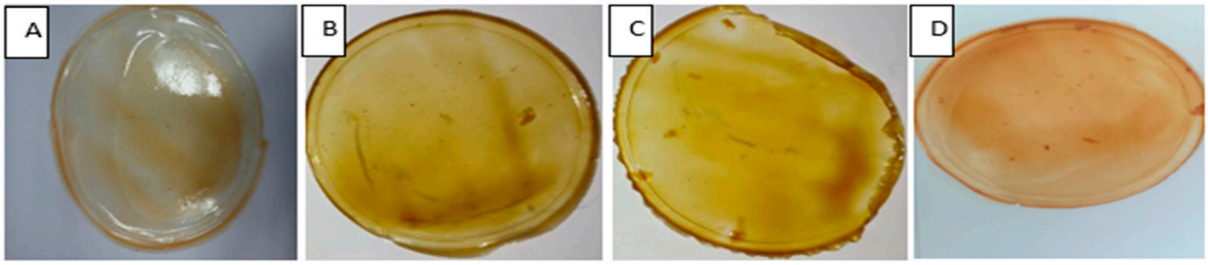

Figure 6. Observed color appearance of chitosan films functionalized with 1% medicinal plant extracts. (**A**) Chitosan-only; (**B**) *Lippia javanica;* (**C**) *Syzygium cordatum;* and (**D**) *Ximenia caffra* extract film.

Table 2. Color attributes, transmittance, and opacity of chitosan films fused with different medicinal plant extracts.

Treatment	b^*	TCD	Yellow Index	White Index	Transmittance (%)	Opacity (A·mm^{-1})
Ch+L	8.1 ± 0.59 ^{c}	8.01 ± 0.3 ^{b}	35.4 ± 1.12 ^{c}	32.2 ± 1.48 ^{b}	31.6 ± 1.74 ^{c}	12.5 ± 0.51 ^{a}
Ch+S	12.2 ± 2.02 ^{b}	10.9 ± 0.24 ^{a}	54.3 ± 0.84 ^{b}	30 ± 0.61 ^{c}	31.8 ± 1.29 ^{c}	10.9 ± 0.17 ^{b}
Ch+X	15.1 ± 1.15 ^{a}	11.5 ± 0.40 ^{a}	61.3 ± 0.61 ^{a}	28.5 ± 0.99 ^{d}	36.6 ± 1.71 ^{b}	9.5 ± 0.12 ^{c}
Ch	4.8 ± 0.44 ^{c}	-	17.3 ± 0.92 ^{d}	39.6 ± 0.59 ^{a}	51.8 ± 0.40 ^{a}	3.3 ± 0.07 ^{d}

Film color attributes, transmittance, and opacity of chitosan films (Ch) fused with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X). Ch: chitosan only, TCD: total color difference. Different letters in the same column are statistically different ($p < 0.05$) according to Duncan's multiple range test. Data presented as mean ± standard error.

Ch+X film had a significantly ($p < 0.05$) higher *YI* and lower *WI* (61.3 and 28.5, respectively), while control films had the lowest *YI* and highest *WI* (17.3 and 39.6, respectively). Arfat et al. [46] stated that the reduced whiteness of the films might prevent visible and ultraviolet from affecting the wrapped food's nutritional content and loss of color and flavor. Nxumalo and Fawole [16] observed that the color of chitosan films is subjective to the concentration of loaded plant material.

3.1.8. Transmittance and Opacity

Chitosan-only films had higher transmittance (51.8%) and lower opacity (3.3 A·mm^{-1}). Fusing the different plant extracts into the chitosan medium significantly ($p < 0.05$) reduced the transmittance and increased the opacity of the films (Table 2). Among the chitosan-based films fused plant extracts, Ch+X films had a significantly ($p < 0.05$) higher transmittance and lower opacity (36.6% and 9.5 A·mm^{-1}, respectively), and the lowest transmittance and highest opacity (316% and 12.5 A·mm^{-1}, respectively) was observed on Ch+L films. It can be hypothesized that the observed higher transmittance and lower opacity of Ch+X films can maintain the native color of packaged food products better than the other treatments.

3.1.9. Film Glossiness

Incorporating different plant extracts into the chitosan compound caused a significant ($p < 0.05$) decrease in the glossiness of the films (Table 3). Notably, an increase in the view angle resulted in increased gloss values. This may be attributed to specular reflection detected as the incidence angle increased [47]. The incorporated plant extracts resulted in significantly ($p < 0.05$) lower glossiness across all the investigated angles when compared with chitosan-only films. Among the treated films, Ch+S films had higher glossiness (45.3, 76.1, and 105.7% at 20, 60, and 80°, respectively), while Ch+L films exhibited the lowest glossiness (39.8, 59.9, and 80.9% at 20, 60, and 80°, respectively). The distribution of the plant extracts into the chitosan medium might have caused increasing irregularities on the film surface [48], thus reducing the film's glossiness. Sánchez-González et al. [49] also observed reduced glossiness with hydroxypropyl methylcellulose films infused with tea essential oil, while Nxumalo and Fawole [16] indicated that lowering the concentration of *B. pilosa* extract resulted in higher glossiness of chitosan films.

Table 3. Gloss values at angles 20°, 60°, and 80° of chitosan films fused with different medicinal plant extracts.

	Angle		
Films	**20°**	**60°**	**80°**
Ch+L	39.8 ± 0.58 ^{d}	59.9 ± 0.91 ^{d}	80.9 ± 0.88 ^{d}
Ch+S	45.3 ± 0.55 ^{b}	76.1 ± 2.38 ^{b}	105.7 ± 1.35 ^{b}
Ch+X	41.2 ± 0.67 ^{c}	61.4 ± 1.39 ^{c}	84.7 ± 0.95 ^{c}
Ch	52.8 ± 0.59 ^{a}	86.3 ± 1.53 ^{a}	113.4 ± 1.22 ^{a}

The glossiness of chitosan films fused with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X). Ch: chitosan only. Different letters in the same column are statistically different ($p < 0.05$) according to Duncan's multiple range test. Data presented as mean ± standard error.

3.2. Release Kinetics of Total Phenolic Content and Antioxidant Capacity

Chitosan-only films peaked and remained constant at 3 mg GAE/g film of total phenolic content (TPC) after 10 min (Figure 7A). However, chitosan films fused with the plant extracts had a steady, significant ($p < 0.05$) upsurge in the release of TPC, and different peak stabilities were observed. Ch+L and Ch+S films peaked and remained constant at 90 min, releasing 74.4 and 80.1 mg GAE/g film of TPC, respectively, while Ch+X films peaked and remained constant at 110 min, releasing 86.2 mg GAE/g film of TPC. It can be hypothesized that Ch+X can release slower TPC over time compared to the other plant extracts in the chitosan compound, which is an integral component of a stable film used to package food.

Regarding the antioxidant properties, the TPC released was subjected to radical scavenging activity (RSA) and ferric ion antioxidant reducing power (FRAP). It was observed that chitosan-only films had 4.2 mg TE/g film and 1.4 mg TE/g film of RSA and FRAP of the released phenolics, respectively. However, Ch+L films peaked at 80 min and remained stable, with the released TPC having 84.2 mg TE/g RSA, while the TPC of Ch+X films peaked at 100 min and remained stable, releasing 97.2 mg TE/g RSA (Figure 7B).

Notably, Ch+X films had a longer TPC release of 85.7 mg TE/g FRAP at 110 min, while Ch+L peaked and remained stable at 90 min, releasing 75.4 mg TE/g FRAP (Figure 7C). According to Bors et al. [50], even though RSA and FRAP are considered to be the antioxidant capacity of compounds, differences in the release of TPC are caused by their different mechanisms. From the results, it can be hypothesized that *L. javanica* extracts in a chitosan matrix (Ch+L) developed weak covalent bonds compared with the other medicinal plants, resulting in a fast release of phenolic content in 50% ethanol. On the other hand, *X. caffra* (Ch+X) developed stronger covalent bonds than the other treatments, slowly releasing the phenolic compounds. Bourtoom and Chinnan [41] and Pan et al. [51] stated that the lengthier release of phenolic and antioxidant composites is essential for the stability of the films under different pH levels.

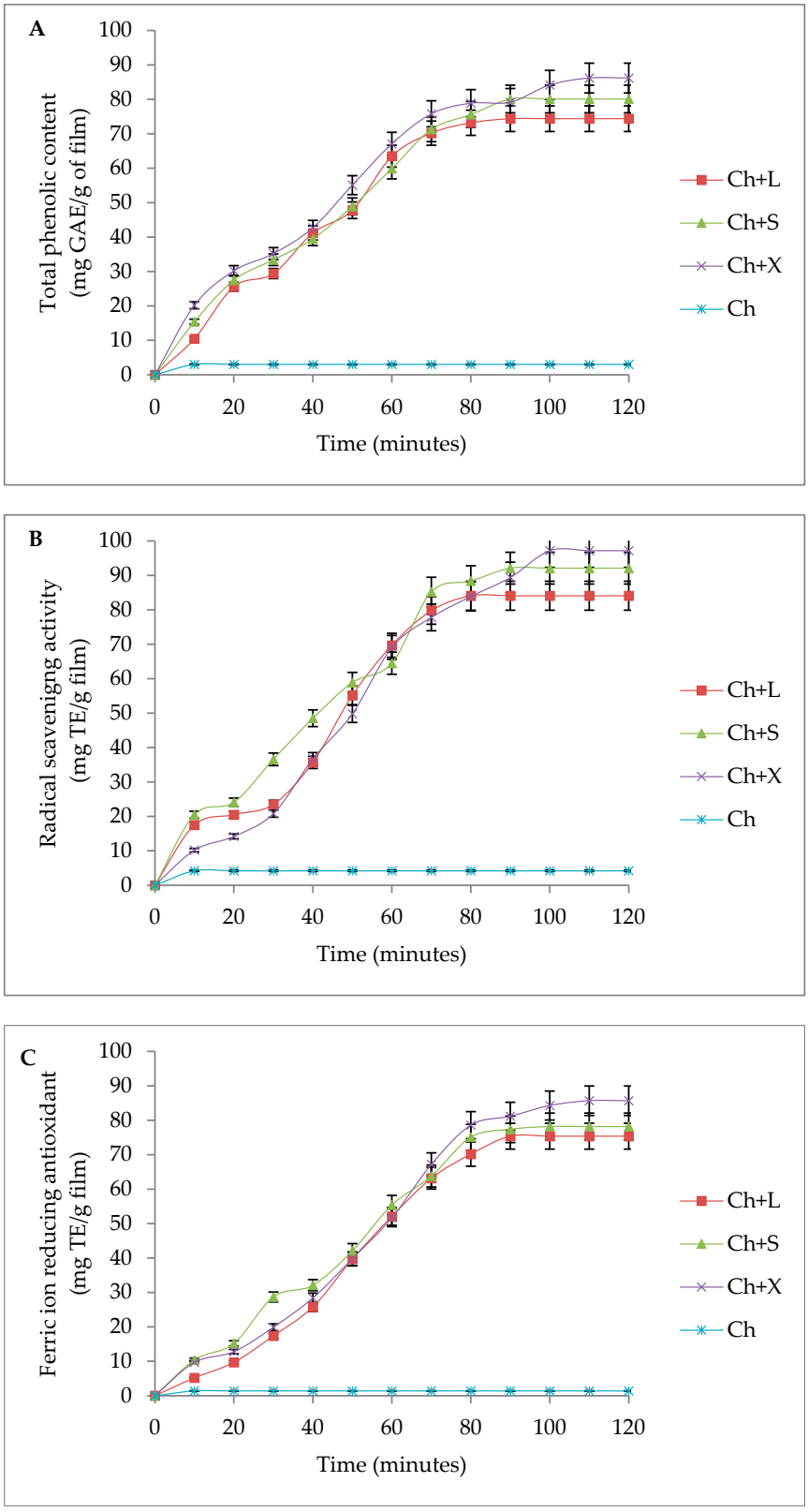

Figure 7. Kinetics release of (**A**) total phenolic content; and antioxidant capacity measured as (**B**) radical scavenging, and (**C**) ferric ion reducing antioxidant power from the chitosan films into 50% ethanol for 120 min. Chitosan films functionalized with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract. Ch: chitosan only. GAE: Gallic acid equivalent, TE: Trolox equivalent. Means ± standard errors (SE) are presented, and error bars denote the SE of the mean.

3.3. Antifungal Activity

The antifungal agent of plant extracts varies depending on their biological composition and biochemical mode of action on distinct positions of the fungal cell [13]. The chitosan films fused with plant extracts were found to have a significantly higher inhibition zone diameter against *Penicillium expansum*, with values ranging from 10 mm to 10.2 mm, which was not significantly different ($p < 0.05$) amongst the plant extracts compared to chitosan-only films, which had a minimum inhibition zone diameter of 3.3 mm (Figure 8A) ($p > 0.05$). This means that the addition of medicinal plant extracts to the chitosan matrix resulted in an improved inhibition zone against *Penicillium expansum*.

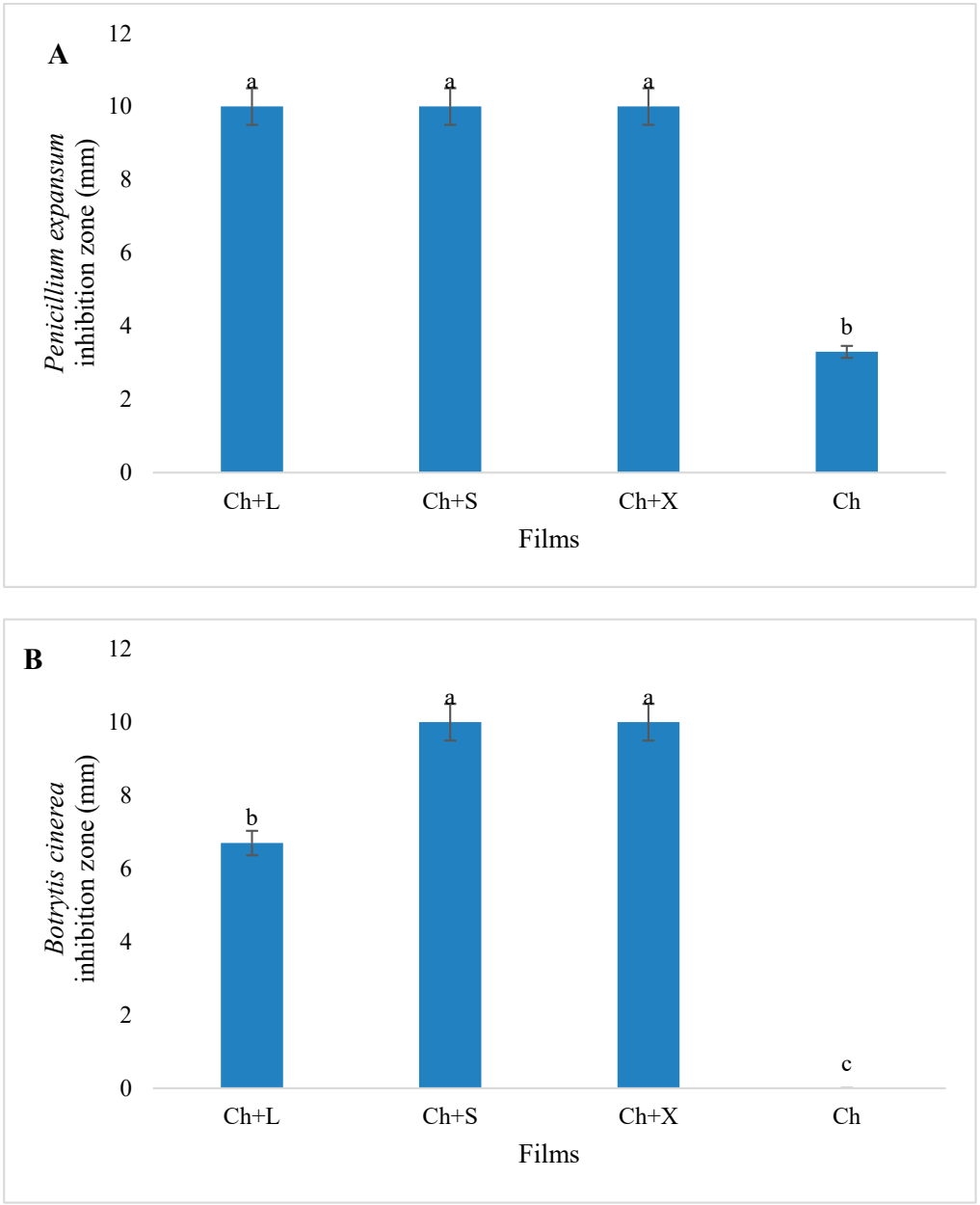

Figure 8. (**A**) Antifungal activity of chitosan films functionalized with 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract against *Penicillium expansum* and (**B**) *Botrytis cinerea*. Ch: chitosan only. Different letters are statistically different ($p < 0.05$). Error bars denote the standard error of the mean.

Similarly, against *Botrytis cinerea*, Ch+S and Ch+X films showed a 10 mm inhibition zone diameter, followed by the Ch+L film with a 6.7 mm inhibition zone diameter. However, chitosan-only films did not exhibit any inhibition zone against the growth of *Botrytis cinerea* (Figure 8B). These findings suggest enriching the chitosan films with different medicinal plant extracts enhanced their antifungal properties against these important postharvest pathogens. This demonstrated the potential of chitosan films functionalized with plant extracts as a promising substitute to synthetic antifungal agents for postharvest preservation of horticultural crops.

3.4. Principal Component Analysis

To obtain a wider scope of the characteristics of the chitosan films fused with plant extracts, statistics attained were analyzed using principal component analysis (PCA). Fawole and Opara [52] reported that the eigenvalue evaluates the magnitude of a component, with eigenvalues $\geq$1 significant, and the uppermost eigenvalues are the utmost significant. The entire variability was described by four factors (F1–F4), with the first two factors of the PCA explaining 91.96% of the variability (Figure 9A). The first factor (F1) contributed 85.1% of the entire dissimilarity, while the second factor (F2) contributed 6.86% of the total variation, signifying that the extreme likely variation in the characterization of the chitosan films was attained from F1 (Figure 8B). Positive scores of F1 linked with chitosan-only films, while the negative scores along F1 matched to Ch+L, Ch+S, Ch+X, and chitosan-only films (Figure 9B). The positive scores along the F1 plane corresponded with the white index (WI), water vapor transmission rate (WVTR), tensile strength, transmittance, water content, L^*, a^*, b^*, and total color difference (TCD). Negative scores along the F1 plane corresponded with water solubility, percent elongation, opacity, swelling degree, density, film thickness, and yellow index (YI). The WVTR of chitosan films along the positive F1 plane can be correlated to high water content. This may be because enriching the chitosan films with the plant extracts resulted in a high capacity of the films to bind with water molecules and improved the hydrophilicity of the films, thus also affecting the WVTR, water content, and eventual tensile strength of the developed films [3]. The observed WVTR, water content, and tensile strength along the F1 plane may be due to the poor hydrophilicity of control chitosan films. Enriching the chitosan-based films with the investigated plant extracts changed the original color of the films; thus, higher TCD values were observed along the positive F1 plane. The L^*, a^*, and b^* along the F1 plane corresponded with the whiteness and transmittance of the films. The medicinal plant extracts in the chitosan matrix resulted in a strong beige color exerted in films, resulting in films along the F2 plane becoming yellow and increased opacity. Dutta et al. [7] stated that the color of films is directly influenced by the type of plant extract incorporated and its concentration. The swelling degree and water solubility of the films indicated that films along the F2 plane had higher water resistance than films along the F1 plane. Thus, the percent elongation, thickness, and density were observed on films along the F2 plane. These properties are desirable for absorbing extra water from the outer surface of high-moisture food [4]. The incorporation of medicinal plant extracts into the chitosan compound altered the physical and mechanical properties of the chitosan-based films at different proportions. For example, the incorporation of *L. javanica* and *X. caffra* extracts into chitosan had a positive impact on the percent elongation and opacity of the developed chitosan films. Conversely, central characteristics of a good film, including swelling degree, density, thickness, and water solubility, are strongly correlated with chitosan films enriched with *Ximenia caffra* extract (Ch+X).

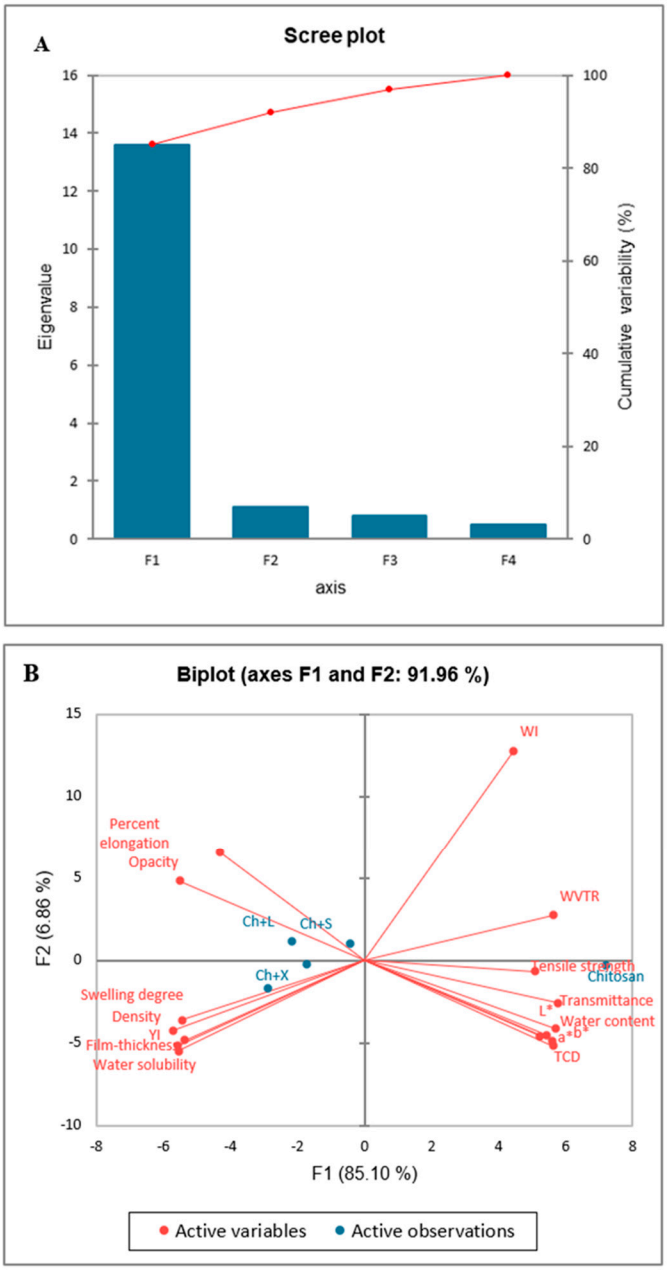

Figure 9. (**A**) Scree plot of variance explained by each factor of the principal component and (**B**) principal component analysis showing active variables and observations for chitosan films functionalized 1% of *Lippia javanica* (Ch+L), *Syzygium cordatum* (Ch+S), and *Ximenia caffra* (Ch+X) extract.

4. Conclusions

In conclusion, incorporating medicinal plant extracts into chitosan-based films enhanced the functional properties. The effectiveness of the films against *Penicillium expansum* and *Botrytis cinerea* varied among the different medicinal plant extracts. The physical, mechanical, and morphological properties of the films were also affected by the incorporation of medicinal plant extracts. These findings suggest that the developed chitosan films have potential practical applications as natural ingredients for improving edible film characteristics. The slower total phenolic content of the medicinal plant extract released over time in the chitosan medium is an integral component of a stable film used to package food. Furthermore, the positive properties of the investigated medicinal plants in the chitosan matrix could potentially be optimized for use in food preservation and packag-

ing. However, depending on their application, future studies should explore the effect of incorporating these plant extracts on food taste and consumer acceptability.

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