Hindawi
Journal of Food Quality
Volume 2023, Article ID 5580928, 18 pages
https://doi.org/10.1155/2023/5580928

Research Article

Chitosan-*Bidens pilosa* Extract-Based Coating with Enhanced Free Radical Scavenging, Antifungal, and Water Barrier Properties: Metabolite Profiling, Film Characterization, and Raspberry Preservation

Kwanele A. Nxumalo and Olaniyi A. Fawole

Postharvest and Agroprocessing Research Centre, Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park, Johannesburg 2006, South Africa

Correspondence should be addressed to Olaniyi A. Fawole; olaniyif@uj.ac.za

Received 10 August 2023; Revised 2 November 2023; Accepted 15 November 2023; Published 30 November 2023

Academic Editor: Alejandro Hernández

Chitosan (Ch) was functionalized with *Bidens pilosa* (BP) extract at 1, 3, or 5% to form Ch-1 BP, Ch-3 BP, and Ch-5 BP, respectively. Ch without plant extract was used as a control. The composite films were characterized and tested for antifungal properties in the agar disc diffusion approach and antioxidant capacity in the 2,2-diphenyl-1-picrylhydrazyl assay. Chitosan films fused with *B. pilosa* extract inhibited the growth of *P. expansum* with a 10 mm inhibition diameter compared to control films (3.33 mm). Ch-1 BP exhibited the highest (79.13%) radical scavenging activity (RSA), and control films had the lowest RSA (50.97%). Liquid chromatography-mass spectrometry analysis identified 20 metabolites, including phenolic acids (9), organic acids (8), monosaccharides (1), amino acids (1), flavonoids (1), and six unknown polyphenols. Chitosan-based composite coating (Ch-1% BP) exhibited the lowest weight loss percentage (16.74%) and decay incidence (17.3%), while the untreated fruit showed higher weight loss and decay incidence (48.33% and 83.2%, respectively). The effectiveness of chitosan-enriched coating in maintaining the postharvest quality of raspberry fruit suggests that this plant could be a postharvest agent for controlling microbial spoilage and protecting against oxidative stress, ultimately resulting in extended storage in horticultural crops.

1. Introduction

In recent decades, the quest for enhancing food safety has pivoted towards the promotion of active food packaging materials that have improved antioxidant scavenging activity, optimized moisture regulation, and efficient gaseous exchange [1]. By integrating functional compounds and elements such as antioxidants and antimicrobial agents, these substances become transformative elements in food preservation [2–4]. With increasing global concerns for food safety, consumer health, and the environment, the pursuit of developing biodegradable natural substances is on the rise.

Among edible packaging materials, chitosan, a natural carbohydrate copolymer, is extensively studied in the food industry [5]. Its unique characteristics include superior film-forming abilities, physical and mechanical properties, biodegradability, nontoxicity, and biocompatibility [5–7]. While chitosan-based films demonstrate efficacy in food preservation, their antioxidant and antimicrobial capacities are somewhat limited [8, 9], highlighting an opportunity to enhance these films for more extensive use in the food industry as an active packaging material.

Plant derivatives are rich in antimicrobial and antioxidant activity and are a natural alternative for integration into edible films [10, 11]. Based on a thorough novel assessment of medicinal plants utilized in indigenous knowledge systems (IKS) for food preservation, one such promising candidate plant is *Bidens pilosa* [12]. *Bidens pilosa* L. (blackjack) is a member of the Asteraceae family and a common annual weed species found in most fields. Its use includes

food and indigenous drugs [13], as a natural biopesticide [14], as weed control, and as a remedy to heavy metal-polluted soils [15, 16], indicating that this plant has a wide range of bioactive compounds such as flavonoids, polyenes, and phenolic and organic compounds that have been reported to have high antibacterial biological activity, and antifungal and antioxidant properties [12, 17, 18]. Research by Ngamo et al. [19] and Goudoum et al. [20] informed that dried *B. pilosa* leaves were customarily used as sauce recipes, food preservation, and tea ingredients [21]. Moreover, extracted essential oils and dried leaves of *B. pilosa* were allegedly efficient at managing stored grain insect pests [20]. The ability to inhibit reactive oxygen species (ROS) and control decay within food, increasing its shelf life, points towards the utility of *Bidens pilosa* in postharvest crop preservation [18, 20].

Our study aimed to harness IKS to fabricate a novel active packaging film with superior antioxidant and barrier properties. This was achieved by blending chitosan films with *Bidens pilosa* extract at varying concentrations. Following the development of the films, an array of film characterization was conducted. The novel chitosan-*B. pilosa* composite was then applied to raspberry fruits to evaluate its effectiveness in postharvest preservation, with emphasis on parameters such as weight loss, decay incidence, and color attributes as a ripening indicator during storage.

2. Materials and Methods

2.1. Sourcing, Extraction, and Phytochemistry Analysis of *Bidens pilosa*

2.1.1. Procurement of Raw Material. Matured and disease free black-jack (*Bidens pilosa*) leaves were gathered from Mafutseni (26°24′21.9″ S, 31°35′05.3″ E), Eswatini, from the Eswatini Institute for Research in Traditional Medicine, Medicinal and Indigenous Food Plants (EIRMIP) farm. A taxonomist from EIRMIP classified the plants and advised on selecting fully matured leaves. Oven-drying at 50°C for 72 h, crushing into a fine powder, and keeping the powdered leaves in a zip-lock bag were done to preserve the phytochemical content and antioxidant potential until further use.

2.1.2. Extraction Procedure. A method by Ramesh et al. [22] and Hassan et al. [23], adopted with minor alterations, was used to extract the powdered plant material. In a nutshell, in 100 mL of 70% ethanol, 20 g of finely ground *B. pilosa* was interspersed in a 250 mL beaker and let to react at room temperature for 30 min. Thereafter, the combination was sonicated at 20°C, 005 Hz, at maximum speed for 1 h, filtered using a Whatman filter paper no. 1, and concentrated under reduced pressure at 45°C using a rotary evaporator (Rotavapor R-200, Buchi Laboratory Equipment, Flawil, Switzerland). Obtained extracts were kept in antiseptic bottles and then refrigerated until further use.

2.1.3. Phenolic Profiling of Bidens pilosa Extract Using Liquid Chromatography-Mass Spectrometry. The metabolomic analysis reported by Magangana et al. [24] with a few adjustments by Magangana et al. [25] was adopted using a Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultraperformance liquid chromatograph (UPLC) (Waters, Milford, MA, USA). The measurement and structural confirmation of some of the metabolites was possible with pure standards (catechin; ellagic acid; epicatechin; gallic acid; punicalagin α; punicalagin β; punicalin α and β; chlorogenic acid; rutin; syringic acid; and quercetin; Sigma-Aldrich, Darmstadt, Germany).

2.2. Fabrication and Characterization of Bidens pilosaExtract-Loaded Chitosan-Based Edible Film

2.2.1. Formulation of Coatings and Film Formation. Chitosan-based films were formed following a procedure by Siripatrawan and Vitchayakitti [26], adopted with minor changes. The chitosan was sourced from Sigma-Aldrich (St. Louis, MO, USA) and was a base ingredient. The formulations were prepared by incorporating 2% (w/v) chitosan, 1% (v/v) acetic acid, 1% (v/v) glycerol, 1% (v/v) canola oil, and 1% (v/v) Tween-20 in 100 mL of distilled water, under continuous magnetic stirring for 60 min. *B. pilosa* extract was dissolved in 70% ethanol at a 1 mg/mL stock concentration by dissolving 1 mg of the dried extract in 1 mL of solvent. This standardized extract was then incorporated into the chitosan matrix at varying concentrations to create composite edible films. Specifically, the films were prepared as follows: Ch-1% BP was formulated by adding 10 mL of the 1 mg/mL *B. pilosa* extract to 990 mL of the coating solution, resulting in a final concentration of 0.01 mg/mL of *B. pilosa* in the film. Similarly, Ch-3% BP was composed of 30 mL of the extract and 970 mL of coating solution, yielding a 0.03 mg/mL final concentration, and Ch-5% BP comprised 50 mL of extract mixed with 950 mL of coating solution to achieve a final concentration of 0.05 mg/mL. These formulations were labeled Ch-1% BP, Ch-3% BP, and Ch-5% BP, respectively, representing the different levels of *B. pilosa* incorporation into the chitosan films. For 10 min, a homogenizer (Separation Scientific, Johannesburg, South Africa) set at 3000 rpm was used to mix the solution thoroughly and then sonicated at 40°C for 1 h. In triplicate, 30 mL aliquots of each film-forming solution were cast onto 90 × 15 mm Petri dishes and conditioned in an oven set at 50°C for 72 h. For 48 h, at room temperature, the subsequent films were conditioned and sealed for future use.

2.2.2. Film Thickness and Density. At three different locations, the film thickness was determined by a digital micrometer (Mitutoyo, Mitutoyo Corporation, Japan) with a sensitivity of 1 μm. Film density (F_d) was obtained by measuring the film weight (f_w) and volume ($_v$) and calculated using the following equation:

$$F_d = \frac{f_w}{v}, \tag{1}$$

where f_w = film weight and v = film volume.

2.2.3. Film Color Attributes. The film color properties were measured using a CR-400 colorimeter (Konica Minolta, Japan), where L^* = lightness, a^* = red green, and b^* = blue yellow. At different sampling points, three readings were taken for each film and the mean was used as a representative of each film color. The total color difference (ΔE), yellow index (YI), and white index (WI) were calculated using the following equations, respectively:

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

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

$$\text{YI} = \frac{142.86 \times b^*}{L^*}, \quad (3)$$

$$\text{WI} = 100 - \sqrt{\left(100 - L^*\right)^2 + a^{*2} + b^{*2}}. \quad (4)$$

2.2.4. Film Transmittance, Opacity, and Glossiness. The film's transmittance procedure was adopted as outlined by Riaz et al. [27]. In triplicates, the films were cut into thin strips of size 1×4 cm strips, and their transparency and opacity were carried out by a UV-Vis spectrophotometer (United Scientific, SP-UV 300, Johannesburg, South Africa) set at 600 nm.

The film's opacity was calculated using the following equation:

$$O = \frac{\text{Abs}_{600}}{L}, \quad (5)$$

where O = opacity; Abs_{600} = absorbance value at 600 nm; and L = film length (mm).

A gloss meter (Multi-Gloss 268, Minolta, Germany) set at a 20, 60, and 80° angle was used to determine the film's glossiness. The gloss values for each angle were obtained in triplicates from random measurements on the film surface.

2.2.5. Film Water Content. A method by Riaz et al. [27] was used to determine the water content (W_c) of the films, adopted with minor adjustments. Briefly, film samples (3×3 cm) were weighed (w_1), oven-dried at 105°C for 48 h, and weighed again (w_2). The water content (%) of the films was calculated using the following equation:

$$W_c\,(\%) = \frac{w_1 - w_2}{w_1} \times 100. \quad (6)$$

2.2.6. Film Solubility and Swelling Degree. In triplicates, film strips of 3×3 cm were used to determine the film solubility and swelling degree, as indicated by Riaz et al. [27]. For film solubility, the pieces were oven-dried at 105°C to constant weight (M_1). Thereafter, the films were soaked in 100 mL beakers filled with 75 mL distilled water, with the top covered with parafilm, and stored at ambient temperature for 24 h. The films were then lightly dried with filter papers for 1 min and then oven-dried at 105°C to constant weight (M_2). The film's solubility was computed using the following equation:

$$\text{Film solubility}\,(\%) = \frac{M_1 - M_2}{M_1} \times 100. \quad (7)$$

The film's swelling degree (%) was determined by weighing the films (M_1) and putting them into 50 mL beakers filled with 40 mL distilled water for 24 h at room temperature. The wet films were then superficially dried with filter papers for 1 min, followed by weighing them again (M_2). The percent swelling degree was obtained by using the following equation:

$$\text{Film swelling degree}\,(\%) = \frac{M_2 - M_1}{M_1} \times 100. \quad (8)$$

2.2.7. Film Water Vapor Transmission Rate. Using the gravimetrical method, the film's water vapor transmission rate (WVTR) was determined following a procedure by Siripatrawan and Vitchayakitti [26] and Moradi et al. [28], adopted with minor alterations. Briefly, an aluminum permeability cell, with an internal diameter of 32 mm, was first filled with 30 mL of distilled water. Samples were mounted on top of the cell and adjusted through a top ring-shaped cover that was fixed in place by tight clamps. The permeability cell was weighed and kept in closed plastic containers. Saturated sodium chloride salt (25 g) in 30 mL of distilled water was placed in 90×15 mm Petri dishes. The cups were periodically weighed every hour for the first 10 h and after 24 h. The WVTR (g m^{-2} 24^{-1} h) of the films was determined using the following equation:

$$\text{WVTR} = \frac{m_{f-m_i}}{d \times s} = \text{g}\,\text{m}^{-2}24^{-1}\text{h}, \quad (9)$$

where m_f = resultant weight of the container with film; m_i = initial weight of the container with film; d = time in hours; s = effective area of the film.

2.2.8. Tensile Strength and Elongation. A procedure by Riaz et al. [27] and Ferreira et al. [29], adopted with minor modifications, was used to evaluate the film's tensile strength (TS) and percentage elongation at break (%E). The experiment was performed at room temperature using a texture analyzer (Agrosta texture analyzer, Calib, France). Film strips (2×3 cm) were cut from each treatment and mounted on the grips in triplicates. The initial grip separation and the detector speed were set at 5 cm and 100 mm/min, respectively. The tensile strength (TS) was calculated by dividing the maximum load ($F_{\max}$) by the initial cross-sectional area (ϕ) of the film sample, as shown in the following equation:

$$\text{TS} = \frac{F_{\max}}{\phi}. \quad (10)$$

The percent elongation at break (%E) of the films was calculated as the ratio of the film extension (Δl) at the point

of sample rupture to the initial length (l_o) of the sample, as shown in the following equation:

$$\%E = \frac{\Delta l}{l_0} \times 100. \quad (11)$$

2.2.9. X-Ray Diffraction. The chitosan-based films were cut into circular pieces (32 mm^2) and were mounted on a zero-background silicon substrate sample holder. Sample analysis was between $2\theta = 5^\circ$ and 60° with a step size $2\theta = 0.017^\circ$ (step time of 2000 s) in a powder X-ray diffractometer X'Pert Pro Panalytical (Almelo, The Netherlands) at 45 kV and 35 mA, using copper Ka radiation ($k = 1.543$ Å) filtered by nickel. The diffractometer was equipped with an X' Celerator detector, a 0.5° divergence slit, and antiscatter slits of 1° and 0.5° in the incident and diffracted beams, respectively.

2.2.10. Scanning Electron Microscopy. Film surface morphology was observed with scanning electron microscopy (SEM, SU8010, Hitachi, Japan) at 10 kV. Films of 10 × 10 mm were cut, dried, and mounted on aluminum stubs using double-sided carbon tape and sputter-coated with gold.

2.2.11. Film Antifungal Activity Property against Postharvest Crop Pathogens. The antifungal property of the films was carried out using the agar disc diffusion method against *Penicillium expansum* and *Botrytis cinerea*, two well-known postharvest pathogens of horticultural crops. Inoculates were obtained from the culture collection at Stellenbosch University, South Africa. The films were cut into a disc form of 6 mm diameter and placed on nutrient agar (Merck, Darmstadt, Germany) plates previously seeded with an inoculum comprising marker fungal strains [26, 30]. The plates were then incubated at 37°C for 4 d. The diameter of the inhibitory zone surrounding film discs and the contact area of film discs with agar surface were measured. If there was no clear zone surrounding the film disc, it was marked as no inhibitory zone. The tests were performed in triplicates.

2.3. Release Kinetics of Total Phenolic Content and Antioxidant Capacity. In triplicates, a procedure by Lian et al. [31], adopted with minor alterations, was employed to determine the film's release kinetics. Briefly, the films were cut into 3 × 3 cm strips, submerged in centrifuge tubes containing 50 mL of 50% ethanol, and centrifuged at 1000 rpm. The release solution was drawn at various times, from 10 to 120 min, and the absorbance was measured using a UV-Vis spectrophotometer.

2.3.1. Total Phenolic Content. A Folin–Ciocalteu method was used to evaluate the total phenolic content of the films, according to a procedure by Lian et al. [31] and Prior et al. [32]. Under dim lights, 450 μL of the 50% ethanol extract released from the film was mixed with 500 μL Folin-C reagent. The reaction was kept in the dark for 2 min before adding 2% sodium carbonate (2% w/v). The sample was then vortex for 30 s and kept in the dark for 2 h, and then, its absorbance was measured using a UV-Vis spectrophotometer set at 760 nm. The released kinetics of TPC was calculated from the standard curve of gallic acid.

2.3.2. Radical Scavenging Activity. Radical scavenging activity (RSA) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as outlined by Siripatrawan and Vitchayakitti [26] and Lian et al. [31]. Briefly, 50% of ethanol extract (15 μL) released from the film was reduced in 735 μL methanolic DPPH solution (0.1 mM) and incubated in the dark at room temperature. The RSA of the films was then measured using a UV-Vis spectrophotometer set at 517 nm and calculated from the standard curve of Trolox and expressed in mg Trolox equivalents (TE)/g film of RSA.

2.3.3. Ferric Reducing Antioxidant Power. The ferric-reducing antioxidant power (FRAP) activity was determined as outlined by Genskowsky et al. [33]. The FRAP working solution (2850 μL) 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 mixed with 150 μL of 50% ethanolic extracts (150 μL) and incubated in the dark for 30 min at room temperature. The reaction was then measured at a wavelength of 593 nm using a UV-Vis spectrophotometer. The FRAP antioxidant activity was calculated from the standard curve of Trolox and expressed in mg Trolox equivalents (TE)/g film FRAP.

2.4. Shelf Life Study of Bidens pilosa Extract-Loaded Chitosan-Based Composite Coating on Raspberry. The effectiveness of the developed *Bidens pilosa*extract-loaded chitosan-based composite as an edible coating was tested on raspberry storage trials for practical application. Raspberry fruit (*Rubus idae*us L. cv. Microprop) of uniform color and size was obtained from the Field Berry Farm (−26°21′30.0″S, 27°55′09.6″E), Johannesburg, South Africa. To disinfect the fruit, they were immersed in 0.01% sodium hypochlorite for 3 min and air-dried using a fan at room temperature for 30 min. Raspberry fruit was immersed for 1 min in either Ch + 1% BP composite coating, chitosan coating alone (Ch), and control (not coated), air-dried and packed in standard commercial punnets (100 mm × 75 mm), and refrigerated at 4 ± 1°C and 90 ± 5% (RH) for 9 d. Each punnet contained 125 g fruits (±48 fruits). Data were taken at 3 d periods ($n = 3$ per interval for each treatment).

2.4.1. Fruit Weight Loss. At each sampling point, weight loss was calculated using the initial weight of three punnets at the beginning of storage (w_i) and obtained the weight of punnets at sampling (w_f) time and calculated using the following equation:

$$W = \frac{w_i - w_f}{w_i} \times 100. \quad (12)$$

2.4.2. Decay Incidence. Fruit decay incidence (%) was visually evaluated. Fruits with decay symptoms were counted and discarded. The percentage of total rots was evaluated using the following equation:

$$\text{Decay incidence}\,(\%) = \frac{\text{number of discarded fruit}}{\text{total number of fruit}} \times 100. \tag{13}$$

2.4.3. Fruit Color Attributes as a Ripening Indicator. Raspberry fruit color was assessed using a colorimeter (Konica Minolta Chroma Meter CR-400, Osaka, Japan) at three distinct points. To calculate chroma (C^*) and hue angle (h°), CIELAB coordinates (L^*, a^*, and b^*) were used as shown in the following equations:

$$C^* = \sqrt{a^{*2} + b^{*2}}, \qquad h^\circ = \tan^{-}\left(\frac{b^*}{a^*}\right). \tag{14}$$

2.5. Statistical Analysis. Data obtained were subjected to statistical analysis using GenStat statistical software (GenStat, 18.2 edition, VSN International, UK), one-way analysis of variance (ANOVA) at a 95% confidence interval. Where significant differences were observed, mean separation was done according to Duncan's multiple range test at $p < 0.05$.

3. Results and Discussion

3.1. Polyphenol Groups Identified in Bidens pilosa Extract and Their Relevance to Food Preservation. The *B. pilosa* extract contained a total of 20 annotated metabolites and six unidentified polyphenols(Table 1). The primary categories included phenolic acids (9), organic acids (8), monosaccharides (1), amino acids (1), and flavonoids (1). These components, particularly phenolic acids, flavonoids, and organic acids, have established roles in food preservation [44–46]. The rich presence of these metabolites validates *B. pilosa*'s potential as a source of diverse phytochemicals and its antioxidant and antimicrobial capabilities [13, 44].

Among the identified polyphenols, phenolic acids were notably abundant in the *B. pilosa* extract. These observations are similar to findings by Idris et al. [47], who reported a high prevalence of phenolic acids and their derivatives in *B. pilosa* extracts obtained through water and 70% ethanolic extraction methods. The medicinal uses of *B. pilosa* in traditional medicine and food preservation could be attributed to this high phenolic acid content [21]. For instance, coumaric acid and its derivatives have been shown to protect food from oxidative stress, enhancing its shelf life [48]. Chatterjee et al. [49] reported that chitosan matrix infused with coumaric acid inhibited *Staphylococcus aureus*, thus validating its efficacy in food preservation.

Other valuable phenolic compounds detected include gallic acid and caffeoylquinic acid derivatives, renowned for their potent antioxidant activities and associated health benefits [50]. Gallic acid, notable for its antioxidant capacity, slows lipid oxidation in stored food [50, 51]. Moreover, bergenin, a known antifungal and antibacterial agents, inhibits enzymes like yeast alcohol dehydrogenase, contributing to the preservation and shelf-life enhancement of beverages like finger millet malt drinks [51, 52]. These observations further emphasize the relevance of *B. pilosa* in indigenous knowledge systems and its potential pharmacological properties, suggesting promising applications in both the pharmaceutical and food industries.

Furthermore, LC-MS assessment of *B. pilosa* extract indicated the occurrence of numerous organic acids (Table 1), a class of compounds renowned for their antioxidant and antimicrobial characteristics, making them instrumental in food preservation [50, 53]. For example, quinic acid, one such organic acid detected in our analysis, exhibits potent antibacterial activity, particularly against common food-borne pathogens such as *Staphylococcus aureus* [54]. It exerts its effect by directly interacting with the genomic DNA of the bacteria, causing a reduction in DNA content, and suggesting *B. pilosa's* effectiveness in food safety and shelf-life enhancement [55]. Another detected compound, hexosyl-O-hexose, serves as a natural catalyst in the food industry. It is commonly employed in cheese and tofu production for its coagulating properties, promoting curd formation for a firmer structure to pasta or noodles [56]. Additionally, its oxygen-scavenging capacity contributes to improved food appearance and extended shelf life [57]. The analysis also identified organic acids, such as citric acid, renowned for their use in the food industry to preserve color by decelerating oxidation rates. Citric acid enhances flavor, functions as an emulsifier, and adds value to processed products like jams and canned food as an antibacterial agent, thus promoting a longer shelf life [58]. The range and potency of polyphenols observed in the *B. pilosa* extract validate their use for health benefits and utility in broader industrial product development.

3.2. Properties of B. pilosaExtract-Loaded Chitosan-Based Film

3.2.1. Film Density and Thickness. The density and thickness of the films increased with the increasing strength of *B. pilosa* extract (Figures 1(a) and 1(b), respectively). The highest film density (2.11 g cm^{-3}) and thickness (0.291 mm) were observed in chitosan films enriched with 5% *B. pilosa* extract (Ch-5B), and the lowest film thickness (0.128 mm) and density (1.08 g cm^{-3}) were observed in chitosan-only films. A similar trend was observed by Riaz et al. [27], who reported that increasing apple peel polyphenols in the chitosan environment increased the film thickness and density. According to Peng and Li [7], increasing the extract concentration increased the interaction between polyphenol compounds and chitosan, which then triggered a tighter composite bonding by shortening the distance between the interacting molecules, thereby increasing the film thickness and density with an increase in the concentration of the applied *B. pilosa* extract into the chitosan matrix.

Table 1: Tentatively identified polyphenolic compounds in *Bidens pilosa* extract through liquid chromatography-mass spectrometry.

Polyphenol class	RT (min)	Experimental *m/z* $(M\text{-}H)^-$	MS^E fragments	Elemental formula	Concentration (μg/g)	Reference
Phenolic acids						
Gallic acid	6.08	169.0130	**169.0051**, 398.9441, 231.0019, 125.0247, 111.0054	$C_7H_6O_5$	4.88	Standard
2,5-Dihydroxybenzoyl hexoside	8.31	315.0714	**315.0690**, 152.0122, 108.0213,	$C_7H_6O_4$	38.30	[34]
Bergenin-O-hydroxybenzoic acid	18.04	477.0643	**477.1777**, 335.1671, 101.0332	$C_{12}H_{13}O_{20}$	369.48	[35]
3,4-Dicaffeoylquinic acid	19.63	515.1201	**515.1196,** 473.0723, 353.1114, 335.0397, 191.0294, 179.0962, 173.3241, 161.2311, 135.3533	$C_{25}H_{23}O_{12}$	665.67	[34, 36, 37]
1,3-Dicaffeoylquinic acid	19.09	515.1187	**515.1252,** 353.0904, 191.0565, 179.0384, 161.3421, 135.0441	$C_{25}H_{23}O_{12}$	348.31	[34, 36, 37]
3,5-Dicaffeoylquinic acid	20.02	515.1202	**515.1206,** 353.0882, 300.0263, 271.0236, 191.0559, 179.0451, 135.0437	$C_{25}H_{23}O_{12}$	76.82	[34, 36, 37]
4,5-Dicaffeoylquinic acid	20.65	515.1190	**515.1210,** 353.0874, 191.0554, 179.0342, 173.2311, 135.0440	$C_{25}H_{23}O_{12}$	407.12	[34, 36, 37]
Bergenin	24.39	327.2160	**327.2171,** 285.0389, 179.0446, 161.0334, 101.0239	$C_{14}H_{16}O_9$	660.68	[35]
Coumaric acid derivative	26.30	265.1465	**265.1456**, 577.2685, 339.2005, 183.0114, 96.9586	$C_{15}H_{21}O_4$	238.30	[38]
∑Phenolic acids					2809.56	
Organic acids						
Gluconic acid	1.59	195.0506	**195.0440**, 96.9686	$C_{13}H_7O_2$ or $C_6H_{11}O_7$ or $C_9H_7O_5$	434.83	[34]
Quinic acid	1.78	191.0557	**191.0560**, 127.0376, 85.0291	$C_7H_{12}O_6$	1477.08	[35, 39]
Citric acid*	3.54	191.0197	**191.0191**, 173.0087, 129.0177	$C_5H_7O_7$	477.08	Standard
Aconitic acid	5.91	173.0072	**173.0078**, 233.0286, 154.9980, 111.0075, 85.0278	$C_6H_5O_6$	646.66	[40]
3-CQA (neochlorogenic acid)	9.94	353.0875	**353.0858**, 353.0445, 179.0347, 191.0559	$C_{12}H_{17}O_9$	231.07	[36, 41, 42]
5-CQA (chlorogenic acid)	12.20	353.0867	**353.0867**, 707.6454, 191.0549	$C_{16}H_{18}O_9$	734.57	[36, 41, 42]
4-CQA (cryptochlorogenic acid)	12.55	353.0862	**353.0862**, 135.0446, 173.0453, 179.0345, 191.0560	$C_{12}H_{17}O_9$	30.31	[36, 41, 42]
Ferulic acid	21.26	193.0505	**284.0188**, 161.0217	$C_5H_5O_8$	154.38	[43]
∑Organic acids					4185.98	
Flavonoids						
Quercetin rutinoside	17.38	609.1471	**609.1494**, 300.0261, 271.0240, 255.0339, 151.0059	$C_{30}H_9O_{15}$	109.72	[41]
∑Flavonoids					109.72	
Monosaccharides						
Hexosyl-O-hexose	1.82	341.1091	**341.1041**, 377.0821, 209.0629, 179.0570, 96.9577, 89.0223	$C_{19}H_{17}O_6$ or $C_{12}H_{21}O_{11}$ or $C_{26}H_{13}O_1$	10.25	[42]
∑Monosaccharides					10.25	
Amino acids						
Tryptophan	9.84	203.0815	**300.9968**, 275.0164, 169.0068, 112.0105	$C_{10}H_3O_5$ or $C_3H_7O_{10}$	14.01	[34]
∑Amino acids					14.01	
Unknown polyphenols						
Unknown 1	5.24	243.0613	**243.0610**, 200.0550, 173.0074, 130.0483	$C_{14}H_{11}O_4$	46.33	—
Unknown 2	11.91	311.0386	**311.0417**, 135.0437, 149.0089, 179.0340, 149.0089, 135.0437	$C_9H_7O_4$	174.79	—
Unknown 3	14.08	387.1655	**387.1639**, 161.0401	$C_{18}H_{27}O_9$, or $C_{25}H_{23}O_4$	74.92	—

Table 1: Continued.

Polyphenol class	RT (min)	Experimental m/z $(M\text{-}H)^-$	MS^E fragments	Elemental formula	Concentration (μg/g)	Reference
Unknown 4	14.55	311.0767	**311.0772**, 179.0333, 149.0450	$C_{14}H_{15}O_8$	82.73	—
Unknown 5	15.44	371.0967	**371.0922**, 301.0225, 249.0653, 169.0222, 121.0273	$C_{23}H_{15}O_5$	12.34	—
Unknown 6	15.77	225.1130	**301.0706**, 293.0905, 286.0516, 284.0226	—	76.27	—
∑Unknown polyphenols				467.38		

Means values were expressed as values of three replicates ($n = 3$); *proven with a pure chemical standard and MSE fragments in bold typeface refer to the base peak (the highest peak) [24].

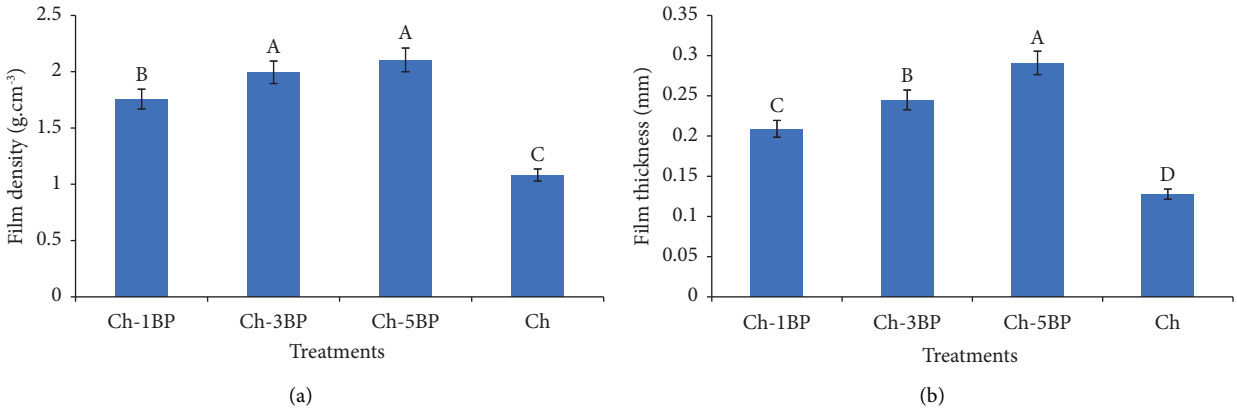

Figure 1: Film density and thickness of chitosan-based films. Different letters indicate a statistically significant difference ($p < 0.05$). Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); chitosan + 5% *Bidens pilosa* (Ch-5 BP); and chitosan only (Ch).

3.2.2. Film Color, Transmittance, and Opacity. Color, transmittance, and opacity are significant variables that influence the film's appearance and marketability and affect consumer acceptance degree [5, 59]. Table 2 shows film color attributes of chitosan-based films enriched with *B. pilosa* extract per the Hunter system (L^*, a^*, and b^* values), the total color difference (ΔE), yellow index (YI), whitish index (WI), transmittance (%), and opacity (A mm^{-1}). Incorporating the different concentrations of *B. pilosa* extract significantly ($p < 0.05$) changed the native color of the chitosan films. After incorporating the *B. pilosa* extract into the chitosan matrix, the b^* and opacity values were significantly ($p < 0.05$) enhanced. The increase was proportional to an increase in *B. pilosa* extract concentration. Therefore, Ch-5 BP films had a significantly ($p < 0.05$) higher b^* value (13.1) and opacity (16.4 A mm^{-1}) compared to the other treated films (Figure 2). Chitosan-only films had the lowest b^* value (4.83) and opacity (3.3 A mm^{-1}). On the other hand, the L^* and a^* values and WI significantly ($p < 0.05$) decreased with an increase in *B. pilosa* extract, indicating low transmittance and consequently a higher YI. Thus, Ch-5 BP films had the highest total color changes (ΔE; 13.14), followed by Ch-3 BP (10.18), and Ch-1 BP had the lowest ΔE (6.21). Therefore, incorporating *B. pilosa* extract into chitosan films at a minimal concentration (Ch-1 BP) is unlikely to affect the overall visual appeal of the coated or wrapped food products. These results are in agreement with findings by Siripatrawan and Harte [59], who reported comparable results for chitosan-based films containing green tea extract. Dutta et al. [5] reported that the color of edible films is subjective to the nature and strength of the incorporated plant material.

3.2.3. Film Glossiness. Gloss values of the chitosan films fused with *B. pilosa* extract at several incidence points are presented in Table 3. According to Hutchings [60], the gloss values increase with an increase in the view angle because of the improvement of specular reflection observed as the incidence angle improves. The glossiness of the films was significantly ($p < 0.05$) reduced after incorporating the *B. pilosa* extract compared to the chitosan-only films. Notably, the gloss values increased with an increase in the view angle and significantly reduced with an upsurge in *B. pilosa* extract. Among the treated films, Ch-1 BP films had higher glossiness 43.2, 74.4, and 99.4% at 20, 60, and 80°, respectively, while Ch-5 BP films exhibited the lowest glossiness (35.3, 56.1 and 85.7% at 20, 60, and 80°, respectively). The extract distribution of the incorporated *B. pilosa* extract into the chitosan matrix might have caused increasing unevenness at the surface of the films, thus resulting in reduced glossiness. Sánchez-González et al. [61] and Bitencourt et al. [4] observed that hydroxypropyl methylcellulose films had reduced glossiness after incorporating tea essential oil. In contrast, gelatin-based films had reduced glossiness after incorporating curcuma extract.

3.2.4. Film Solubility and Swelling Degree. The solubility and swelling degree of the chitosan films fused with *B. pilosa* extract are presented in Figures 3(a) and 3(b), respectively. These properties affect the film's water resistance properties [62]. The chitosan-only films exhibited the lowest solubility and swelling (18.9% and 31.9%, respectively). Incorporating the *B. pilosa* extract into the chitosan medium resulted in a significant ($p < 0.05$) increase in the water solubility and swelling degree of the films, and it improved with an increase in *B. pilosa* strength. Therefore, Ch-5 BP films had the higher film solubility and swelling degree (44.43% and 75.4%, respectively), and control film had the lowest film solubility and swelling degree of 18.9% and 31.9%, respectively. According to Liu et al. [62], the enhanced film solubility and swelling degree might be attributed to the hydrophilic groups of the extract's interaction with water molecules. Mayachiew and Devahastin [63] reported that the rate of solubility and swelling degree of chitosan films differs on the drying temperature and the nature and the number of intermolecular chain interactions in the chitosan matrix.

Table 2: Color, transmittance, and opacity of chitosan-based films enriched with *Bidens pilosa* extract at different concentrations.

Treatment	L^*	a^*	b^*	ΔE	YI	WI	Transmittance (%)	Opacity (A mm^{-1})
Ch-1 BP	36.6 ± 0.74^{b}	2.2 ± 0.58^{b}	9.93 ± 0.20^{c}	6.21 ± 0.62^{c}	38.76 ± 0.39^{c}	35.79 ± 0.62^{b}	44.69 ± 0.66^{b}	8.67 ± 0.95^{c}
Ch-3 BP	33 ± 0.79^{c}	1.77 ± 0.19^{c}	12.1 ± 0.18^{b}	10.18 ± 0.58^{b}	52.38 ± 0.41^{b}	31.89 ± 0.64^{c}	35.89 ± 0.52^{c}	11.7 ± 0.0411^{b}
Ch-5 BP	29.9 ± 0.81^{d}	1.51 ± 0.15^{d}	13.1 ± 0.45^{a}	13.14 ± 0.63^{a}	62.59 ± 0.43^{a}	28.67 ± 0.65^{d}	23.6 ± 0.58^{d}	16.4 ± 0.0409^{a}
Ch	39.93 ± 0.93^{a}	3.4 ± 0.28^{a}	4.83 ± 0.44^{d}	—	17.28 ± 0.92^{d}	39.64 ± 0.59^{a}	51.78 ± 0.40^{a}	3.3 ± 0.066^{d}

Values are expressed as mean ± standard deviation. Different letters indicate a statistically significant difference ($p < 0.05$). Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); chitosan + 5% *Bidens pilosa* (Ch-5 BP); and chitosan only (Ch).

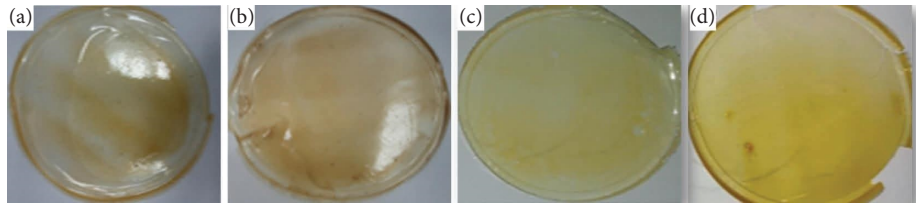

Figure 2: Physical color appearance of chitosan-based films enriched with *B. pilosa* extract at different concentrations. (a) Chitosan only; (b) chitosan + 1% *Bidens pilosa* (Ch-1 BP); (c) chitosan + 3% *Bidens pilosa* (Ch-3 BP); and (d) chitosan + 5% *Bidens pilosa* (Ch-5 BP).

Table 3: Gloss values at angles 20°, 60°, and 80° of chitosan films enriched with *B. pilosa* extract.

Film	Angle		
	20°	60°	80°
Ch-1 BP	43.2 ± 1.63^{b}	74.4 ± 0.82^{b}	99.4 ± 1.45^{b}
Ch-3 BP	39.8 ± 0.58^{c}	69.9 ± 0.91^{c}	90.9 ± 0.88^{c}
Ch-5 BP	35.3 ± 0.55^{d}	56.1 ± 2.38^{d}	85.7 ± 1.35^{d}
Ch	52.8 ± 0.59^{a}	86.3 ± 1.53^{a}	113.4 ± 1.22^{a}

Values are expressed as mean ± standard deviation. Figures in the same column followed by different letters are statistically significant from each other ($p < 0.05$). Chitosan + 1% *Bidens pilosa* (Ch-1BP); chitosan + 3% *Bidens pilosa* (Ch-3BP); chitosan + 5% *Bidens pilosa* (Ch-5BP); and chitosan only (Ch).

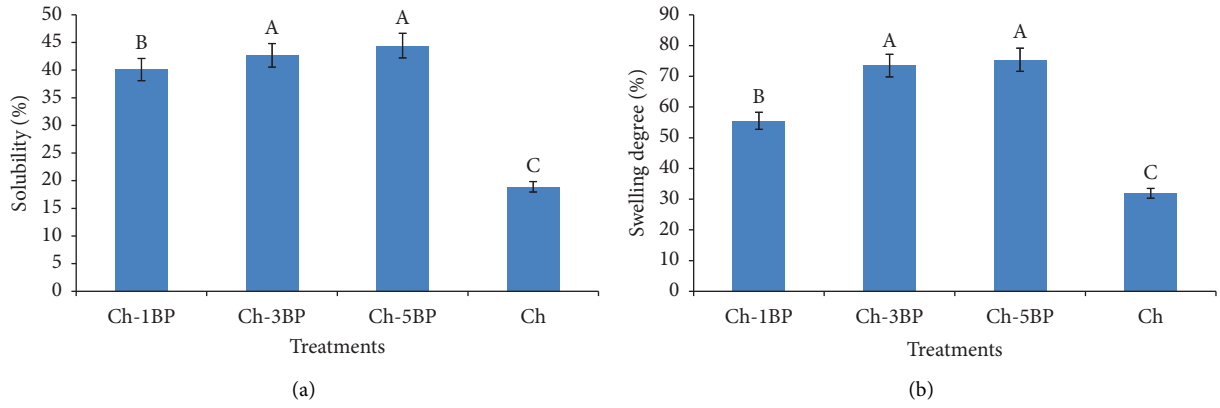

Figure 3: (a) Film solubility and (b) swelling degree of chitosan films fused with *B. pilosa* extract. Different letters indicate a statistically significant difference ($p < 0.05$). Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); chitosan + 5% *Bidens pilosa* (Ch-5 BP); and chitosan only (Ch).

3.2.5. Water Vapor Transmission Rate and Water Content. The importance of a film is to control water movement from the food and the adjacent environment. Therefore, the water vapor transmission rate (WVTR) of the film should be minimal [64]. Increasing the application of *B. pilosa* extract into the chitosan environment decreased the WVTR of the film (Table 4). Considerable differences ($p < 0.05$) were detected in employed treatments. Chitosan-only films had the highest WVTR (17.15 g m^{-1} 24 h^{-1}) followed by Ch-1 BP (9.56 g m^{-1} 24 h^{-1}), Ch-3 BP (9.21 g m^{-1} 24 h^{-1}), and Ch-5 BP (8.92 g m^{-1} 24 h^{-1}). The decrease in WVTR could be accredited to the interplay between the chitosan and the applied *B. pilosa* extracts, thereby lowering the accessibility of the hydrophilic groups in the chitosan and reducing its interaction with water [28].

Table 4: Water vapor transmission rate (WVTR) and water content of chitosan-based films.

Treatment	WVTR (g m^{-1} $24\,h^{-1}$)	Water content (%)
Ch-1 BP	9.56 ± 2.62^{b}	27.7 ± 0.67^{b}
Ch-3 BP	9.21 ± 2.42^{b}	25.4 ± 0.75^{c}
Ch-5 BP	8.92 ± 2.31^{c}	23.1 ± 0.77^{d}
Ch	17.15 ± 2.54^{a}	33.3 ± 0.57^{a}

Values are expressed as mean ± standard deviation. Different letters in each column are statistically different ($p < 0.05$) according to Duncan's multiple range test. Chitosan + 1% *Bidens pilosa* (Ch-1BP); chitosan + 3% *Bidens pilosa* (Ch-3BP); chitosan + 5% *Bidens pilosa* (Ch-5BP); and chitosan only (Ch).

The water content of the chitosan films was significantly ($p < 0.05$) altered by an increase in *B. pilosa* extract concentration (Table 1). Chitosan films incorporated with 5% *B. pilosa* extract (Ch-5 BP) had the lowest moisture content (23.1%), followed by Ch-3 BP (25.4%), Ch-1 BP (27.7%), and chitosan-only films had higher water content (33.3%). Adding *B. pilosa* extract increased the ability of the chitosan to bind with water and thus enhanced its hydrophilic properties. Similarly, Wang et al. [3] stated that the moisture content of chitosan films incorporated with tea polyphenols (TP) declined with an increase in TP concentration. As Riaz et al. [27] suggested, plant extracts, such as *B. pilosa* extract, can establish relations with chitosan molecules through potential hydrogen bonding, limiting the interactions involving hydrophilic groups of chitosan and water molecules due to the competitive binding effect.

3.2.6. Tensile Strength and Elongation. The mechanical properties of chitosan-based films enriched with *B. pilosa* are presented in Figures 4(a) and 4(b). Incorporating the *B. pilosa* extract into the chitosan matrix significantly ($p < 0.05$) altered the tensile strength and elongation of the chitosan films. The tensile strength of the films decreased from 23.4 MPa (chitosan only) to 13.1 MPa (Ch-5 BP) (Figure 4(a)), while the percent elongation increased from 25.5% (chitosan only) to 41.3% (Ch-5 BP) (Figure 4(b)). The results also indicated that increasing the strength of *B. pilosa* in the chitosan environment significantly ($p < 0.05$) reduced the tensile strength of the film, while the elongation percentage significantly ($p < 0.05$) increased. These findings disagree with the results by Shen and Kamden [65], who reported that the tensile increased with increasing citronella essential oil concentration, but elongation decreased with an increase in essential oil concentration. The interactions between the *B. pilosa* extract produced a cross-linking effect, thereby decreasing the flexibility of the chitosan polymer and leading to reduced tensile strength and increased elongation of the chitosan with an increase in *B. pilosa* concentration.

3.2.7. X-Ray Diffraction. Figure 5 shows that incorporating *B. pilosa* into the chitosan matrix affected the degree of crystallinity of the films. The crystallinity of the chitosan films increased with an upsurge in *Bidens pilosa* extract concentration (Figure 5). Four major peaks (peaks I, II, III, and IV) were observed. The reflection around $2\theta = 10^{\circ}$ (peak I) is associated with the moisturized crystalline structure of chitosan films, which contain bound water even when extensively dried [66]. Peak II (reflection around $2\theta = 20^{\circ}$), which corresponds to the major peak of crystallinity, was observed in all films, with Ch-5 BP films exhibiting the highest crystallinity (21115.33 a.u.) and chitosan-only films exhibiting the lowest crystallinity (16409.76 a.u.). The crystalline peak centered at around 20° (peak II) is attributed to the hydrated crystalline structure of chitosan, as noted by Zhang et al. [67]. The degree of crystallinity gradually declined in all films thereafter, with only Ch-1 BP films exhibiting a crystalline peak (peak III and IV) at $2\theta = 30^{\circ}$ and $2\theta = 55^{\circ}$ with a value of 20120 a.u. and 7874.53, respectively. Similarly, Rubilar et al. [68] observed that chitosan films fused using carvacrol and grape seed extract exhibited reflections around $2\theta = 10^{\circ}$ and $2\theta = 20^{\circ}$. Souza et al. [66] stated that variations in the degree of crystallinity observed among the films can be ascribed to differences in their oxygen permeability.

3.2.8. Scanning Electron Microscopy. Results indicated that incorporating the *B. pilosa* extract into the chitosan environment influenced the final microscopic structure of the chitosan films (Figure 6). Microstructures of the control films (chitosan only) had evenly distributed visible pores without cracks; however, different surface morphology was observed after incorporating the *B. pilosa* extract into the chitosan matrix (Figures 6(b)–6(d)). At 1% *B. pilosa* (Ch-1BP), the extract was evenly dispersed in the chitosan matrix without apparent aggregation (Figure 6(b)). With the increase of *B. pilosa* extract strength from 3% to 5%, the film surface became more heterogeneous, and an increase in roughness on the surface was observed (Figures 6(c) and 6(d)). The observed increase in roughness on the morphology of the films with an increase in *B. pilosa* concentration might be due to increased hydrophilicity of the *B. pilosa* extract. These findings agree with Riaz et al. [27], who observed that incorporating apple peel polyphenols (APP) into the chitosan matrix altered the surface morphology of the films when viewed under SEM. The authors also reported that the increase in the APP intensity resulted in different surface morphology of the chitosan films.

3.2.9. Antifungal Activity against Postharvest Crop Pathogens. The chitosan-based films were tested for antimicrobial activity against postharvest pathogens (*Penicillium expansum* and *Botrytis cinerea*), and the results are shown in Figures 7(a) and 7(b). Chitosan films without *B. pilosa* extract had a significantly ($p < 0.05$) low inhibition zone (3.33 mm). In contrast, chitosan films enriched with the different concentrations of *B. pilosa* all had an inhibition zone of 10 mm against *P. expansum* (Figure 7(a)), suggesting that incorporating *B. pilosa* extract into the chitosan environment enhanced additional antifungal properties of the chitosan film against

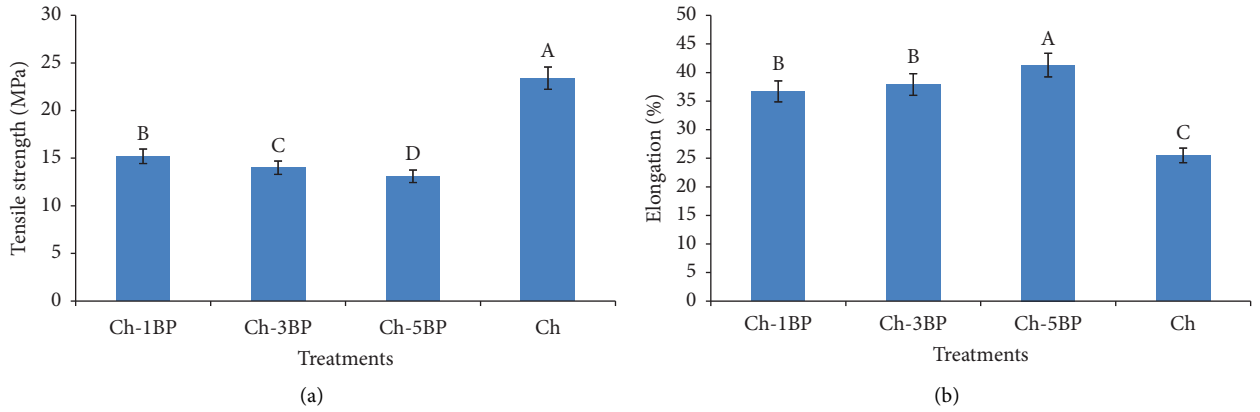

Figure 4: (a) Tensile strength and (b) elongation of chitosan-based films. Different letters indicate statistically significant differences ($p < 0.05$). Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); chitosan + 5% *Bidens pilosa* (Ch-5 BP); and chitosan only (Ch).

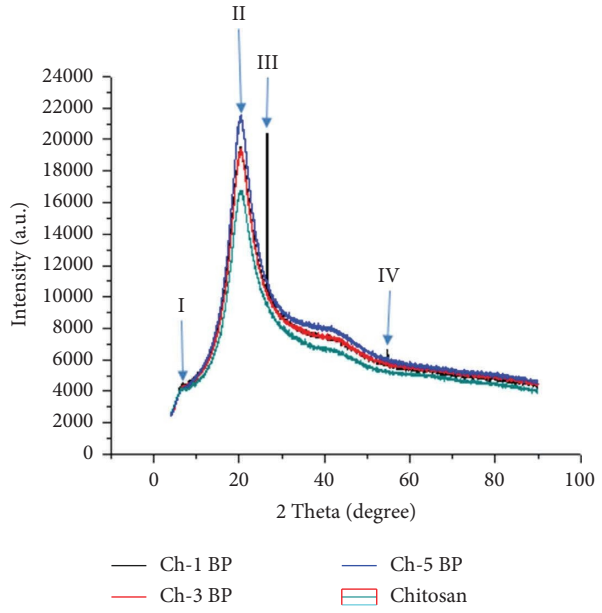

Figure 5: X-ray diffraction patterns of the chitosan films fused with *Bidens pilosa* extract. Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); and chitosan + 5% *Bidens pilosa* (Ch-5 BP).

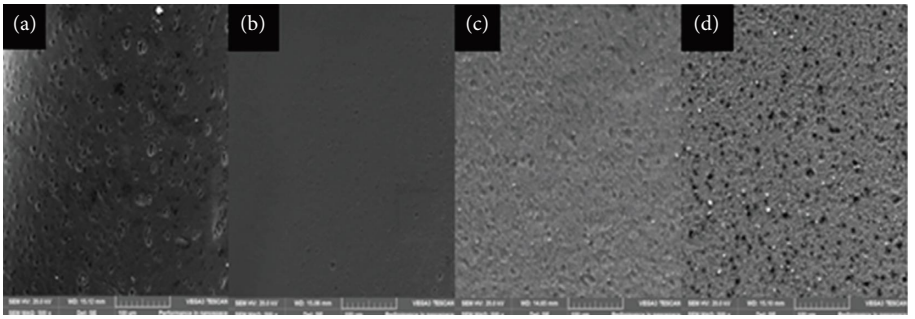

Figure 6: Scanning electron microscopy micrographs (magnification: 1.50 k×) of chitosan-based films with different *B. pilosa* extract concentrations. Key: (a–d) correspond to cross-sectional morphology chitosan only; chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); and chitosan + 5% *Bidens pilosa* (Ch-5 BP), respectively.

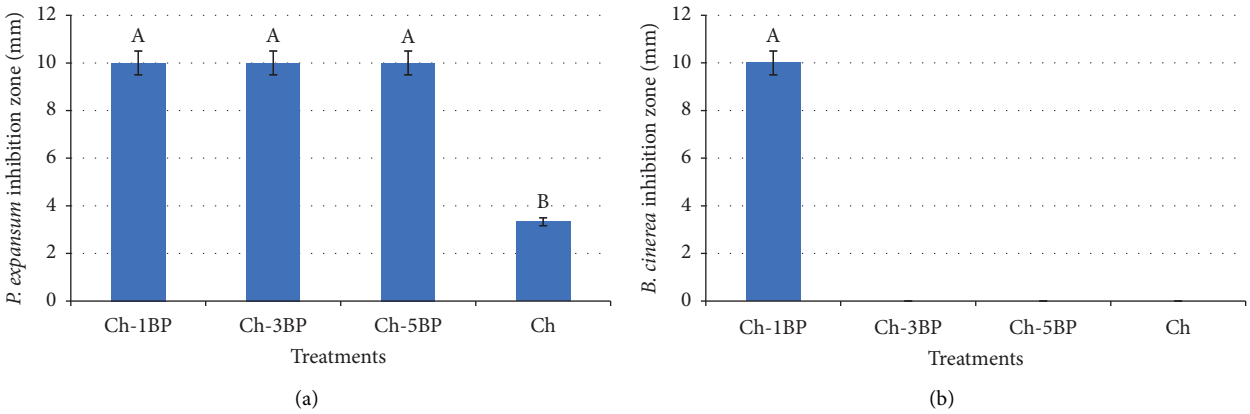

Figure 7: (a) Antimicrobial activity of chitosan films against *P. expansum* and (b) *B. cinerea*. Different letters indicate a statistically significant difference ($p < 0.05$). Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); chitosan + 5% *Bidens pilosa* (Ch-5 BP); and chitosan only (Ch).

P. expansum. Furthermore, only chitosan film enriched with 1% *B. pilosa* extract (Ch-1 BP) significantly ($p < 0.05$) inhibited (10 mm) the spread of this postharvest pathogen (Figure 7(b)). The observed selectivity of Ch-1 BP in inhibiting *B. cinerea* suggests that a 1% concentration of *B. pilosa* extract may be optimal for the efficient release and dispersion of specific antifungal compounds (against *B. cinerea*) in the chitosan matrix. Our research does not agree with Torlak and Sert [69], who reported no antifungal activity with chitosan films in the agar diffusion method.

3.3. Release Kinetics of Bidens pilosa Extract from Chitosan-Based Films. The degree of release of total phenolic content (TPC) in food simulant (50% ethanol) is shown in Figure 8(a). Chitosan-only films peaked and remained constant at 3 mg GAE/g film of total phenolic content (TPC) after 10 min. However, chitosan-based films enriched with *B. pilosa* extract at different concentrations had a steady and significant ($p < 0.05$) increase in the release of TPC, and different peaks and stabilities were observed. Raising the concentration of *B. pilosa* extract in the chitosan matrix resulted in a lower release of TPC. Therefore, Ch-1 BP films significantly ($p < 0.05$) released higher phenolic compounds (78.9 mg GAE/g film) compared to Ch-5 BP films (59.1 mg GAE/g film) after 120 min. It was observed that Ch-3 BP and Ch-5 BP films had a constant release of phenolic compounds at 90 min, while Ch-1 BP films had a constant release of phenolic compounds after 110 min. Notably, control films (chitosan) had a constant release and very low TPC (3 mg GAE/g film) after 120 min. This confirms that chitosan films have very low TPC, thus the low release kinetics observed in this study.

Regarding the release of antioxidants, it correlated with the release of phenolic compounds. The radical scavenging showed a fast increase in the first 70 min in all chitosan films enriched with *B. pilosa* extract before it reached a linear curve. Increasing the concentration of *B. pilosa* extract in the chitosan matrix showed a fast release of radical scavenging activity of the chitosan films (Figure 8(b)). However, at the end of the 120 min, the lowest concentration of *B. pilosa* (Ch-1BP) significantly ($p < 0.05$) exhibited the highest release of radical scavenging activity at the end of 120 min compared to the other treatments. Therefore, Ch-1 BP had the highest released radical scavenging activity (95.2 mg TE/g film), followed by Ch-3 BP (82.5 mg TE/g film) and Ch-5 BP (76.2 mg TE/g of film). Notably, chitosan-only films exhibited a constant radical scavenging activity (4.2 mg TE/g film). The FRAP kinetics displayed a trend similar to that observed in the radical scavenging activity of the films. However, the time required to attain a steady state differed. Increasing the concentration of *B. pilosa* extract into the chitosan matrix resulted in an early constant release (70 min) of FRAP compared to the other treatments (Figure 8(c)). Notably, the constant time for Ch-1 BP and Ch-3 BP films was 90 min. At the end of the 120 min, it was observed that Ch-1 BP films exhibited a significantly ($p < 0.05$) higher FRAP release rate (79.9 mg TE/g film), followed by Ch-3 BP films (61.1 mg TE/g film) and Ch-5 BP films had the lowest (49.6 mg TE/g film). Untreated films exhibited a constant release of FRAP (1.4 mg TE/g film). Even though RSA and FRAP are considered antioxidant capacities of compounds, the observed difference in their kinetics could be linked to their distinct action. DPPH is based on the reduction of nitrogen radicals, while FRAP is involved in electron transfer and, therefore, reduces ferric to ferrous iron [70]. It can be hypothesized that increasing the concentration of *B. pilosa* extract in the chitosan matrix might have developed a weak covalent bond compared to a lower concentration of *B. pilosa* extract, thus resulting in a fast release of phenolic and antioxidant compounds. Therefore, molecules were quickly released in the simulated food stimulant, causing the chitosan film to swell and dissolve and easily releasing the phenolic and antioxidant compounds from the film [61, 71]. The slower release of *B. pilosa* phenolic and antioxidant compounds from the chitosan films is essential for the stability of the composite film.

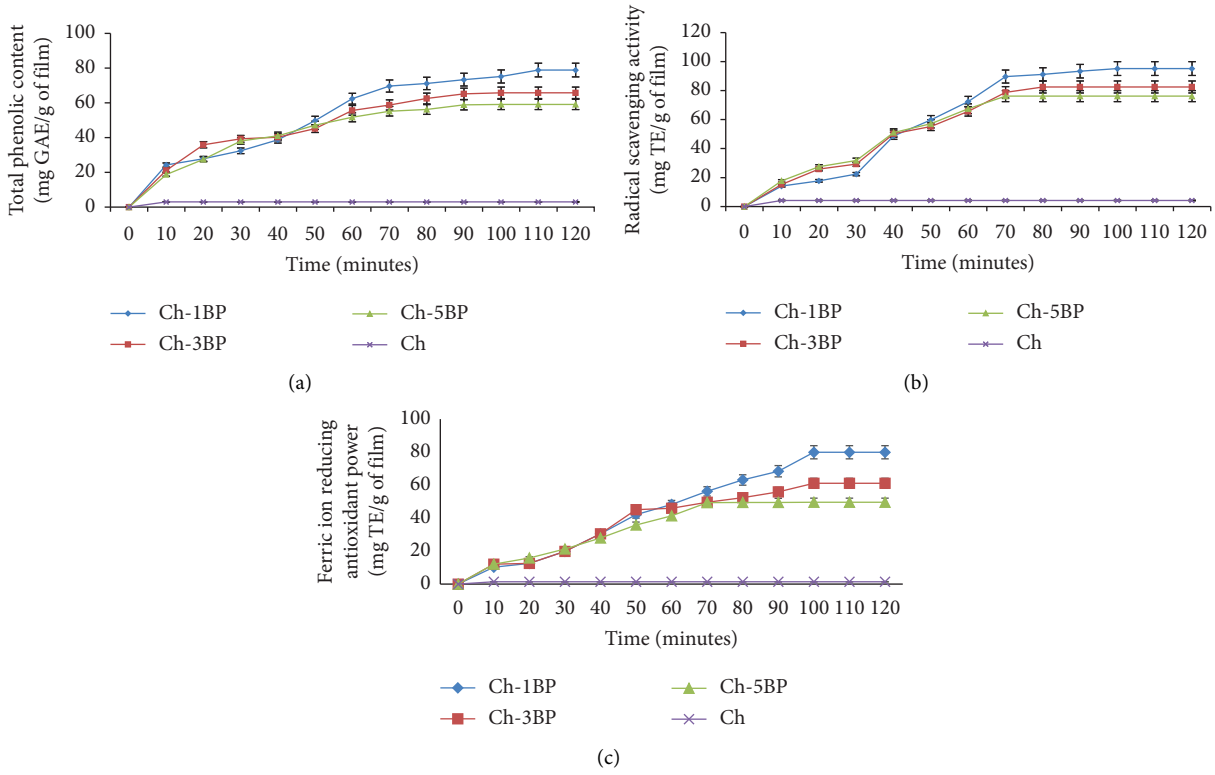

Figure 8: Kinetics of the release of (a) total phenolic content, antioxidant capacity as (b) DPPH radical scavenging, and (c) ferric ion antioxidant power from the films into 50% ethanol at different dissolving times. Chitosan + 1% *Bidens pilosa* (Ch-1 BP); chitosan + 3% *Bidens pilosa* (Ch-3 BP); chitosan + 5% *Bidens pilosa* (Ch-5 BP); and chitosan only (Ch). Means ± standard errors are presented, and error bars represent the standard error (SE) of the mean. SE: standard error; GAE: gallic acid equivalent; TE: trolox equivalent.

3.4. *Effect of Bidens pilosaExtract-Loaded Chitosan-Based Edible Coating on Raspberry Preservation*

3.4.1. Fruit Weight Loss. The interaction between the treatments and the storage time had a significant ($p < 0.0001$) impact on the weight loss of raspberry fruit (Figure 9). It was observed that fruit coating with chitosan alone and chitosan enriched with *Bidens pilosa* extract substantially reduced the weight loss of raspberry fruit compared to uncoated fruit. Chitosan-coated fruits have a strong water barrier that reduces the fruit's metabolic rate, reducing water movement [72]. The raspberry fruit coated with the composite coating (Ch-1% BP) exhibited the lowest weight loss percentage (16.74%), while the untreated fruit had higher weight loss (48.33%). Incorporating *B. pilosa* extract into the chitosan environment augmented the barrier characteristics of the fruit. Similarly, Han et al. [73] observed that raspberry fruit immersed in chitosan alone had lower weight loss than the control.

3.4.2. Decay Incidence. The susceptibility of raspberry fruit to fungal decay is a major factor limiting its shelf life, owing to its high water content and metabolic rate activity [72]. A significant ($p < 0.0001$) interaction between the storage time and applied coatings to decay incidence was observed (Figure 10). It was observed that the decay incidence increased with a prolonged storage period, with control fruit exhibiting signs of decay as early as 3 days of storage. However, coated fruit showed signs of decay after 6 days of storage, with a decay incidence ranging between 17.3% (Ch-1% BP) and 83.2% (control) at 9 d of storage. Chitosan coating exerted a high barrier to water movement and reduced the fruit's metabolic rate, consequently reducing the decay incidence [74]. The composite coating (Ch-1% BP) further improved the coating's ability to control decay incidence, suggesting that incorporating *B. pilosa* extract into the chitosan matrix can serve as a potential eco-friendly alternative to synthetic fungicides in controlling decay incidence in raspberry fruit. Similarly, Moreno et al. [75] reported that gelatin-coating fused with Argentinian propolis extract was efficient in controlling decay incidence in raspberry fruit compared to the control. This supports the potential commercial application of edible coatings in controlling decay incidence in raspberry fruit.

3.4.3. Raspberry Color Change. The assessment of raspberry fruit maturity and quality is often based on the fruit's color, and it is one of the essential indicators for the fruit's marketability (Figure 11). Table 5 depicts the color changes of raspberry fruit fused with *B. pilosa* extract during the 9-d storage period. The fruit redness (a^*) was significantly ($p < 0.0001$) influenced by treatments and storage period. The initial a^* value was 18.5, which generally increased across all the treatments in the first 6 d of storage; after that,

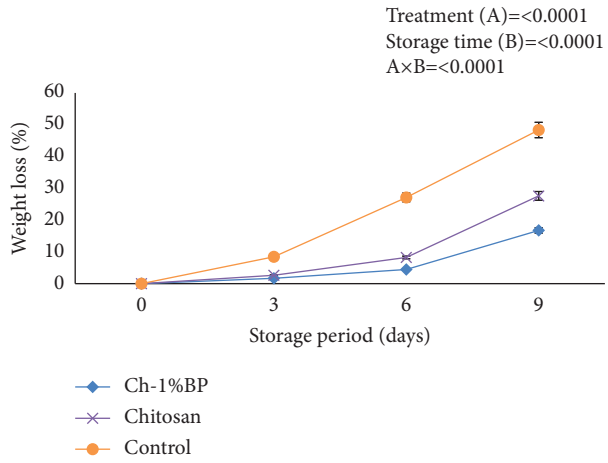

Figure 9: Changes in weight loss of raspberry fruit during storage for 9 days at 4 ± 1°C and 90 ± 5% relative humidity. Each bar represents the mean ± standard error (SE) of the mean. Factorial ANOVA was performed for the main factors, treatment, and storage period. Ch-1% BP-chitosan + 1% *Bidens pilosa*.

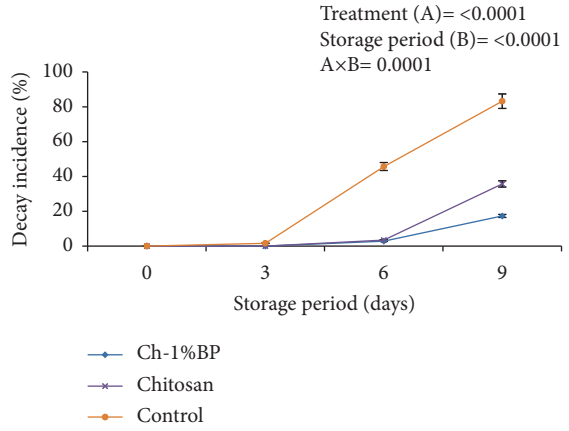

Figure 10: Decay incidence of raspberry fruit during storage for 9 days at 4 ± 1°C and 90 ± 5% relative humidity. Each bar represents the mean ± standard error (SE) of the mean. Factorial ANOVA was performed for the main factors, treatment, and storage time. Ch-1% BP-chitosan + 1% *Bidens pilosa*.

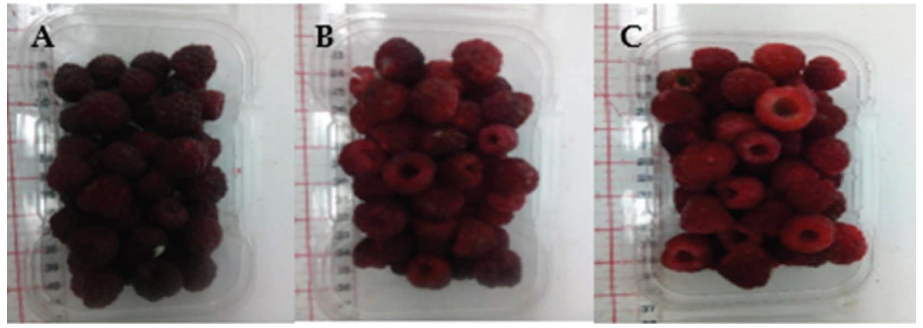

Figure 11: Color changes of raspberry fruit after 9 days of storage. (A) Uncoated (control); (B) chitosan alone; and (C) chitosan + 1% *B. pilosa* (Ch-1% BP).

it started to decline. Control raspberry fruit exhibited the highest a^* value (36.6) after 6 d of storage. Ch-1% BP coated fruit had the highest a^* value (32.2), while the lowest a^* value (29.3) was observed in the control fruit at the end of the storage period. The fruit color intensity/chroma (C^*) was also influenced by treatments ($p < 0.0001$) and storage period ($p < 0.014$). The C^* increased from 24.6 at harvest to 43.6 (control) and 39.8 (chitosan only) and then decreased at 6 d of storage. Coating raspberry fruit with the composite coating (Ch-1% BP) resulted in a steady increase in the fruit

Table 5: Redness (a^*), chroma (C^*), and hue angle (h°) of raspberry fruit coated with chitosan fused with *Bidens pilosa* extract, after 9 d of storage at 4°C and 90 ± 5% relative humidity.

Variable(s)	Factor(s)	Initial data at harvest	Storage time (days)			Factor(s) level of significance		
			3	6	9	Factor (A)	Factor (B)	$A \times B$
a^*		18.5 ± 0.42				<0.0001	<0.0001	<0.162
	Ch-1% BP		29.1 ± 0.53^{d}	32.3 ± 0.47^{c}	31.1 ± 0.47^{cd}			
	Chitosan		28.4 ± 0.47^{e}	33.1 ± 0.57^{b}	30.5 ± 0.67^{cd}			
	Control		32.3 ± 0.66^{c}	36.6 ± 0.67^{a}	29.3 ± 0.71^{d}			
C^*		24.6 ± 0.41				<0.0001	<0.014	<0.125
	Ch-1% BP		34.2 ± 0.67^{e}	37.9 ± 0.63^{c}	37.6 ± 0.53^{c}			
	Chitosan		33.5 ± 0.78^{e}	39.8 ± 0.58^{b}	36.1 ± 0.82^{d}			
	Control		39.3 ± 0.92^{b}	43.6 ± 0.71^{a}	38.4 ± 0.82^{bc}			
h°		41.2 ± 0.82				<0.237	<0.049	<0.679
	Ch-1% BP		35.2 ± 0.75^{a}	31.5 ± 0.82^{bc}	30.9 ± 0.78^{c}			
	Chitosan		32.5 ± 0.76^{b}	32.1 ± 0.81^{b}	30.7 ± 0.87^{c}			
	Control		34.6 ± 0.71^{a}	32.8 ± 0.71^{b}	30.1 ± 0.78^{c}			

Data expressed as mean ± SE. Different letters across treatments and storage duration for each attribute differ significantly ($p < 0.05$) according to Duncan's multiple range test. Factor (A) = treatments; factor (B) = storage time. Factorial ANOVA was performed for the main factors, treatment, and storage period. Ch-1% BP (chitosan + 1% *Bidens pilosa*).

C^* throughout the storage period (37.6). The less pronounced a^* and C^* values during the storage period in coated fruit were due to the delayed ripening and reduced enzyme activity associated with anthocyanin degradation in raspberry fruit [76]. The hue angle (h°) of the raspberry fruit was significantly influenced ($p = 0.049$) by the storage period. Generally, the hue angle decreased as the raspberry fruit changed from light red to a redder hue or purity during storage [76].

4. Conclusions

Characteristics of chitosan-based films were significantly affected by incorporating *Bidens pilosa* extract. Different microstructural and physical properties were observed due to the incorporation of *B. pilosa* extracts at different concentrations. For instance, the film thickness and density improved with increased applied *B. pilosa* extract, resulting in higher water vapor transmission rate and water content (%) of control films. The phytochemical content, antioxidant, and antimicrobial activity of the chitosan films against *Penicillium expansum* improved after incorporating the *B. pilosa* extract. However, against *Botrytis cinerea*, only Ch-1 BP films were effective in controlling the inhibition zone of this postharvest pathogen. At a lower concentration of *B. pilosa* extract (Ch-1 BP), the kinetics of release of total phenolic content, DPPH radical scavenging, and ferric ion antioxidant power from the films into 50% ethanol at various dissolving times improved as compared to a higher concentration. Furthermore, the incorporation of *Bidens pilosa* extract at 1% enhanced the antimicrobial and water vapor barrier properties of the film, while maintaining its mechanical integrity. Therefore, Ch-1 BP film is worth optimizing for food preservation. The comprehensive analysis of the metabolite profiles of *Bidens pilosa,* using liquid chromatography-mass spectrometry, revealed a diverse range of bioactive compounds present in this medicinal plant. The variations in the content of individual polyphenols indicate that this medicinal plant may offer significant benefits as a functional food and hold promising food preservation potential. *Bidens pilosa*extract-loaded chitosan-based edible coating (Ch-1% BP) reduced weight loss and decay incidence and maintained color attributes such as redness (a^*), chroma (C^*), and hue angle (h°) of raspberry fruit, indicating that the individual polyphenols of *Bidens pilosa* were effective in delaying ripening and maintaining the postharvest attributes of the examined fruit.

Data Availability

The data supporting the current study are available from the corresponding author upon request.

Disclosure

The opinions, findings, conclusions, or recommendations expressed are those of the author(s) alone, and the NRF accepts no liability whatsoever in this regard.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

O.A.F. conceptualized the study; K.A.N. performed methodology; O.A.F. validated the manuscript; K.A.N. performed the formal analysis; K.A.N. investigated the manuscript; O.A.F. collected resources; K.A.N. prepared the original draft of the manuscript; O.A.F. reviewed and edited the manuscript; O.A.F. visualized the manuscript; O.A.F. supervised the manuscript; O.A.F. administrated the project; O.A.F. performed funding acquisition. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This work was based on the research supported wholly or partly by the National Research Foundation of South Africa (Grant number: 129295). Open Access funding was enabled and organized by SANLiC Gold.

References

[1] V. Coma, "Bioactive packaging technologies for extended shelf life of meat-based products," *Meat Science*, vol. 78, no. 1-2, pp. 90–103, 2008.

[2] A. Muñoz-Bonilla and M. Fernández-García, "Polymeric materials with antimicrobial activity," *Progress in Polymer Science*, vol. 37, no. 2, pp. 281–339, 2012.

[3] L. Wang, Y. Dong, H. Men, J. Tong, and J. Zhou, "Preparation and characterization of active films based on chitosan incorporated tea polyphenols," *Food Hydrocolloids*, vol. 32, no. 1, pp. 35–41, 2013.

[4] C. M. Bitencourt, C. S. Fávaro-Trindade, P. J. A. Sobral, and R. A. Carvalho, "Gelatin-based films additivated with curcuma ethanol extract: antioxidant activity and physical properties of films," *Food Hydrocolloids*, vol. 40, pp. 145–152, 2014.

[5] P. K. Dutta, S. Tripathi, G. K. Mehrotra, and J. Dutta, "Perspectives for chitosan based antimicrobial films in food applications," *Food Chemistry*, vol. 114, no. 4, pp. 1173–1182, 2009.

[6] S. I. Park and Y. Zhao, "Incorporation of a high concentration of mineral or vitamin into chitosan-based films," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 7, pp. 1933–1939, 2004.

[7] Y. Peng and Y. Li, "Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films," *Food Hydrocolloids*, vol. 36, pp. 287–293, 2014.

[8] E. Portes, C. Gardrat, A. Castellan, and V. Coma, "Environmentally friendly films based on chitosan and tetrahydrocurcuminoid derivatives exhibiting antibacterial and antioxidative properties," *Carbohydrate Polymers*, vol. 76, no. 4, pp. 578–584, 2009.

[9] M. B. Vásconez, S. K. Flores, C. A. Campos, J. Alvarado, and L. N. Gerschenson, "Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings," *Food Research International*, vol. 42, no. 7, pp. 762–769, 2009.

[10] K. Ncama, L. S. Magwaza, A. Mditshwa, and S. Z. Tesfay, "Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: a review," *Food Packaging and Shelf Life*, vol. 16, pp. 157–167, 2018.

[11] K. A. Nxumalo, A. O. Aremu, and O. A. Fawole, "Potentials of medicinal plant extracts as an alternative to synthetic chemicals in postharvest protection and preservation of horticultural crops: a review," *Sustainability*, vol. 13, no. 11, p. 5897, 2021.

[12] M. Tomczykowa, K. Leszczyńska, M. Tomczyk, E. Tryniszewska, and D. Kalemba, "Composition of the essential oil of *Bidens tripartita* L. roots ant its antibacterial and antifungal activities," *Journal of Medicinal Food*, vol. 14, no. 4, pp. 428–433, 2011.

[13] W. C. Yang, "Botanical, Pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant *Bidens pilosa.* L," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 698617, 14 pages, 2014.

[14] B. Li, J. Xie, F. Xie et al., "Polyacetylenes from *Bidens pilosa* linn., promising insecticides with anti-inflammatory effects on HaCaT cells," *Industrial Crops and Products*, vol. 171, Article ID 113929, 2021.

[15] T. D. Xuan, T. Shinkichi, T. D. Khanh, and I. M. Chung, "Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview," *Crop Protection*, vol. 24, no. 3, pp. 197–206, 2005.

[16] Y. Sun, Q. Zhou, W. Liu, J. An, Z. Xu, and L. Wang, "Joint effects of arsenic and cadmium on plant growth and metal bioaccumulation: a potential cd-hyperaccumulator and as-excluder *Bidens pilosa* L," *Journal of Hazardous Materials*, vol. 165, no. 1-3, pp. 1023–1028, 2009.

[17] Y. Chiang, D. Chuang, S. Wang, Y. Kuo, P. Tsai, and L. Shyur, "Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*," *Journal of Ethnopharmacology*, vol. 95, no. 2-3, pp. 409–419, 2004.

[18] F. Lima Silva, D. C. Fischer, J. Fechine Tavares, M. Sobral Silva, P. Filgueiras de Athayde-Filho, and J. M. Barbosa-Filho, "Compilation of secondary metabolites from *Bidens pilosa* L," *Molecules*, vol. 16, no. 2, pp. 1070–1102, 2011.

[19] L. S. T. Ngamo, A. Goudoum, M. B. Ngassoum, P. M. Mapongmetsem, and T. Hance, "Chronic toxicity of essential oils of 3 local aromatic plants toward *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae)," *African Journal of Agricultural Research*, vol. 2, no. 4, pp. 164–167, 2007.

[20] A. Goudoum, L. Ngamo Tinkeu, M. B. Ngassoum, and C. M. Mbofung, "Antioxidant activities of essential oils of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Labiateae) plants used against stored grains insects in North Cameroon," *International Journal of Brain and Cognitive Sciences*, vol. 3, no. 3, pp. 567–577, 2009.

[21] A. P. Bartolome, I. M. Villaseñor, and W. C. Yang, "*Bidens pilosa* L. (Asteraceae): botanical properties, traditional uses, phytochemistry, and pharmacology," *Evidence-based Complementary and Alternative Medicine*, vol. 2013, Article ID 340215, 51 pages, 2013.

[22] P. Ramesh, A. Rajendran, and M. Meenakshisundaram, "Green synthesis of zinc oxide nanoparticles using flower extract *Cassia Auriculata*," *Journal of Nanoscience and Nanotechnology*, vol. 2, pp. 41–45, 2014.

[23] M. H. Hassan, M. A Zayton, and S. A El-Feky, "Role of green synthesized zno nanoparticles as antifungal against post-harvest gray and black mold of sweet bell pepper," *Journal of Biotechnology and Bioengineering*, vol. 3, no. 4, pp. 8–15, 2019.

[24] T. P. Magangana, N. P. Makunga, C. la Grange, M. A. Stander, O. A. Fawole, and U. L. Opara, "Blanching pre-treatment promotes high yields, bioactive compounds, antioxidants, enzyme inactivation and antibacterial activity of 'Wonderful' pomegranate peel extracts at three different harvest maturities," *Antioxidants*, vol. 10, no. 7, p. 1119, 2021a.

[25] T. P. Magangana, N. P. Makunga, O. Amos Fawole, and U. L. Opara, "Effect of solvent extraction and blanching pre-treatment on phytochemical, antioxidant properties, enzyme inactivation and antibacterial activities of "Wonderful" pomegranate peel extracts," *Processes*, vol. 9, no. 6, p. 1012, 2021b.

[26] U. Siripatrawan and W. Vitchayakitti, "Improving functional properties of chitosan films as active food packaging by incorporating with propolis," *Food Hydrocolloids*, vol. 61, pp. 695–702, 2016.

[27] A. Riaz, S. Lei, H. M. S. Akhtar et al., "Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols," *International Journal of Biological Macromolecules*, vol. 114, pp. 547–555, 2018.

[28] M. Moradi, H. Tajik, S. M. Razavi Rohani et al., "Characterization of antioxidant chitosan film incorporated with *Zataria multiflora* boiss essential oil and grape seed extract," *LWT- Food Science and Technology*, vol. 46, no. 2, pp. 477–484, 2012.

[29] A. S. Ferreira, C. Nunes, A. Castro, P. Ferreira, and M. A. Coimbra, "Influence of grape pomace extract incorporation on chitosan films properties," *Carbohydrate Polymers*, vol. 113, pp. 490–499, 2014.

[30] A. C. Seydim and G. Sarikus, "Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils," *Food Research International*, vol. 39, no. 5, pp. 639–644, 2006.

[31] H. Lian, Y. Peng, J. Shi, and Q. Wang, "Effect of emulsifier hydrophilic-lipophilic balance (HLB) on the release of thyme essential oil from chitosan films," *Food Hydrocolloids*, vol. 97, Article ID 105213, 2019.

[32] R. L. Prior, X. Wu, and K. Schaich, "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 10, pp. 4290–4302, 2005.

[33] E. Genskowsky, L. A. Puente, J. A. Pérez-Álvarez, J. Fernandez-Lopez, L. A. Muñoz, and M. Viuda-Martos, "Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*)," *LWT- Food Science and Technology*, vol. 64, no. 2, pp. 1057–1062, 2015.

[34] A. T. Ramabulana, P. A. Steenkamp, N. E. Madala, and I. A. Dubery, "Application of plant growth regulators modulates the profile of chlorogenic acids in cultured *Bidens pilosa* cells," *Plants*, vol. 10, no. 3, p. 437, 2021.

[35] J. Qiu, X. Chen, P. Liang et al., "Integrating approach to discover novel bergenin derivatives and phenolics with antioxidant and anti-inflammatory activities from bioactive fraction of *Syzygium brachythyrsum*," *Arabian Journal of Chemistry*, vol. 15, no. 1, Article ID 103507, 2022.

[36] K. Masike, B. S. Khoza, P. A. Steenkamp, E. Smit, I. A. Dubery, and N. E. Madala, "A metabolomics-guided exploration of the phytochemical constituents of *Vernonia fastigiata* with the aid of pressurized hot water extraction and liquid chromatography-mass spectrometry," *Molecules*, vol. 22, no. 8, p. 1200, 2017.

[37] K. Masike and N. Madala, "Synchronized survey scan approach allows for efficient discrimination of isomeric and isobaric compounds during LC-MS/MS analyses," *Journal of Analytical Methods in Chemistry*, vol. 2018, Article ID 2046709, 8 pages, 2018.

[38] O. R. Pereira, M. D. Catarino, A. F. Afonso, A. M. S. Silva, and S. M. Cardoso, "*Salvia elegans, Salvia greggii* and *Salvia officinalis* decoctions: antioxidant activities and inhibition of carbohydrate and lipid metabolic enzymes," *Molecules*, vol. 23, no. 12, p. 3169, 2018.

[39] M. Yasir, B. Sultana, and M. Amicucci, "Biological activities of phenolic compounds extracted from Amaranthaceae plants and their LC/ESI-MS/MS profiling," *Journal of Functional Foods*, vol. 26, pp. 645–656, 2016.

[40] N. J. Goosen, D. Oosthuizen, M. A. Stander, A. I. Dabai, M. Pedavoah, and G. O. Usman, "Phenolics, organic acids and minerals in the fruit juice of the indigenous African sourplum (*Ximenia caffra*, Olacaceae)," *South African Journal of Botany*, vol. 119, pp. 11–16, 2018.

[41] C. Hamany Djande, L. A. Piater, P. A. Steenkamp, N. E. Madala, and I. A. Dubery, "Differential extraction of phytochemicals from the multipurpose tree, *Moringa oleifera*, using green extraction solvents," *South African Journal of Botany*, vol. 115, pp. 81–89, 2018.

[42] N. I Kashchenko and D. N. Olennikov, "Phenolome of Asian agrimony tea (*Agrimonia asiatica* Juz., Rosaceae): LC-MS profile, α-glucosidase inhibitory potential and stability," *Foods*, vol. 9, no. 10, p. 1348, 2020.

[43] Y. Chen, J. Luo, Q. Zhang, and L. Kong, "Identification of active substances for dually modulating the renin–angiotensin system in *Bidens pilosa* by liquid chromatography–mass spectrometry–based chemometrics," *Journal of Functional Foods*, vol. 21, pp. 201–211, 2016.

[44] A. E. M. Abdalla, S. M. Darwish, E. H. E. Ayad, and R. M. El-Hamahmy, "Egyptian mango by-product 2: antioxidant and antimicrobial activities of extract and oil from mango seed kernel," *Food Chemistry*, vol. 103, no. 4, pp. 1141–1152, 2007.

[45] M. M. Tajkarimi, S. Ibrahim, and D. O. Cliver, "Antimicrobial herb and spice compounds in food," *Food Control*, vol. 21, no. 9, pp. 1199–1218, 2010.

[46] J. B. Gurtler and T. L. Mai, "Preservatives traditional preservatives – organic acids," in *Encyclopedia of Food Microbiology*, C. A. Batt and M. L. Tortorello, Eds., pp. 119–130, Academic Press, Oxford, UK, 2nd edition, 2014.

[47] O. A. Idris, N. Kerebba, S. Horn, M. S. Maboeta, and R. Pieters, "Phytochemical-based evidence of the health benefits of *Bidens pilosa* extracts and cytotoxicity," *Chemistry Africa*, vol. 2023, 2023.

[48] L. R. Ferguson, S. T. Zhu, and P. J. Harris, "Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells," *Molecular Nutrition and Food Research*, vol. 49, no. 6, pp. 585–593, 2005.

[49] N. S. Chatterjee, S. K. Panda, M. Navitha, K. K. Asha, R. Anandan, and S. Mathew, "Vanillic acid and coumaric acid grafted chitosan derivatives: improved grafting ratio and potential application in functional food," *Journal of Food Science and Technology*, vol. 52, no. 11, pp. 7153–7162, 2015.

[50] M. P. Kähkönen, A. I. Hopia, H. J. Vuorela et al., "Antioxidant activity of plant extracts containing phenolic compounds," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 10, pp. 3954–3962, 1999.

[51] D. Zhou, X. Qin, Z. R. Zhang, and Y. Huang, "Physicochemical properties of bergenin," *Die Pharmazie*, vol. 63, no. 5, pp. 366–371, 2008.

[52] H. B. Pushpalatha, K. Pramod, R. Devanathan, and R. Sundaram, "Use of bergenin as an analytical marker for standardization of the polyherbal formulation containing *Saxifraga ligulata*," *Pharmacognosy Magazine*, vol. 11, no. 42, pp. S60–S65, 2015.

[53] H. B. Coban, "Organic acids as antimicrobial food agents: applications and microbial productions," *Bioprocess and Biosystems Engineering*, vol. 43, no. 4, pp. 569–591, 2020.

[54] J. Just, B. J. Deans, W. J. Olivier, B. Paull, A. C. Bissember, and J. A. Smith, "New method for the rapid extraction of natural products: efficient isolation of shikimic acid from star anise," *Organic Letters*, vol. 17, no. 10, pp. 2428–2430, 2015.

[55] J. Bai, Y. Wu, K. Zhong et al., "A comparative study on the effects of quinic acid and shikimic acid on cellular functions of *Staphylococcus aureus*," *Journal of Food Protection*, vol. 81, no. 7, pp. 1187–1192, 2018.

[56] V. Verma, G. Singhal, S. Joshi, M. Choudhary, and N. Srivastava, "Chapter 10- plant extracts as enzymes," in *Plant Extracts: Applications in the Food Industry*, S. A. Mir, A. Manickavasagan, and M. A. Shah, Eds., pp. 209–223, Academic Press, Cambridge, MA, USA, 2022.

[57] T. Shintani, "Food industrial production of monosaccharides using microbial, enzymatic, and chemical methods," *Fermentation*, vol. 5, no. 2, p. 47, 2019.

[58] M. M. Silva and F. C. Lidon, "Food preservatives– an overview on applications and side effects," *Emirates Journal of Food and Agriculture*, vol. 28, no. 6, pp. 366–373, 2016.

[59] U. Siripatrawan and B. R. Harte, "Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract," *Food Hydrocolloids*, vol. 24, no. 8, pp. 770–775, 2010.

[60] J. B. Hutchings, *Food and Colour Appearance*, Chapman and Hall Food Science Book, Aspen Publication, Gaithersburg, MD, USA, 2nd edition, 1999.

[61] L. Sánchez-González, M. Cháfer, C. González-Martínez, A. Chiralt, and S. Desobry, "Study of the release of limonene present in chitosan films enriched with bergamot oil in food simulants," *Journal of Food Engineering*, vol. 105, no. 1, pp. 138–143, 2011.

[62] J. Liu, C. Meng, Y. Yan, Y. Shan, J. Kan, and C. Jin, "Protocatechuic acid grafted onto chitosan: characterization and antioxidant activity," *International Journal of Biological Macromolecules*, vol. 89, pp. 518–526, 2016.

[63] P. Mayachiew and S. Devahastin, "Effects of drying methods and conditions on release characteristics of edible chitosan films enriched with indian gooseberry extract," *Food Chemistry*, vol. 118, no. 3, pp. 594–601, 2010.

[64] T. Panrong, T. Karbowiak, and N. Harnkarnsujarit, "Thermoplastic starch and green tea blends with LLDPE films for active packaging of meat and oil-based products," *Food Packaging and Shelf Life*, vol. 21, Article ID 100331, 2019.

[65] Z. Shen and D. P. Kamdem, "Development and characterization of biodegradable chitosan films containing two essential oils," *International Journal of Biological Macromolecules*, vol. 74, pp. 289–296, 2015.

[66] B. W. S. Souza, M. A. Cerqueira, J. T. Martins, A. Casariego, J. A. Teixeira, and A. A. Vicente, "Influence of electric fields on the structure of chitosan edible coatings," *Food Hydrocolloids*, vol. 24, no. 4, pp. 330–335, 2010.

[67] C. Zhang, Y. Ding, Q. Ping, and L. L. Yu, "Novel chitosan-derived nanomaterials and their micelle-forming properties," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 22, pp. 8409–8416, 2006.

[68] J. F. Rubilar, R. M. S. Cruz, H. D. Silva, A. A. Vicente, I. Khmelinskii, and M. C. Vieira, "Physico-mechanical properties of chitosan films with carvacrol and grape seed extract," *Journal of Food Engineering*, vol. 115, no. 4, pp. 466–474, 2013.

[69] E. Torlak and D. Sert, "Antibacterial effectiveness of chitosan-propolis coated polypropylene films against foodborne pathogens," *International Journal of Biological Macromolecules*, vol. 60, pp. 52–55, 2013.

[70] W. Bors, C. Michel, and K. Stettmaier, "Structure-activity relationships governing antioxidant capacities of plant polyphenols," *Methods in Enzymology*, vol. 335, pp. 166–180, 2001.

[71] Y. Pan, K. Wang, S. Huang et al., "Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel," *Food Chemistry*, vol. 106, no. 3, pp. 1264–1270, 2008.

[72] H. Hassanpour, "Effect of *Aloe vera* gel coating on antioxidant capacity, antioxidant enzyme activities and decay in raspberry fruit," *LWT- Food Science and Technology*, vol. 60, no. 1, pp. 495–501, 2015.

[73] C. Han, Y. Zhao, S. Leonard, and M. Traber, "Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus ideaus*)," *Postharvest Biology and Technology*, vol. 33, no. 1, pp. 67–78, 2004.

[74] S. C. Riva, U. O. Opara, and O. A. Fawole, "Recent developments on postharvest application of edible coatings on stone fruit: a Review," *Scientia Horticulturae*, vol. 262, Article ID 109074, 2020.

[75] M. A. Moreno, A. Vallejo, A. R. Ballester et al., "Antifungal edible coatings containing Argentinian propolis extract and their application in raspberries," *Food Hydrocolloids*, vol. 107, Article ID 105973, 2020.

[76] D. Valero, H. M. Díaz-Mula, P. J. Zapata et al., "Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage," *Postharvest Biology and Technology*, vol. 77, pp. 1–6, 2013.