

Article

Poly (Lactic Acid) Fibrous Film with Betalains from Pitaya (*Stenocereus thurberi*) by Electrospinning for Potential Use as Smart Food Packaging

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Abstract: The incorporation of biopolymers and natural colorants in smart packaging has garnered significant attention in the food packaging industry. This study investigates the design and characterization of novel fibrous films incorporating betalain extract (BE) from *Stenocereus thurberi* in poly (lactic acid) (PLA). An electrospinning technique was developed with varying PLA concentrations (2%–12% *w/v*) and BE concentrations (8%–12% *w/v*) to create a colorimetric freshness indicator. BE was characterized by quantifying its phytochemical content and assessing its antioxidant capacity. Morphological and structural analyses included scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), polydispersity index (PI), mechanical properties, and functional characteristics such as ammonia sensitivity and total antioxidant activity. The results indicated that the incorporation of BE significantly influenced the average diameter of the nanofibers, ranging from 313 ± 74 nm to 657 ± 99 nm. SEM micrographs showed that PLA12-BE12 films exhibited smooth surfaces without bead formation. The FTIR analysis confirmed effective BE incorporation, revealing intermolecular interactions between the betalain molecules and the PLA matrix, which contributed to enhanced structural and functional stability. The mechanical properties analysis revealed that moderate BE additions (8%–10% *w/v*) enhanced the Young's modulus and tensile strength, while higher BE concentrations (12% *w/v*) disrupted the polymer network, reducing these properties. Additionally, the strain at break decreased significantly with BE incorporation, reflecting limited molecular chain mobility. Increasing BE concentration notably improved antioxidant activity, with the BE concentration of 12% (*w/v*), the ABTS•+, DPPH•, and FRAP radical scavenging activities at the highest values of $84.28 \pm 1.59\%$, $29.95 \pm 0.34\%$, and 710.57 ± 28.90 $\mu\text{M ET/g}$, respectively. Ammonia sensitivity tests demonstrated a significant halochromic transition from reddish-pink to yellow, indicating high sensitivity to low ammonia concentrations. The possible mechanism is alkaline pH induces aldimine bond hydrolysis and generates betalamic acid (yellow) and cyclo-DOPA-5-O- β -glucoside (colorless). The fibrous films also exhibited reversible color changes and maintained good color stability over 30 days, emphasizing their potential for use in smart packaging applications for real-time freshness monitoring and food quality assessment.

Keywords: poly (lactic acid); betalain extract; electrospinning; antioxidant capacity; freshness indicator

1. Introduction

Intelligent packaging is defined by the European Commission as materials and articles that monitor the condition of food and its environment when in contact with food [1]. Such packaging is capable of performing intelligent functions, such as sensing, recording, communicating, etc., in relation to the condition of the food and provides an early warning to the consumer and/or food producers about the quality of the food during transport and storage [2,3]. They are classified as follows: indicators, sensors and radio frequency identifiers (RFID) [4]. Freshness indicators are widely used and can effectively monitor food freshness in real time due to their simplicity, accuracy, and non-invasive characteristics [5–8]. On the other hand, active packaging is a type of food packaging with an additional function, in addition to providing a protective barrier against external influence, according to European Union Commission of European Communities. Active packaging absorbs chemicals derived from food or the environment within the packaging, or releases substances into the food or the surrounding environment. These substances include preservatives, antioxidants, and flavorings and are incorporated into a polymeric matrix or coatings, labels, pads, or sachets [1]. The bioactive compounds applied, such as phenolic compounds, can be derived from various sources including different anatomical parts of plants, such as the root, stem, seed, flowers, or fruits, herbs as well as algae and vegetables [9], which are capable of detecting changes in the food or its environment, as [10–14]. A new term in the food packaging industry is smart packaging. Based on the above definitions, active and intelligent packaging can act synergistically [15], combining the diverse capabilities. Smart packaging encompasses both active and intelligent systems designed to provide comprehensive and accurate information about the condition of and changes in a food product and its surrounding environment. Additionally, it actively responds to these changes by enhancing the protection of the food through, for instance, the incorporation of antioxidant or antimicrobial agents [9–16].

During storage, spoilage of meat and seafood products as a result of microbial growth and metabolite production occurs with a noticeable increase in pH due to the production of volatile compounds such as ammonia and amines (dimethylamine, trimethylamine, etc.) [17]. Hence, ammonia is frequently chosen as a model gas to simulate the changes induced by bacteria in spoiled food, allowing for the approximate assessment of the detection capabilities of indicator films and showing halocromic shifts in freshness indicators [18].

Recent research highlights a preference for biopolymers over those derived from non-renewable sources because of their exceptional biocompatibility and biodegradability using natural pigments such as alizarin, curcumin, anthocyanins, betalains, etc. [4,19–22], rendering them ideal for the development of freshness indicators.

The latest progress in the study of betalains has led to the diversification of their potential applications, particularly as natural food colorants. The use of natural colorants has grown significantly due to their health benefits over synthetic alternatives, which are associated with harmful effects. Betalains, the most widely used natural food colorants, are predominantly derived from red beetroot [23]. However, their application is limited by the earthy odor caused by geosmin and pyrazines, which become noticeable at high concentrations. As a result, there is a growing need to explore alternative sources of betalains. Notable candidates include amaranth and cactus fruits, which have garnered significant interest for their technological and nutritional potential as natural colorants [24–27].

Betalains are water-soluble nitrogen compounds that are synthesized from the amino acid tyrosine and consist of two structural groups which are betacyanins (red coloration) and betaxanthins (yellow coloration). These molecules have demonstrated stability within a pH range of 3.0–6.0. At pH > 6, they demonstrate a degradation of color [28,29]. In alkaline conditions, betanin broke down, leading to the liberation of betalamic acid and cyclodopa-5-O-glucoside [30]. For optimal stability of beetroot pigments under anaerobic conditions, pH 4.0–5.0 is recommended; under aerobic conditions, pH 5.5–5.8 is preferred [31,32].

Recent research has explored the use of betalains for synthesizing freshness indicators through various techniques. Qin et al. [33] incorporated betalains from *Hylocereus*

polyrhizus peel into a starch and polyvinyl alcohol (PVOH) matrix. Kanatt [20] utilized gelatin and PVOH enriched with betalains extracted from *Amaranthus* spp. Additionally, Nagdhi et al. [8] developed a starch-based film infused with *Bougainvillea glabra* extract, and Castro [34] synthesized nanoparticles in a glutenin matrix on a PLA film as a food sensor, which did not change color but increased the shelf life of fish fillets (*Scomber scombrus*) due to their antioxidant and antimicrobial activity. These investigations have demonstrated the usefulness of biopolymers as a matrix for the development of these indicator systems, but most of these have been carried out using the casting technique, where in some cases, as they are too dense, their sensitivity is limited.

On the other hand, Li et al. [17] developed nanofibrous films via the electrospinning technique with PLA and anthocyanins extracts, which have been found to be highly sensitive with the ability to detect 10^2 CFU/mL denoted by color change. Aghaei et al. [35] developed a halochromic sensor based on cellulose acetate and alizarin nanofibers, which they evaluated on rainbow trout fillets. They found color changes in the sensor after 6 days of storage related to changes in pH, total volatile basic nitrogen (TVB-N), and total bacterial counts. Therefore, nanofibrous films as quality indicators have several advantages compared to traditional methods, some of them being the following: (a) greater sensitivity and specificity—due to their nanometric structure, the contact surface between the films and the analytes increases, which improves the ability to detect it in minimum concentrations [36,37]; (b) rapid response—the materials react in a shorter reaction time to environmental stimuli such as pH, humidity, temperature, and the production of volatile compounds [38,39]; (c) variability in design—they can be functionalized with enzymes, nanoparticles, conductive polymers, bioactive molecules, etc. [36,40]; (d) improved mechanical properties—they are flexible, light, and rigid and facilitate their integration as adhesive labels [37,39]; (e) sustainability—utilizing biodegradable materials, which reduces the environmental impact compared to those made from non-renewable resources [4]; (f) less invasive—no exclusive direct contact with the food for the detection of compounds such as amines, ethanol, etc., depending on the food matrix [37,38].

The use of betalains has been limited due to their susceptibility to various factors affecting stability including chemical structure, pigment content, matrix, additives, light, temperature, water activity (aw), metals, enzymes, exposure to oxygen, etc. [41]. Such instability can be minimized with the use of encapsulation technology [42,43].

A variety of technologies are used for the production of freshness indicators, wherein the bioactive compounds are encapsulated either on the surface or in interaction with the polymeric matrix. Technologies used are the absorption method, sol-gel, extrusion, layer-by-layer, extrusion, thermoforming, electrochemical writing, inkjet printing, electro-spraying, and electrospinning [44,45]. The preparation of pH-sensitive fibrous films using the electrospinning technique offers several advantages, including high absorption capacity, increased surface area, enhanced porosity, and uniform dye absorption, compared to other methods. Some drawbacks of alternative methods include co-solubility, high temperature requirements, increased density, reduced contact surface area, and dye leaching [46].

Electrospinning is a novel technique where a high potential electric field is applied to a polymer solution, causing it to overcome molecular cohesive forces and surface tension, resulting in the formation of fibers that migrate to a collector [47]. In comparison to traditional encapsulation techniques for bioactive compounds, electrospinning offers the fabrication of polymeric nano or micro-sized fibers [48] and offers an opportunity to meet the desired requirements for colorimetric detection, including high sensitivity, rapid detection time, and uniform color change [49]. Moreover, electrospinning is cost-effective and operates at low temperatures, making it suitable for encapsulating temperature sensitive molecules such as betalains.

There has been a growing interest in the development of active and intelligent packaging materials using biopolymers such as starch, gelatin, chitosan, zein, collagen, polyvinyl alcohol, polycaprolactone, and poly lactic acid [50]. For instance, natural pigments were immobilized in PLA nanofibers, and the color of the fibrous film gradually changed [17,41]

with varying environmental pH. As a substitute for petroleum-based polymers, PLA is a hydrophobic, biodegradable, and biocompatible aliphatic polyester with good mechanical properties approved by the FDA (Food and Drug Administration) and produced from renewable natural materials and can be easily processed to fabricate fibrous films [51].

In conclusion, with the exponential growth of the population, the food industry needs to produce food according to food legislation. This has led to acquiring safe and quality products, avoiding economic losses and food poisoning. The use of new technology for the development of smart packaging will monitor food quality and freshness in real time, avoiding food poisoning and reducing the consumption of spoiled food.

The main objective of the present work is to design a fibrous film indicator by the electrospinning technique for ammonia colorimetric detection and antioxidant activity using PLA and betalains as the indicator pigment and bioactive compound. Firstly, we investigate the optical characteristics of the betalain extract, some of the parameters of the polymeric solutions, and the basic structural properties and interactions of the film and finally evaluate the sensibility, stability, and reusability characteristics of the fibrous film indicator for potential application as smart packing in the food industry.

2. Materials and Methods

2.1. Materials and Reagents

Fresh red pitaya from cacti (*Stenocereus thurberi*) was purchased from the town of Carbó located in the center of Sonora, México (29°41'05" N 110°57'16" O). The fruits were harvested in May and June 2022, and those that were completely closed and without signs of senescence were selected. Poly lactic acid (4060D, Mn = 119,000 g mol^{−1}) was obtained from Nature Works® LLC (Minnetonka, MN, USA), and N,N-dimethylformamide (DMF), acetone (Ac), ammonium hydroxide, acetic acid, sodium hydroxide, and hydrochloric acid were obtained from Meyer (SA de CV, Cd. De México) and millyQ water.

2.2. Betalain Extraction

The pitayas were washed, disinfected, and stored in polyethylene bags at −5 °C until further use. The extraction process followed the methodology proposed by Rodríguez et al. [52], with some modifications. Two grams of freeze-dried and seedless pulp (Labconco Freezone 6, Kansas City, MO, USA) were weighed, and 34 mL of Milli-Q water was added. The mixture was placed in a 57 °C water bath and sonicated (Branson M3800H, Mexico City, Mexico) for 27 min. Subsequently, it was shaken in the dark for 20 min using a horizontal shaker (VWR Mini Blot Mixer, USA). To facilitate separation, the pulp extract was then centrifuged at 5082 × g and 5 °C for 20 min using a refrigerated centrifuge (Thermo Fisher Scientific, Asheville, NC, USA). The resulting supernatant was decanted, lyophilized, and stored in amber containers at −40 °C until further use.

2.3. Characterization and Antioxidant Capacity of Betalains Extract

2.3.1. Photometric Quantification of Betalains

The quantification of total betalains, betaxanthins, and betacyanins was carried out by measuring the absorbance at 538 nm for betacyanins and 483 nm for betaxanthins using a UV-Vis spectrophotometer (Varian, Cary 50, Melbourne, Australia). The amount of betalains was calculated by Equation (1) [52] as follows:

$$\left[B \left(\frac{\text{m}}{\text{g}} \right) \right] = \frac{A \times DF \times MW \times V}{\epsilon \times W \times L} \quad (1)$$

where B refers to the betacyanins or betaxanthins concentration, A is absorbance of the sample, DF is dilution factor, MW is molecular weight (betanin 550 g/mol) (indicaxanthin 308 g/mol), V is volume, ϵ is molar extinction coefficient (for betanin, it is 60,000 mol/L × cm and for indicaxanthin, 48,000 mol/L × cm), W is the weight of the sample (g), and L is the length of the cuvette (1 cm). The total betalains was obtained with the sum of betacyanins and betaxanthins.

2.3.2. Total Phenolic Content

Spectrophotometric quantification of total phenol content was determined by Prior [53] methodology, using the Folin-Ciocalteu (FC) reagent in a 96-well microplate system. Here, 10 μL of sample was mixed with 25 μL of the 1N FC reagent. The mixture was left for 5 min at room temperature, and subsequently, 25 μL of 20% Na_2CO_3 and 140 μL of deionised water were added. The reaction was left for 30 min, and then absorbance was determined at a wavelength of 760 nm on a microplate reader (Thermo Fisher Scientific Inc. Multiskan GO, NY, USA). A standard calibration curve for the gallic acid standard was performed, and the results were expressed as milligrams of gallic acid equivalents for grams of dry sample (mg GAE/g).

2.3.3. Antiradical Capacity by ABTS•+ Method

The assay was performed by the method proposed by Re et al. [54]. The ABTS•+ radical was prepared by dissolving 19.3 mg of ABTS•+ in water (5 mL). From a $\text{K}_2\text{S}_2\text{O}_8$ (0.0378 g/mL) solution, an aliquot of 88 μL was added to the ABTS•+ solution and allowed to stand in the dark for 16 h. The radical was adjusted to an absorbance of 0.7 ± 0.01 at 734 nm. For the reaction, 270 μL of the radical was mixed with 20 μL of the sample. After a darkness incubation time of 30 min, the assay was measured at 734 nm in a microplate reader (Thermo Fisher Scientific Inc., Multiskan GO, NY, USA). The determinations were in triplicate. The results were expressed as micromoles of Trolox equivalent per gram of dry sample ($\mu\text{M TE/g}$), and the concentration of the extract at which 50% of the radical is inhibited (IC50) was determined using the following equation:

$$\%Inhibition = \left[\frac{A_0 - (Absorbance\ Sample - Absorbance\ Blank\ Sample)}{A_0} \times 100 \right] \quad (2)$$

where A_0 is absorbance of reagent + H_2O , *Absorbance sample* is the absorbance of the reagent + sample, *Absorbance blank sample* is the absorbance of H_2O + sample. From the % inhibition, the IC50 was calculated.

2.3.4. Antiradical Capacity by DPPH• Method

The assay was performed by the method proposed by Molyneux [55]. The DPPH• radical was prepared at 0.03 mg/mL in methanol. In the reaction system, 200 μL of the radical was mixed with 20 μL of the sample in a microplate reader (Thermo Fisher Scientific Inc. Multiskan GO, NY, USA) and incubated for 30 min in darkness. The radical solution was previously diluted to an absorbance of 0.7 ± 0.01 at 515 nm. The blank and the determinations at 515 nm were the same as in the ABTS assay as well as the expression of the results obtained.

2.3.5. Ferric Reducing Antioxidant Power (FRAP) Method

The protocol for the determination of FRAP was described by Benzie [56]. The assay was based on the reaction between Fe^{3+} and TPTZ, measured at 638 nm. First, stock solutions were prepared: sodium acetate buffer (300 mmol/L, pH 3.6), iron (III) chloride (FeCl_3 , 20 mmol), and a TPTZ solution (10 mM) were dissolved in HCl (40 mM). Subsequently, the FRAP working solution was formulated in a 10:1:1 ratio (buffer: FeCl_3 : TPTZ). A volume of 20 μL of the sample was mixed with 280 μL of the FRAP solution, and the mixture was placed in a microplate reader (Thermo Fisher Scientific Inc., Multiskan GO, NY, USA). After an incubation time of 30 min, the absorbance was measured at 638 nm. The blank and the determinations at 638 nm were the same as in the ABTS assay, and the results were expressed as micromoles of Trolox equivalent per gram of dry sample ($\mu\text{M ET/g}$).

2.3.6. pH Sensitivity Property

The sensitivity of BE (3 mg/mL) was assessed using a UV-Vis spectrophotometer (Varian, Cary 50, Melbourne, AU) [57]. Spectra of the betalain solution were recorded over a wavelength range of 200–800 nm for solutions with pH values reaching from 1.0 to 14.0. The pH adjustments were made by 0.1 M of NaOH and 0.1 M of HCl solutions, and the pH

values were measured with a potentiometer (Orion 420 A, Thermo Electron Corporation, Waltham, MA, USA). Photographs were taken to visually capture the color changes in the solutions at different pH values.

2.4. Development of the Color Indicator Fibrous Film

2.4.1. Preparation of the Electrospinning Solutions

Firstly, polymeric solutions were prepared by dissolving pellets of PLA at 2, 4, 6, 8, 10, and 12% (*w/v*) concentrations in a binary solvent system of Ac and DMA in a 1:1 ratio by magnetic stirring for 24 h at 25 °C to determine the polymer concentration from which fiber formation is promoted. Once the best concentration of PLA was selected (according to the morphological characteristics of the defect-free fibers), the BE solution was developed by dissolving 8, 10, and 12% (*w/v*) (selected from a preliminary evaluation, where at a lower concentration of 8% (*w/v*) of BE, the fibers did not show a characteristic tones) in DMF for 3 h at 25 °C by a magnetic stirrer (IKA, Staufen, Germany) and subsequently added to the best fibers, forming solutions of PLA. Both solutions were homogenized in a magnetic stirrer (IKA, Germany) for 2 h to disperse well and then sonicated (Branson, M3800H, Mexico City, Mexico) for 90 min to eliminate air bubbles, which are named as PLA12-BE8%, PLA12-BE10%, and PLA12-BE12% (*w/v*).

2.4.2. Characterization of the Electrospinning PLA-BET Solutions

Viscosity

The viscosity of the electrospinning PLA-BE (8, 10, 12% *w/v*) solutions was determined using an Anton Paar (Ostfildern, Germany) rheometer, model MCR-102, equipped with concentric cylinder geometry [58]. The measurements were conducted with a 1 mm gap between the cylinder and the base, at a constant shear rate of 100 s^{−1}, and at a controlled temperature of 25 °C. Each determination was performed in triplicate to ensure accuracy.

Electrical Conductivity

Electrical conductivity was measured following the methodology described by Prietto [59] using a HANNA instrument model HI 2550 (pH, ORP & EC/TDS/NaCl meter, Woonsocket, RI, USA) conductometer. The tests were performed at 25 °C. All determinations were carried out in triplicate to ensure reliable results.

2.4.3. Electrospinning of Fibrous Film

Figure 1 depicts the general diagram on the development of the nanofibrous film. The PLA-BE electrospinning solution was loaded in a syringe (12.48 mm inner diameter) with a metal needle of 20 gauge (0.60 mm inner diameter). The fibrous film was prepared using a syringe pump, and the flow velocity was 1 mL/h (Scientific, KD, USA) at 25 °C and 30%–40% relative humidity. A collector made of aluminum of 49 cm² square was used to receive the fibrous film, and the distance between the collector and the tip of the needle was 10 cm. A high voltage power supply (Spellman, CZE 1000R, New York, NY, USA) was used for the application of 16 kV to the polymer solution.

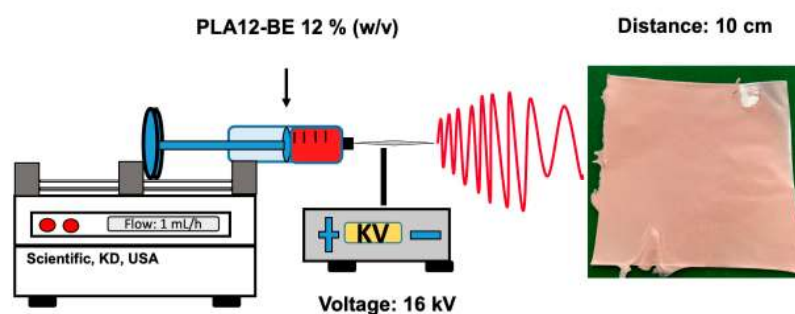


Figure 1. Schematic diagram of the production of PLA12-BE12 nanofibrous film by electrospinning technique.

2.5. Physico-Chemical and Mechanical Characteristics of Fibrous Film

2.5.1. Scanning Electron Microscopy (SEM)

Once the fibrous film nanofibers films were obtained—PLA films of 2, 4, 6, 8, 10, and 12% (*w/v*) concentrations and the combinations that result in the best morphology with BE 8, 10, and 12% (*w/v*) at different concentrations—morphological characterization was performed using a scanning electron microscope (JEOL 5410LV, Pleasanton, CA, USA). For the assay, the samples were coated with a gold film, and a 20 kV intensity electron beam was used at high vacuum with 1000 and 5000 $\times$ magnifications. The fiber sizes of PLA and PLA-BE fibrous films were determined from SEM micrographs using ImageJ version 1.34 e Software (NIH, Bethesda, MD, USA). A total of 100 nanofibers were randomly selected, and their diameters were measured in nanometers (nm). The average diameter and standard deviation were calculated to determine the polydispersity index (PDI) using the following equation:

$$PDI = \frac{\sigma}{\bar{X}} \quad (3)$$

where

σ is the standard deviation of the nanofiber diameters;
 $\bar{X}$ is the average diameter of the nanofibers.

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectra were obtained using a Perkin Elmer Frontier model, where the attenuated total reflectance (ATR) technique was used for the films obtained, and the spectrum was measured between 4000 and 500 cm^{-1} in transmittance mode.

2.5.3. Mechanical Properties

The elastic modulus (EY), elongation at break (EB), and tensile strength (TS) of the PLA and PLA-BE fibrous film were measured by stress versus strain curves using a mechanical testing machine (ElectroForce 5110, TA Instruments, New Caste, DE, USA) [60]. It was equipped with a 200N load cell and a crosshead rate of 1 mm/s until they reached the point of failure and WinTest 7 software to analyze the results. The nanofibers were cut into strip-like shapes measuring 10 mm length by 5 mm in width with a range of 0.1–0.15 mm of thickness. Determinations were carried out at least five times for each sample.

2.6. Functional Properties of the Color Indicator Fibrous Film

2.6.1. Color

The color of the films was measured using tristimulus colorimetry within CIE $L^*a^*b^*$ color space, with the use of a colorimeter (HunterLab MiniScan, Resto, VA, USA). The L^* parameter corresponded to lightness, the a^* parameter to red/green, and b^* to yellow/blue in reflectance mode with a port opening of 0.5 cm. The calibration was according to the manufacturer's manual. The color difference (ΔE) was calculated by Equation (3) [16] as follows:

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (4)$$

where L_0^* , a_0^* , and b_0^* are the color values of the PLA12 film, and L^* , a^* , b^* are the color values of the film after adding BE (8, 10, and 12% *w/v*).

2.6.2. Ammonia Gas Sensitivity

To investigate the indicator film color responsiveness to ammonia gas, were carried out using the method described by Li [17] with some modifications. First, indicator films based on PLA12-BE12 nanofibers of a size of 1.5 cm by 1.5 cm were obtained, which were placed in a covered Petri dish and then 15 mL of NH_4OH solution was added. The indicator film was exposed to a headspace reaction time of 10 and 20 min with NH_4OH solutions of concentration 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1 M in a sealed system. The film's color values before and after reacting were recorded by the method described above. The color

difference (ΔE) was calculated by Equation (2) described above, and the color change (ΔL , Δa , Δb) was calculated as follows:

$$\begin{aligned}\Delta L &= |L^* - L0^*| \\ \Delta a &= |a^* - a0^*| \\ \Delta b &= |b^* - b0^*|\end{aligned}\quad (5)$$

where $L0^*$, $a0^*$, and $b0^*$ are the initial color values of the film in a petri dish headspace containing 0 M ammonia solution, and L^* , a^* , b^* are the color values of the film after reacting with ammonia. Measurement was performed at 25 °C. Determinations were carried out in triplicate.

2.6.3. Antioxidant Activity

Antioxidant capacity was determined using the method outlined by Duan [61] with some modifications. This capacity was characterized by the scavenging rate of ABTS, DPPH• radicals expressed in % of inhibition, and FRAP in $\mu\text{M TE/g}$ of fiber. A 0.05 g sample of the fibrous film was placed in a conical 50 mL plastic tube with 5 mL of deionized water. The nanofibrous film in water was agitated for 15 min and subsequently centrifuged (Thermo Fisher Scientific, Asheville, NC, USA) for 10 min at 8170 g. Subsequently, an aliquot of the liquid medium containing the nanofibrous film was taken at different reaction times: 0.5 h, 1 h, and 1.5 h. The collected samples were processed according to the protocol described in the antioxidant capacity section for each assay (23.3, 23.4, and 23.5).

2.6.4. Reusability

The reusability of the color-indicating fibrous film was assessed following the methodology outlined by Li [17] with minor modifications. This evaluation involved subjecting the film to alternating conditions of a highly basic environment using ammonium hydroxide (pH 13.8) and an extremely acidic atmosphere generated by acetic acid (pH 2.5).

First, the 1.5 cm $\times$ 1.5 cm fibrous film indicator of PLA12-BE12 was placed in a covered petri dish containing 15 mL of the ammonium hydroxide solution for 30 s, and immediate measurements of the color values (L^* , a^* , b^*) were analyzed. And subsequently, the same indicator fibrous film was evaluated by the same process in an acetic acid atmosphere containing a 15 mL solution. The same evaluation was carried out for a total of four cycles. The measurement was performed at 25 °C. Determinations were carried out in triplicate.

2.6.5. Color Stability

The stability color of the PLA-BE fibrous film was determined during storage for 30 days at 25 °C and 4 °C. To explore the color stability of the PLA12-BE12 indicator, the first batch of 1.5 cm $\times$ 1.5 cm indicators were sealed in a petri dish and maintained at 25 °C and 30%–40% relative humidity in an oven (Thermo Fisher Scientific). The second batch was placed in refrigeration (Frigidaire, model GLFC1526FW, Mississauga, ON, Canada) equipment at 4 °C and 50%–60% relative humidity. The films color values and total color change values (ΔE) were analyzed as described above. The Chroma (C) (Equation (6)) and Hue angle (H) (Equation (7)) were calculated for 2 days each and performed in triplicate for a month as follows [17,62]:

$$C = \sqrt{[a^{*2} + b^{*2}]} \quad (6)$$

$$H = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (7)$$

2.7. Statistical Analysis

All measurement were carried out in triplicate and expressed as the mean value $\pm$ standard deviations. Data were analyzed by a one-way analysis of variance (ANOVA) and Tuckey comparison tests, with a significance level of $p < 0.05$ using NCSS 2023 (LLC,

Kaysville, UT, USA) software. Image J software (LOCI, University of Wisconsin) was used for fiber size measurement. All figures were processed using Origin 7.0 software (Origin Laboratories).

3. Results and Discussion

3.1. Characterization of Betalain Content and Antioxidant Activity

3.1.1. Photometric Quantification of Betalains

Table 1 presents the betalain content of the pitaya extract. These values were similar to those obtained by Castro [42], who reported values of 2.54 mg/g for the same fruit. In species such as *Stenocereus pruinosus* and *Stenocereus stellatus*, lower values of betalains of 0.559 mg/g and 0.571 mg/g, respectively, have been reported [63]. The betalain content depends on the cultivar or variety, maturity, climate, and origin of the fruit [64].

Table 1. Phytochemical content and antioxidant capacity by ABTS, DPPH• and FRAP assays on extracts of pitaya (*Stenocereus thurberi*) (BE).

Phytochemical		
Betalains (mg/g)	3.70 ± 0.37	
Phenols (mg GAE/g)	24.53 ± 0.45	
Method	(a) µM TE/g	(b) IC ₅₀ (mg/mL)
ABTS	1756.69 ± 41.72	0.165
DPPH	964.02 ± 7.51	1.019
FRAP	439.35 ± 3.36	-

Results are shown in (a) Trolox equivalents. (b) IC₅₀ is the concentration at which 50% of the radical is reduced by the pitaya extract (*Stenocereus thurberi*) (BE). Data (mean ± SD) are from three independent experiments.

Perez [65] analyzed the betalain content in *Stenocereus stellatus* pitayas and compared it with 32 samples of other cactus fruits to classify the fruits as poor, good, and excellent sources of betalains. They determined three confidence intervals for the total betalain content as low (lower 1208 µg/g), medium (2935–3288 µg/g), and high (4488–9248 µg/g). So considering this classification, the betalain content in *Stenocereus thurberi* pitaya pulps is defined as a good source of betalain.

3.1.2. Total Phenols

Phenolic compounds are the most abundant secondary metabolites in plants. Their chemical structure consists of an aromatic ring and one or more hydroxyl substituents. These substances are considered of great importance as they prevent different processes related to oxidative stress [66]. As indicated in Table 1, the total phenol content of BE was 24.53 ± 0.45 mg GAE/g of dry sample. In the same genus and species, values of 11.3 mg GAE/g fresh weight, 13.89 GAE/g dry weight, and 1.9 mg GAE/g fresh weight have been previously reported [25,42,67]. In other species, such as *S. pironosus*, lower phenolic content values have been reported in red and orange variety fruits, with 0.07 mg GAE/g and 0.05 mg GAE/g dry weight, respectively [68]. In *S. stellatus* species, great variability in phenol content has been reported in red (mg GAE/g fresh weight) (0.1 to 13.8), yellow (3.7 to 21.2), white (0.1 to 23.9), orange (0.15), and purple (15.5) pitaya [65,69–71]. These differences can be attributed to species, ecotype, and different ways of extraction [72]. In an investigation conducted by Muy [73], it was noted that harvesting pitaya fruit before 42 days post-flowering prevents the fruit from achieving physiological maturity, resulting in a diminished production of certain phytochemical compounds.

3.1.3. Antioxidant Capacity and Antiradical Capacity by ABTS•+, DPPH•, and FRAP Methods

Fruits of the cacti of the genus *Stenocereus* exhibit a high antioxidant potential due to their content of betalains and various phenolic compounds with the ability to donate an electron or hydrogen atom to neutralize oxidizing compounds [74]. In Table 1, the

antioxidant capacity of BE, which was evaluated by ABTS•+, DPPH•, and FRAP assays can be observed. The results are expressed in $\mu\text{M TE/g}$ and % inhibition and of dry samples. The results indicate that BE exhibits high antioxidant capacity. When comparing these findings with previously reported values for the same fruit using the ABTS•+ assay, the antioxidant capacity varied according to the fruit's variety and color. The measured values were $32 \mu\text{M TE/g}$ for red, $55 \mu\text{M TE/g}$ for orange, $32 \mu\text{M TE/g}$ for yellow, and $30 \mu\text{M TE/g}$ for dark red fruits, which were all on a wet basis [25]. A similar value of $1130.6211 \mu\text{M TE/g}$ was noted by Figueroa [27]. In other fruits, *Stenocereus pruinosus* had an antioxidant capacity with values of $740\text{--}1090 \mu\text{M TE/g}$ and *Stenocereus stellatus* from 57.2 to $128.6 \mu\text{M TE/g}$ [69,75]. Another cactus fruit that has been assessed using the ABTS•+ method is the prickly pear (*Opuntia ficus-indica*), which shows an antioxidant capacity in a range of $146.33\text{--}316.63 \mu\text{M TE/100 g}$ of dry samples [76].

The antioxidant capacity of the DPPH• radical in BE is greater than in the red pitaya (*Hylocereus polyrhizus*), and the value obtained was $1.664 \mu\text{M TE/g}$ [27]. In the same fruit, Smeriglio et al. [76], reported values from 2.20 to $3.08 \mu\text{M TE/g}$. Additionally, a recent report by Martínez et al. [24] showed the antioxidant activity by FRAP assay of yellow and red prickly pears (*Opuntia ficus-indica*; 4.80 and 25.32 TE/mL , respectively), and yellow and red pitaya *S. pruinosus* (8.65 and 27.44 TE/mL , respectively). However, the values that we showed were higher in BE and possibly associated with the species of the fruit that we studied.

On the other hand, the concentration at which 50% of the ABTS•+ and DPPH• radicals are reduced is known as IC₅₀. Barkociova et al. [77], reported IC₅₀ values in the DPPH• radical in 25 cactaceous plants, obtaining values in DPPH• radicals ranging from 22.47 mg/mL to 776.81 mg/mL , which are higher in comparison with that of BE obtained in the present study. On the other hand, Alkayari et al. [78], obtained (*Beta vulgaris*) a $0.84 \pm 0.04 \text{ mg/mL}$ value and IC₅₀ value in the ABTS•+ radical of $1.46 \pm 0.42 \text{ mg/mL}$ in red beet extract. These values are similar to those obtained in the present study, which are comparable with ascorbic acid (IC₅₀ 0.02 mg/mL) that is considered to be a potent antioxidant [77].

The literature indicates that each radical exhibits different affinities for the sample, which leads to variations in the measured antiradical activity. It is important to note that depending on its reaction mechanism, the ABTS•+ radical shows a higher affinity for reacting with both hydrophilic and lipophilic compounds, particularly those involved in hydrogen atom donation [53,57]. In contrast, DPPH has a lower affinity for compounds containing only hydroxyl groups on aromatic rings but a higher affinity for lipophilic compounds [79]. Additionally, some antioxidants function by reducing oxidizing compounds, such as ferric ions (Fe^{3+}), to their more stable ferrous form (Fe^{2+}). Methods such as DPPH, ABTS, and FRAP measure distinct antioxidant mechanisms, providing a more comprehensive assessment of the overall antioxidant capacity of plant extracts.

Centered on their structure, betalains are molecules with high antioxidant activity and are able to donate electrons and protons due to the presence of the 5-O-glucosyl-catechol system and 1,7 diazaheptamethine [80]. BE showed better antioxidant activity in electron and hydrogen transfer-based assays (ABTS•+, DPPH•), followed by the electron transfer-based assay (FRAP). Given that pitaya fruits have a higher concentration of betaxanthins, it is reasonable to hypothesize that these compounds contribute to the elevated antioxidant activity observed, particularly when comparing these findings with the results of the phytochemical characterization.

The differences in free radical scavenging activities between betaxanthins and betacyanins can be attributed to the unique physicochemical properties of these two classes of betalains. Notably, betanin—the predominant betacyanin found in pitaya pulp extracts—demonstrates greater hydrophilicity than indicaxanthin. Additionally, betanin has the capacity to accept 14 hydrogen bonds, while indicaxanthin can accept only 7 [76].

3.1.4. pH-Sensitive Property

Figure 2 shows the effect of pH on BE and its UV-vis absorption spectrum (a,b) and color variations (c). Due to the conjugated dienes of the 1,7-diazaheptamethine structure, betalains exhibit absorption in both UV and visible regions [81]. In Figure 2a, a high-definition peak is observed between 400 and 500 nm. According to Khan et al. [82], this peak corresponds to the absorption of betaxanthins, which exhibit a maximum absorption at 471 nm. Additionally, a lower-definition peak is observed after 500 nm corresponding to the absorption of betacyanins, which Martinez et al. [83] have an absorption maximum of 541 nm. These findings align with the quantification of betalains in the pitaya fruit used in the present study.

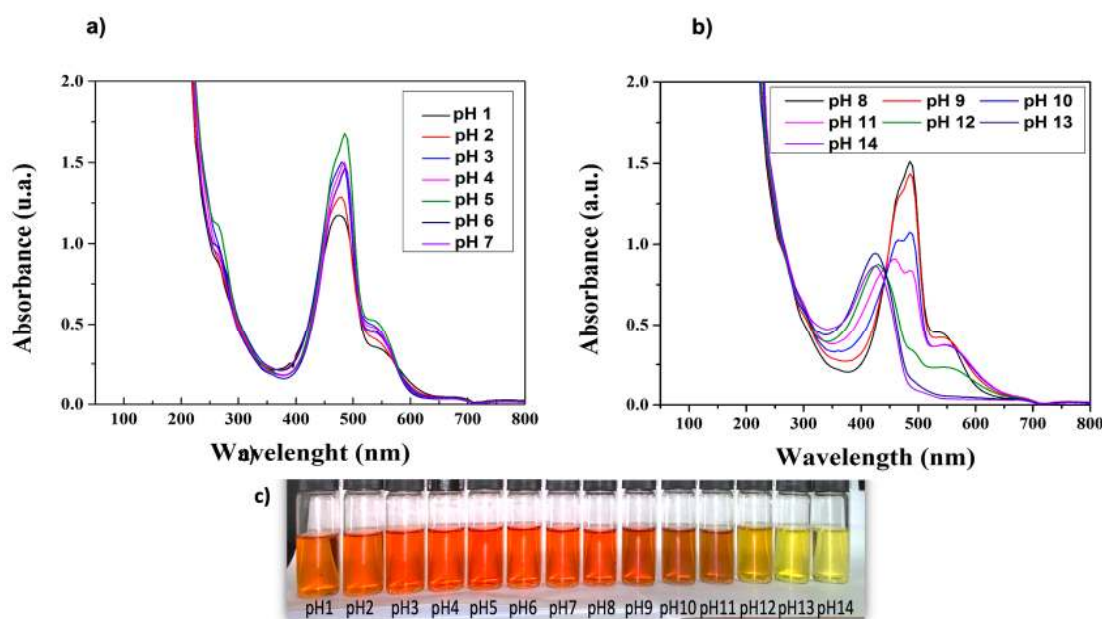


Figure 2. UV-vis spectra (a,b) and photograph of color variations (c) in pH 1–14 range of betalain solutions.

The absorption peak decreases as the pH value gradually decreases. It has been reported in the literature that at low pH values, an isomerization of C15 of betanin and betanidin occurs into isobetanin and isobetanidin, respectively [80]. This could confirm that betalains are stable at pH 3–7 [64].

Therefore, under alkaline conditions (Figure 2b), the color of the solutions is orange (pH 8–11) and subsequently turns yellow (pH 12–14) (Figure 2c). The intensities of both peaks decreased when the pH exceeded seven. The peak observed at 540 nm, characteristic of betacyanins, began to disappear as the pH approached 10, suggesting that betacyanins in the extract are gradually degraded in alkaline solutions. At pH values greater than eight, hypochromic shifts were observed, indicating the release of betalamic acid. An alkaline pH induces aldimine bond hydrolysis and generates betalamic acid and absorbs at 410 nm, and a colorless cyclo-DOPA-5-O- β -glucoside [84].

3.2. Characterization of the Electrospinning PLA-BET Solutions

3.2.1. Viscosity

Table 2 summarizes the viscosity and electrical conductivity of the corresponding electrospinning solutions. As the BE content increased from 8% to 12% (*w/v*), the viscosity of the solutions increased significantly ($p < 0.05$). According to Duan [61], increasing the viscosity of polymer solutions generates an increase in the viscoelasticity of the polymer jet, which in turn hinders the stretching of the fibers and causes an increase in the diameter of the fibers. It has been documented [47] that achieving extremely low viscosity values of PLA

solutions hinders the formation of continuous fibers and instead favors the development of irregular beaded structures or particles. The hydrogen bonding between PLA and BE is a factor that contributes to the increase in solution viscosity and the larger diameter of the nanofibers formed. This behavior was previously reported by Xu [85] in PLA polymeric solutions with anthocyanin extract leading to an increase in the chain-to-chain entanglement capacity.

Table 2. Viscosity and electrical conductivity of electrospinning solutions.

Electrospinning Solutions PLA-BE % (w/v)	Viscosity (mPa·s)	Electrical Conductivity ($\mu\text{S}/\text{cm}$)
PLA12	241.29 ± 18.59^a	3.56 ± 0.11^a
PLA12-BE8	376.65 ± 5.08^b	141.06 ± 0.40^b
PLA12-BE10	402.55 ± 4.06^b	152.30 ± 1.73^c
PLA12-BE12	491.35 ± 29.77^c	168.16 ± 2.48^d

Data with different letters on the same column refer to significant differences ($p < 0.05$). Data (mean $\pm$ SD) are from three independent experiments.

3.2.2. Electrical Conductivity

The electrical conductivity of polymeric solutions influences the morphology of the nanofibers, which must possess the appropriate conductivity for establishing the repulsion force to overcome the surface tension of the drop, promoting stable jets [86]. It has been previously reported that PLA has intrinsically low levels of conductivity [47], which would seem to indicate that the number of electrical charges found on the surface of the polymer solution controls its tendency to form nanofibers or nanoparticles. The PLA electrospinning solution conductivity was $3.56 \pm 0.11 \mu\text{S}/\text{cm}$, which was attributed to the fact that PLA is a non-electrolyte biopolymer [61]. The significant increase ($p < 0.05$) in the conductivity of spinning solutions after blending with BE can be attributed to the presence of the ionized forms of betalains. The pK of betalains is lower than the pH of the polymeric solutions (6.5) [87], which are in ionic form in solution and consequently influence the conductivity. In conclusion, for both polymer characteristics, increased PLA concentration and betalain extract (BE) addition raise the viscosity through enhanced chain entanglement while also increasing conductivity due to the ionized betalains. These effects influence jet stability and fiber formation, requiring careful optimization for uniform nanofibers.

3.3. Characteristics of the Fibrous Film

3.3.1. Scanning Electron Microscopy

Micrographs of SEM images on the surface of the fibrous film are shown in Figure 3, assessing different polymer concentrations captured at $1000\times$ and $5000\times$ magnifications. This was performed to understand at which concentration the formation of fibers is favored. The results show that particles were collected using a PLA 2% (w/v) solution. This was due to the low density and viscosity of the polymer solution, which in turn represents a lower level of crosslinking of the polymer chain [47,88,89].

This behavior is similar to that previously reported by Ibili [90], where at the same PLA polymer concentration, they obtained particles. On increasing the polymer concentration to 4%–6% (w/v), more nanofibers were produced even though a lot of beads defects were still present, as show in Figure 3b,c. The PLA nanofibers with 8% (w/v) (Figure 3d) also contained some beads. Above 10% (w/v) (Figure 3e), nanofibers with less defects could be collected, and 12% (w/v) displayed smooth, uniform, and defect-free nanofibers. Figure 3f depicts when the solution concentration increased to the same as the viscosity of the solution. The change in fiber morphology with increasing concentration can be attributed to the competition between the surface tension and the viscosity of the fibers. An increase in polymer concentration causes greater crosslinking of the chains, leading to increases in the viscosity of the solution and thus an increase in the viscoelastic force, which counteracts the Coulombic stretching force and thus results in nanofibers with fewer microspheres [91,92].

Similarly, it is important that the polymer solution has adequate electrical conductivity to establish a charge repulsion force that overcomes the surface tension of the droplet to form the fiber. Otherwise, it will be difficult to form uniform fibers [93]. The results indicate that nanofiber films with fewer defects and improved morphology were obtained at a PLA concentration of 8%–12% (*w/v*). Therefore, the effects of incorporating BE at concentrations of 8%, 10%, and 12% (*w/v*) were analyzed.

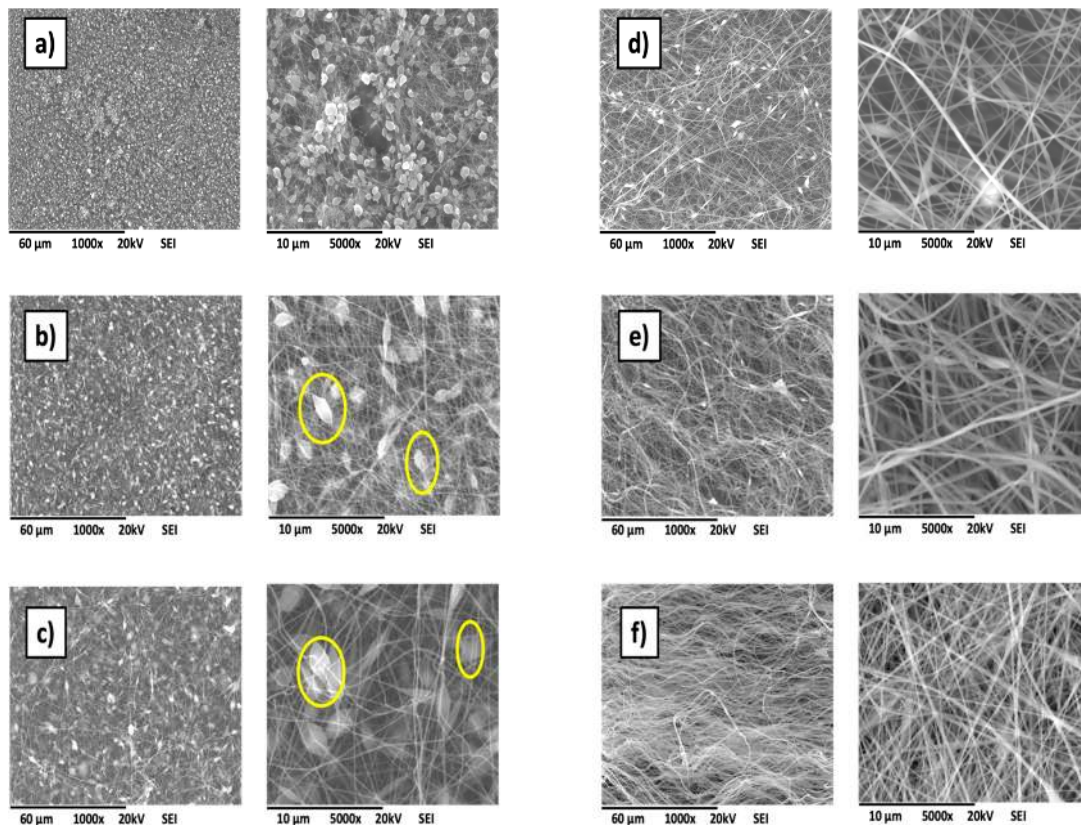


Figure 3. SEM micrographs of PLA films at 2 (a), 4 (b), 6 (c), 8 (d), 10 (e), and 12 (f) % (*w/v*) fiber. Magnifications images are 1000 $\times$ and 5000 $\times$. Yellow circles show fibers defects.

Figures 4–6 present micrographs illustrating the morphology characteristics of PLA 8, 10, and 12% (*w/v*) with the addition of BE at 8, 10, and 12% (*w/v*) and nanofibers films at 1000 $\times$ and 5000 $\times$ magnifications. It was observed that the addition of the extract to PLA 8 and 10% (*w/v*) films resulted in the formation of fibers with small numbers of beads (which are indicated). The addition of BE at 8 and 10% (*w/v*) did not completely reduce defects in the nanofibers, and it was found that the most uniform nanofibers are formed from PLA 12% (*w/v*) with BE 12% (*w/v*).

This phenomenon can be attributed to the increased conductivity of the polymer solutions, which is caused by the charge provided by BE to the nanofibers. To investigate this effect, a study of nanofiber diameter was conducted. The results, shown in Figure 7, include both the fiber diameter measurements and the distribution histogram. The data reveal an increase in fiber diameter with higher BE concentrations. For PLA 12% (*w/v*), PLA12-BE 8% (*w/v*), PLA12-BE 10% (*w/v*), and PLA12-BE 12% (*w/v*), the diameters were 323 ± 74 nm, 370 ± 76 nm, 500 ± 120 nm, and 657 ± 99 nm, respectively. This increase in diameter is likely due to the rise in solution viscosity, which makes it more difficult for the polymer solution to exit through the needle and stretch, resulting in thicker fibers [17]. A similar effect was reported by Palak [89], who developed a freshness indicator using a PLA matrix with anthocyanin extract, achieving fiber diameters ranging from 327 ± 101 nm to 629 ± 86 nm. Similarly, Li [17] reported slightly larger diameters. These differences could

be attributed to variations in the electrospinning equipment settings or the nature of the polymer used. Table 3 summarizes the nanofiber characteristics, where the polydispersity index (PDI) values provide insights into polymer dispersion. A PDI value close to one indicates a highly polydisperse polymer, while a value near zero suggests a monodisperse polymer [58].

Table 3. Electrospun fibrous film diameter size and polydispersity index.

Electrospinning Solutions PLA-BE % (w/v)	Average Diameter (nm)	Polydispersity Index
PLA12	313 ± 74 nm	0.236
PLA12-BE8	370 ± 75 nm	0.205
PLA12-BE10	500 ± 120 nm	0.241
PLA12-BE12	657 ± 99 nm	0.150

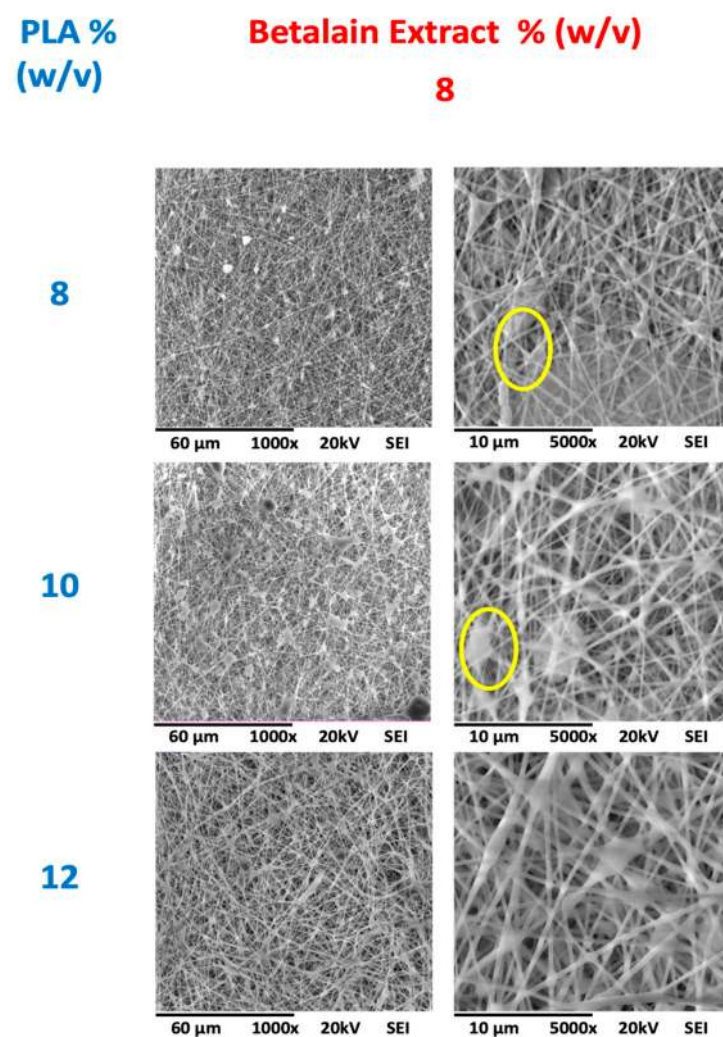


Figure 4. SEM micrographs of PLA 8, 10, and 12% (w/v) fibrous film incorporated with BE at 8% (w/v). The magnification images are 1000× and 5000×. Yellow circles show fibers defects.

A higher polydispersity index typically reflects a wider range of molecular weights among the resulting nanofibers. According to Table 2, increases in viscosity and electrical conductivity resulted in the formation of larger diameter nanofibers. These findings are consistent with those previously reported [46,59,81,89] on the formation of nanofibers as natural pH indicators. The polydispersity index (PDI) strongly influences nanofiber formation by reflecting the uniformity of molecular weight distribution in polymer solutions. In

Table 3, lower PDI values, such as 0.150 for PLA12-BE12, are associated with more uniform nanofiber diameters, suggesting that reduced molecular weight variability promotes consistent fiber morphology. Conversely, higher PDI values, like 0.241 for PLA12-BE10, may lead to broader diameter distributions due to uneven polymer chain lengths, highlighting the importance of PDI in achieving homogeneous nanofibrous structures.

In summary, PLA 12-BET 12 has uniform beadless nanofibers, which could mean greater precision, stability, and sensitivity for potential use as an indicator of food freshness.

Figure 8 illustrates the physical appearance and color values of PLA 8, 10, and 12% (w/v) with the addition of BE at 8, 10, and 12% (w/v). In general, L^* values range from 0 (black) to 100 (white), a^* values range from -100 (greenness) to 100 (redness), and b^* values range from -100 (blueness) to 100 (yellowness) [94]. The PLA film was colorless with the highest L^* value (99.05 ± 0.22) but the lowest with a^* (-0.11 ± 0.01) and b^* (-0.38 ± 0.04) values. PLA-BE nanofibers films showed a pink color due to the presence of betacyanins in the films. A similar color was observed when pitaya peel extract was added to glucomannan-PVA film [95], starch-PVA film [33], and chitosan-starch film [96] by the casting technique.

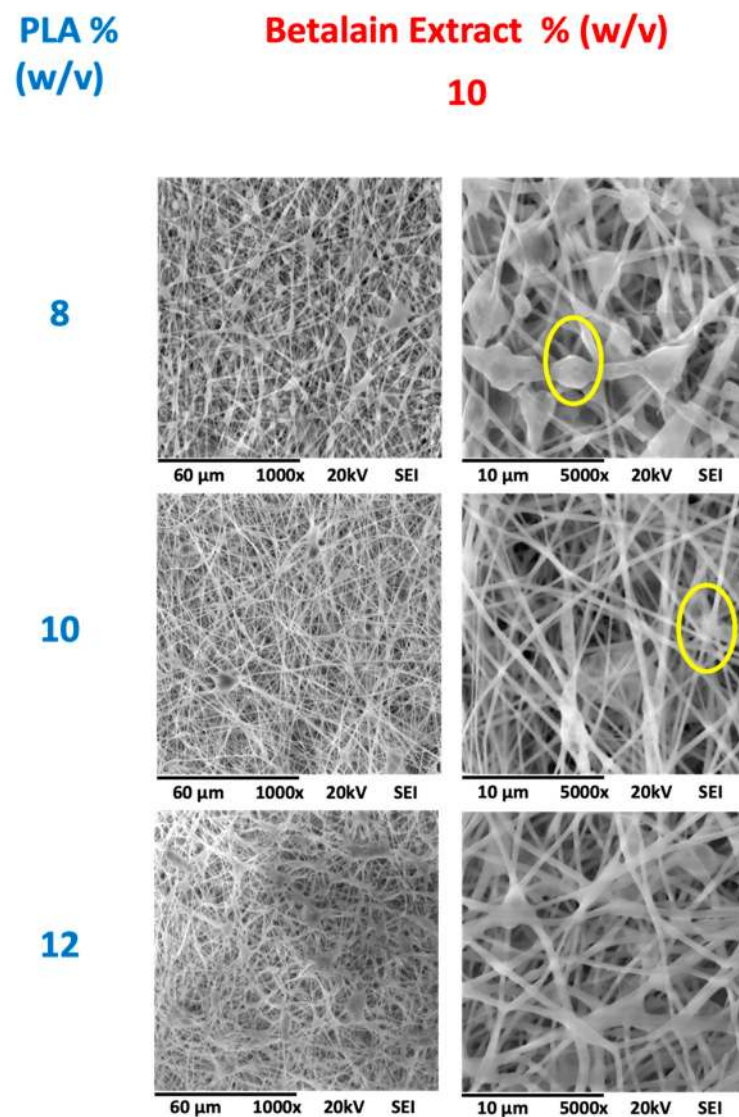


Figure 5. SEM micrographs of PLA 8, 10, and 12% (w/v) film incorporated with BE at 10% (w/v). The magnification images are $1000\times$ and $5000\times$. Yellow circles show fibers defects.

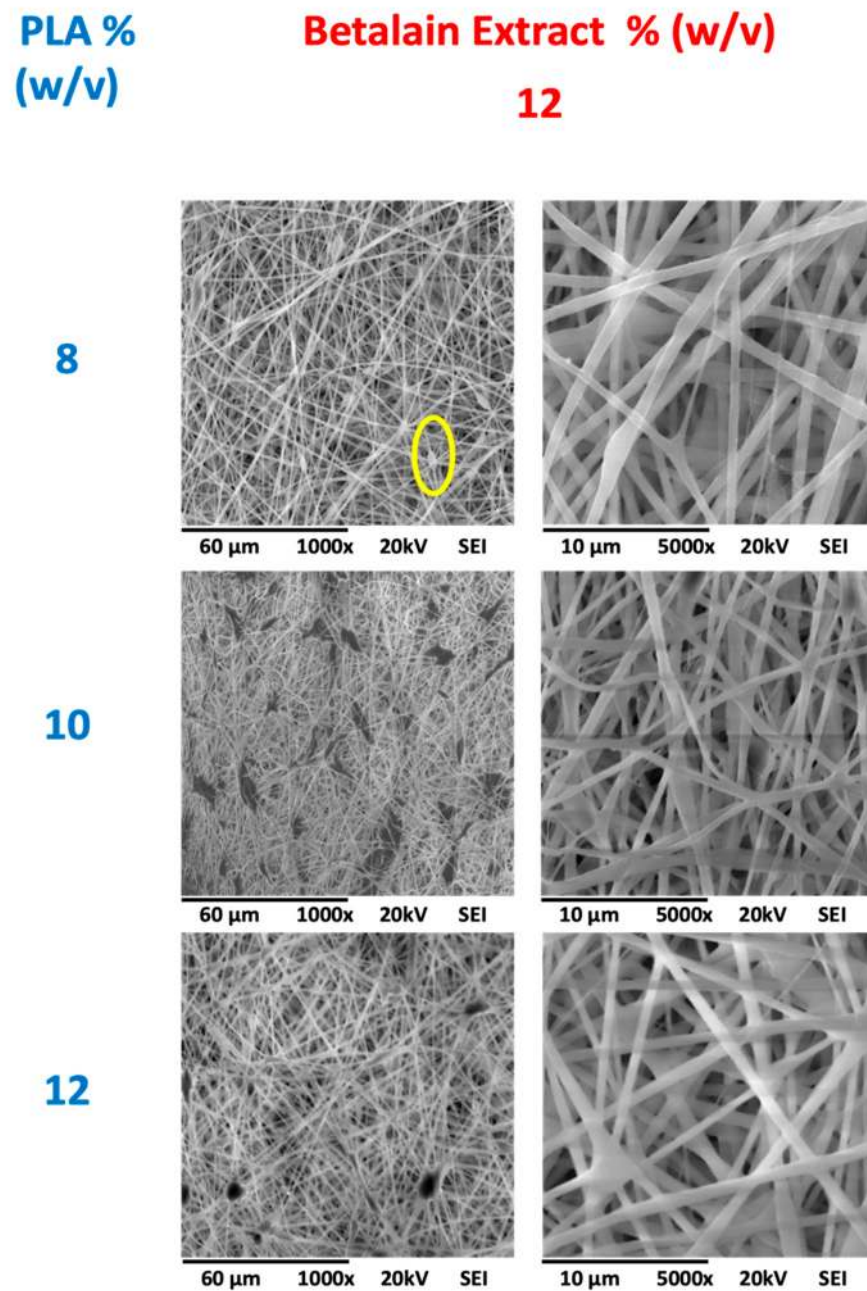


Figure 6. SEM micrographs of PLA 8, 10, and 12% (*w/v*) fibrous films incorporated with BE at 12% (*w/v*). The magnification images are 1000 $\times$ and 5000 $\times$. Yellow circles show fibers defects.

PLA-BE fibrous films showed lower L^* (81.31 ± 0.84 – 87.79 ± 0.13) but higher a^* (10.99 ± 0.08 – 16.60 ± 0.22), b^* (6.05 ± 0.04 – 13.66 ± 0.35), and ΔE (14.12 ± 0.14 – 23.84 ± 0.24) values than the PLA fibrous films. As the BE content increased, the a^* , b^* , and ΔE values increased proportionally, which is consistent with the gradually more intense color of the films. Similar behavior was previously reported by Qin [33] and indicates that the incorporation of pitaya peel extract caused a significant ($p < 0.05$) increase in a^* , b^* , and ΔE values in starch-PVA films.

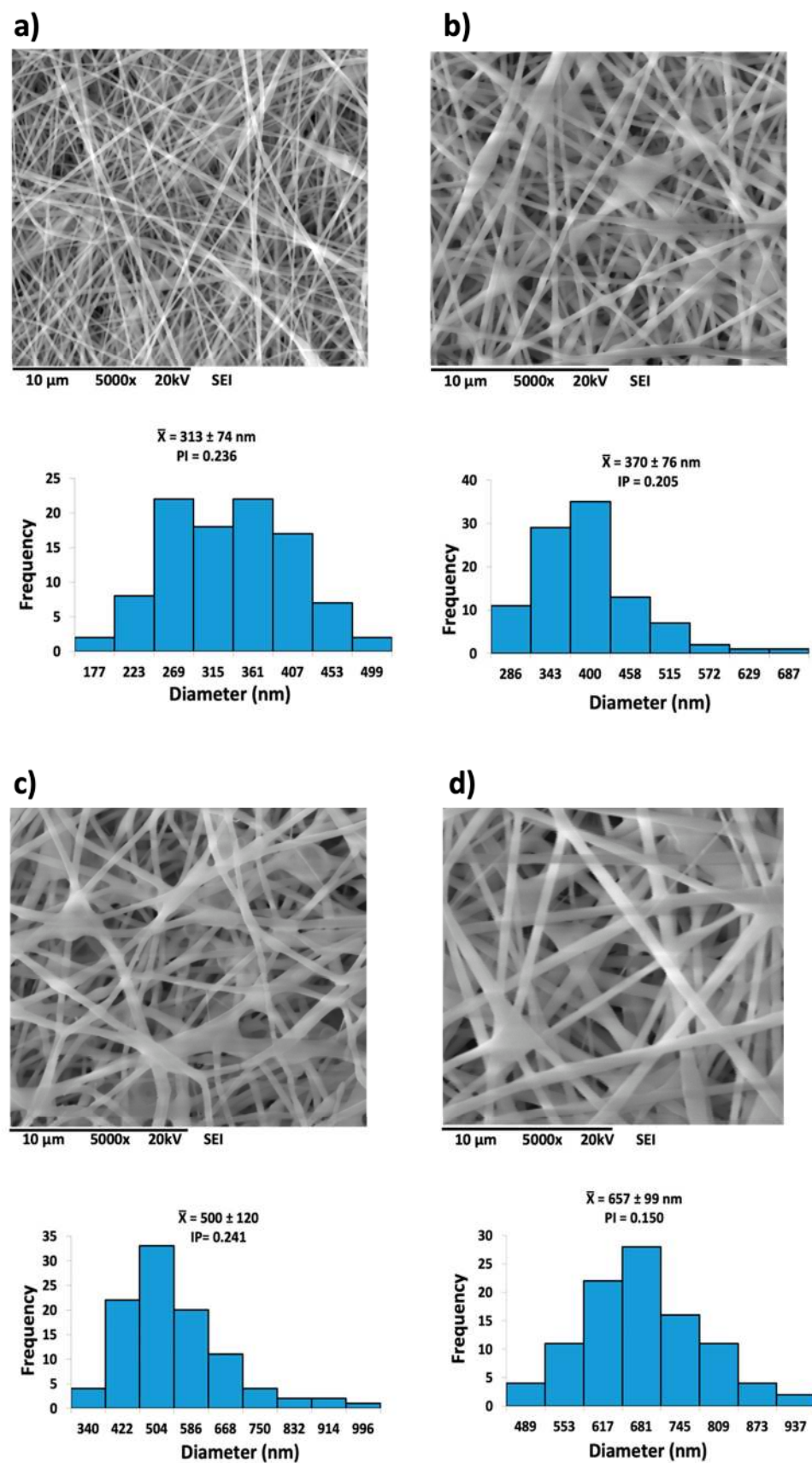


Figure 7. SEM micrographs of PLA 12% (a), PLA12%-BE8% (b), PLA12%-BE10% (c), and PA12%-BE12% (d) (*w/v*) fibrous films and their corresponding histograms. The magnification images are 5000 $\times$.

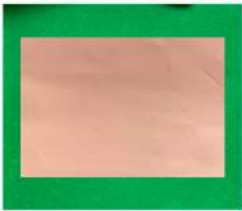
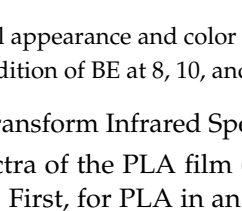
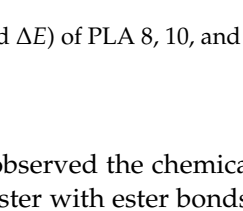
		Betalain Extract % (w/v)					
		8 _a		10 _b		12 _c	
PLA % (w/v)	8 _A		L 87.39 ± 0.16 a 10.99 ± 0.08 b 6.05 ± 0.04 ΔE 14.12 ± 0.14		L 85.60 ± 0.32 a 11.68 ± 0.22 b 7.44 ± 0.13 ΔE 16.30 ± 0.30		L 82.71 ± 0.61 a 13.16 ± 0.34 b 10.76 ± 0.09 ΔE 20.44 ± 0.49
			L 86.34 ± 0.31 a 10.50 ± 0.37 b 9.33 ± 0.11 ΔE 16.64 ± 0.24		L 85.54 ± 1.05 a 14.28 ± 0.20 b 12.69 ± 0.14 ΔE 21.04 ± 0.81		L 82.36 ± 0.84 a 15.93 ± 0.14 b 13.89 ± 0.35 ΔE 22.68 ± 0.61
			L 87.79 ± 0.13 a 11.23 ± 0.13 b 10.37 ± 0.06 ΔE 16.28 ± 0.10		L 86.39 ± 0.29 a 12.33 ± 0.08 b 12.25 ± 0.07 ΔE 18.57 ± 0.19		L 81.73 ± 0.45 a 16.60 ± 0.22 b 11.52 ± 0.12 ΔE 23.84 ± 0.24
			L 87.79 ± 0.13 a 11.23 ± 0.13 b 10.37 ± 0.06 ΔE 16.28 ± 0.10		L 86.39 ± 0.29 a 12.33 ± 0.08 b 12.25 ± 0.07 ΔE 18.57 ± 0.19		L 81.73 ± 0.45 a 16.60 ± 0.22 b 11.52 ± 0.12 ΔE 23.84 ± 0.24

Figure 8. Physical appearance and color value (L , a^* , b^* and ΔE) of PLA 8, 10, and 12% (w/v) fibrous films with the addition of BE at 8, 10, and 12% (w/v).

3.3.2. Fourier Transform Infrared Spectroscopy

In the spectra of the PLA film (Figure 9), we observed the chemical nature groups of the polymer. First, for PLA in an aliphatic polyester with ester bonds, the nanofibers showed the carbonyl (C=O) stretching absorption band at a wavelength of 1762 cm^{-1} . The asymmetric stretches of the C-O-C bond were present at 1090 cm^{-1} . The signals for the asymmetric and symmetric stretches of the -CH₃ group were found at 2996 and 2946 cm^{-1} , respectively, and the bands for the asymmetric and symmetric C-H strain vibrations in the same group were found at 1456 – 1368 cm^{-1} , and the C-O-C stretch vibration was at 1090 cm^{-1} [57].

BE exhibited a band at 3267 cm^{-1} , which was attributed to the stretching vibrations of hydroxyl groups (O-H) in the betalains structure and phenolic compounds, as previously reported for this type of extract [27,52]. A stretching band for the C=O bond was observed at 1712 cm^{-1} . Additionally, a band at 1591 cm^{-1} corresponded to the stretching vibrations of C=N and C=C bonds within the aromatic ring of the molecule. Bands associated with asymmetric stretches of the C-O-C bond were observed between 1405 and 1360 cm^{-1} , while a distinct band appeared at 1016 cm^{-1} .

Specifically, the band of the carbonyl group (C=O) present in PLA, BE and the nanofibrous film, specifically in BE, is located at 1712 cm^{-1} and shifted towards 1754 cm^{-1} in the film, at a higher wavenumber, indicating a higher energy bond. This variation is due to the effect of the addition of the extract in the PLA nanofiber (1762 cm^{-1}), affecting the polarization of the bond (carbon-oxygen) and leading to the formation of a hydrogen bond. It is important to note that a band at a wavelength of 1591 cm^{-1} was observed from BE. It is offset at 1586 cm^{-1} and is of lower intensity in the nanofiber film, which is attributed to the stretching of C=N at a lower wavenumber and generally indicates a decrease in the vibrational energy of the associated bond as a product to the formation of hydrogen bond. In general, the main functional groups of the materials and of the fibrous film were observed to be modified due to the interactions formed, giving signals mainly by the formation of hydrogen bond, which indicates that BE was successfully immobilized in the PLA12-BE12 fibrous film.

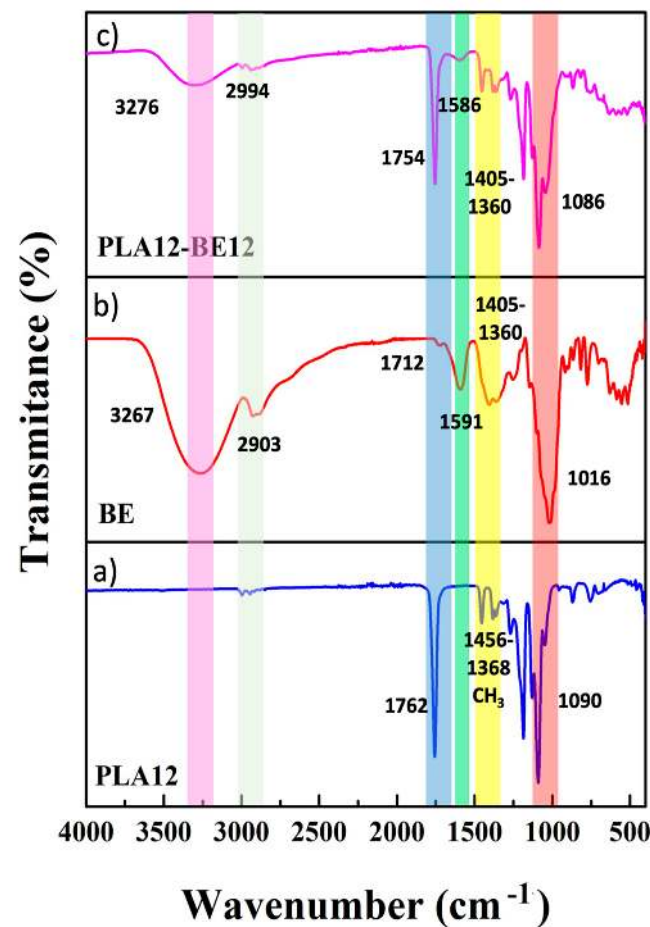


Figure 9. FTIR spectra of the fibrous film: PLA 12% (a), BE (b), PLA12-BE12 (c).

3.3.3. Mechanical Properties

The mechanical properties of electrospun nanofibers play an important role in determining their applications [83], and specifically food packaging films reflect their ability to maintain integrity and sustainability throughout the food supply chain [97]. The Young's modulus (YM), strain at break (SB) and tensile strength (TS) of the PLA and PLA-BE 8–12 (*w/v*) fibrous films were calculated by stress-strain curves, and properties data are shown in Table 4.

The YM, SB, and TS of PLA films were 15.91 ± 3.71 MPa, $100.68 \pm 7.02\%$, and 2.87 ± 0.27 MPa, respectively, which are similar to results with previous data in the literature [60,98]. After blending with BE (8% *w/v*), the flexibility decreased significantly ($p < 0.05$), and the YM and TS were enhanced.

Table 4. Mechanical properties of PLA and PLA-BE fibrous films.

Fibrous Film (% <i>w/v</i>)	Elastic Modulus (MPa)	Strain at Break (%)	Tensile Strength (MPa)
PLA12	15.91 ± 3.71^a	100.68 ± 7.02^a	2.87 ± 0.27^a
PLA12-BE8	25.44 ± 2.76^b	77.77 ± 5.11^c	3.09 ± 0.35^a
PLA12-BE10	28.94 ± 2.31^b	85.31 ± 8.16^b	3.53 ± 0.47^a
PLA12-BE12	11.87 ± 3.81^a	$67.44 \pm 4.43^{b,c}$	2.00 ± 0.14^b

Data with different letters on the same column refer to significant differences ($p \leq 0.05$). Data (mean $\pm$ SD) are from five independent experiments.

The reason for this observation could be due to the interaction of PLA with BE promoting the formation of hydrogen bridge bonds. This interaction enhances the density of

the cross-linking network with the biopolymer, resulting in improved mechanical properties that are observed in the spectroscopy analysis. However, conversely, such behavior limited the movements of the molecular chain segments, causing a reduction of SB [99]. On the other hand, Lui et al. [100] pointed out that the addition of phenolic compounds could hinder the chain-to-chain interactions in the film matrix and thus reduce flexibility. Such behavior has been previously reported in the elaboration of electrospun nanofiber films with anthocyanins [97]. When 10% (*w/v*) BE was added, the film's YM, TS, and SB increased and had no significant difference ($p > 0.05$). Then at 12% (*w/v*) BE, they disrupted the biopolymer network, leading to a significant ($p < 0.05$) decrease in the YM and TS and a not significant decrease ($p > 0.05$) in SB, in which betalains could promote a plasticizing effect. However, it is important to note that the addition of higher BE content is important to obtain better morphology and tones for a smart fibrous film. Similar behavior was observed in anthocyanin loaded with PLA/chitosan electrospun nanofiber film [85].

3.4. Functional Properties of the Color Indicator Fibrous Film

3.4.1. Ammonia Response of PLA-BET Fibrous Films

The behavior of the intelligent fibrous film indicator is displayed in Figure 10a, which detected changes gradually from red to yellow color, with increases in ammonia concentration indicating that the film is sensitive to ammonia. The coloration changes of the film after reaction with ammonia (ΔE , ΔL , Δa , Δb) can be seen in Figure 10b. The observed behavior in the nanofibers may be attributed to the diffusion of ammonia gas into the films, where it undergoes hydrolysis to produce hydroxyl ions, creating an alkaline environment within the indicator films [101]. This process involves the dissociation of NH_3 in water into NH_4^+ and OH^- ions, which contribute to the alkaline conditions observed in the films [102]. The total color difference index value, ΔE , is the index that objectively evaluates the nanofibers indicator film, which is defined in terms of the amount of color change that occurs in the indicator compared to its original color. However, the quantified definition for the human eye's ability to perceive ΔE is as follows (Table 5) [95].

Table 5. Relationship between observer judgment and color difference of ΔE .

ΔE Value by Equipment Measure	ΔE Value by Sensory Perceived
0.0–0.05	No color difference
0.5–1.0	Perceptible by experienced observers
1.0–2.0	Minimal color difference
2.0–4.0	Noticeable color difference
4.0–5.0	Significant color difference
>5.0	Color difference

Adapted from Klinke et al. [103].

Figure 10b shows that the light red color of the films faded when they were exposed to the ammonia solution for 10 min, discoloring to until reaching yellow tones in all different ammonia concentrations. Consequently, significant changes are observed ($p < 0.05$) in the parameters ΔE , ΔL , Δa and Δb , likewise a value of $\Delta E = 6.54$ (Figure 10b) is observed in relation to the first concentration of ammonia evaluated, and that in relation to the above-mentioned classification the color change is perceptible to the human eye at $\Delta E > 5.0$ [104]. Similarly, at the reaction time of 20 min (Figure 11a,b); significant changes ($p < 0.05$) were observed in the parameters ΔE , ΔL , Δa and Δb , likewise a value of $\Delta E = 8.60$ is observed, which is perceptible to the human eye, which indicates that the indicator fibrous film has an excellent response time. The color changes of the film are attributed to the degradation of betacyanins by hydrolysis in the aldimine bond, which generates betalamic acid which presents a yellow coloration and the CICLO-DOPA B glucoside molecule which turns out to be colorless.

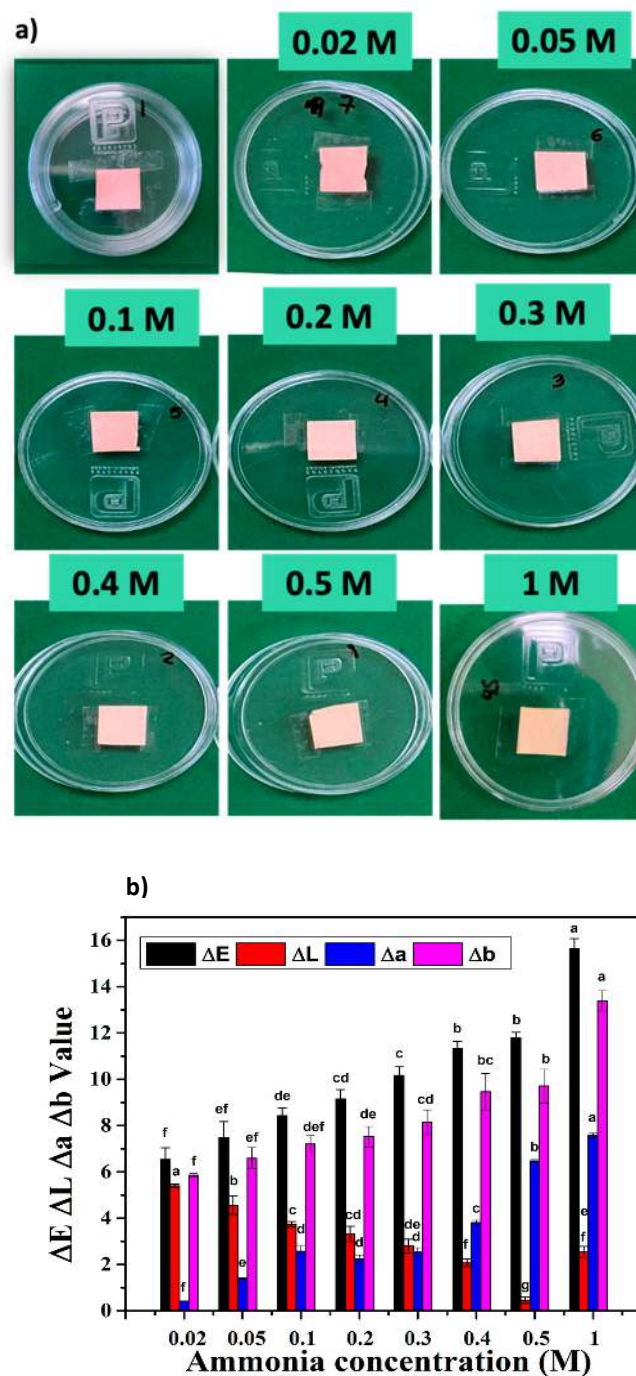


Figure 10. Color response of PLA12-BET12 indicator fibrous film at different ammonia concentrations: photographs after reaction with ammonia (a) and color values of the film after reaction with ammonia (ΔE , ΔL , Δa , Δb) (b) at 10 min of reaction. Data with different letters on the same column color refer to significant differences ($p < 0.05$). Data (mean $\pm$ SD) are from three independent experiments.

Several studies have reported the preparation of smart indicator films using betalain extract through the casting technique [33,101,105]. Although this method is simple, the resulting films have a dense structure, which limits their responsiveness and sensitivity. In contrast, films prepared using the electrospinning technique exhibit a high surface area and porosity [46,59,61,86,106–108]. These characteristics significantly increase the contact area between the active substance and the target molecule, thereby enhancing the responsiveness and sensitivity of the fibrous films. Qin et al. [33], developed a multifunctional film incorporating betalains from pitaya (*Hylocereus polyrhizus*) peel in starch and PVA,

evaluating the sensitivity of the film in 5 M ammonia solution, which compared to the one structured in the present study are less sensitive and accuracy. Our results suggested PLA12-BE12 fibrous films could be used as a potential intelligent packaging film for high protein content food.

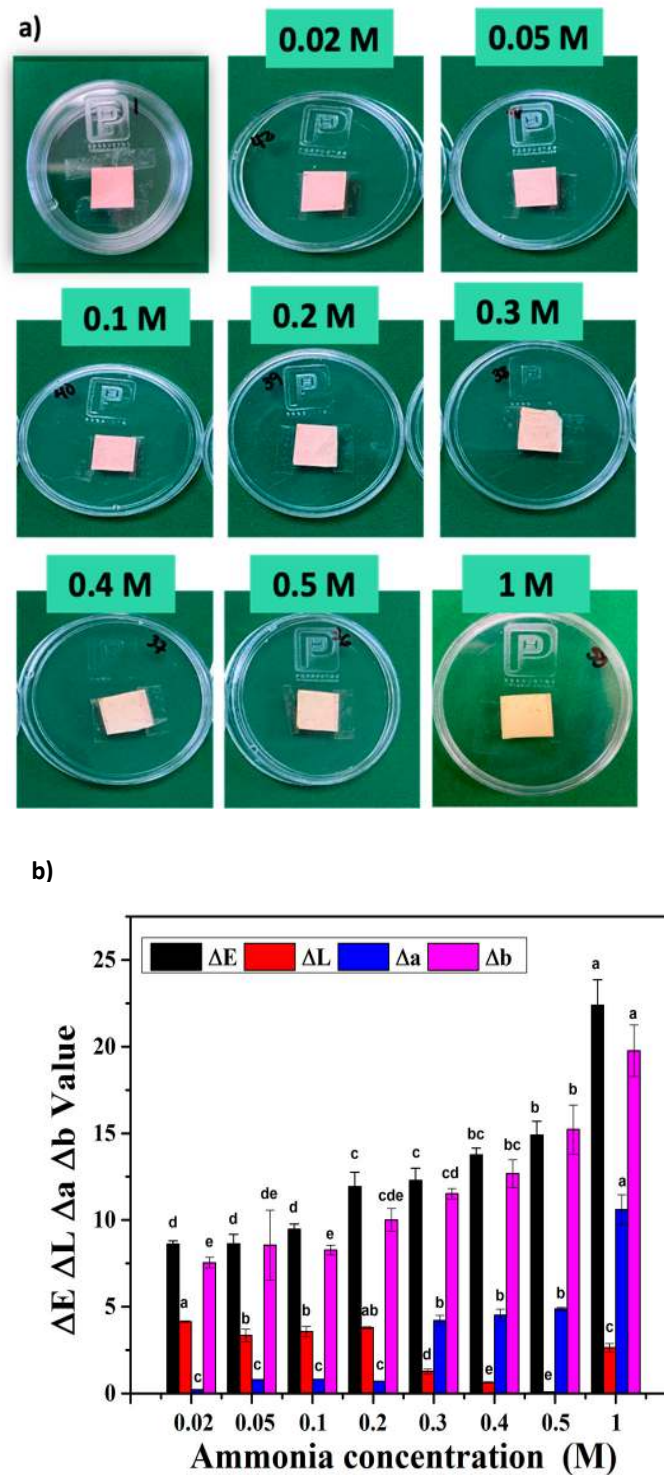


Figure 11. Color response of PLA12-BE12 fibrous film, at different ammonia concentrations: photographs after reaction with ammonia (a), color values of the film after reaction with ammonia (ΔE , ΔL , Δa , Δb) (b), at 20 min of reaction. Data with different letters on the same column color reference to significant differences ($p < 0.05$). Data (mean $\pm$ SD) are from three independent experiments.

3.4.2. Antioxidant Activities Analysis in Fibrous Film

Throughout the food processing chain, foods may experience various transformations, including oxidation reactions that result in detrimental changes to their sensory and physiological characteristics such as color, flavor, aroma, texture, and nutritional value [85]. Consequently, the development of smart food packaging, particularly those designed to inhibit the propagation of free radicals, is crucial for protecting foods from oxidative degradation [109].

Figure 12 displays the results of the antioxidant activities of the fibrous film, as measured by the ABTS•+, DPPH•, and FRAP methods and expressed as percentages of radical scavenging. For the PLA fibrous film, the determined values were $13.72 \pm 1.03\%$, $11.48 \pm 0.40\%$, and $158.60 \pm 4.59 \mu\text{M ET/g}$.

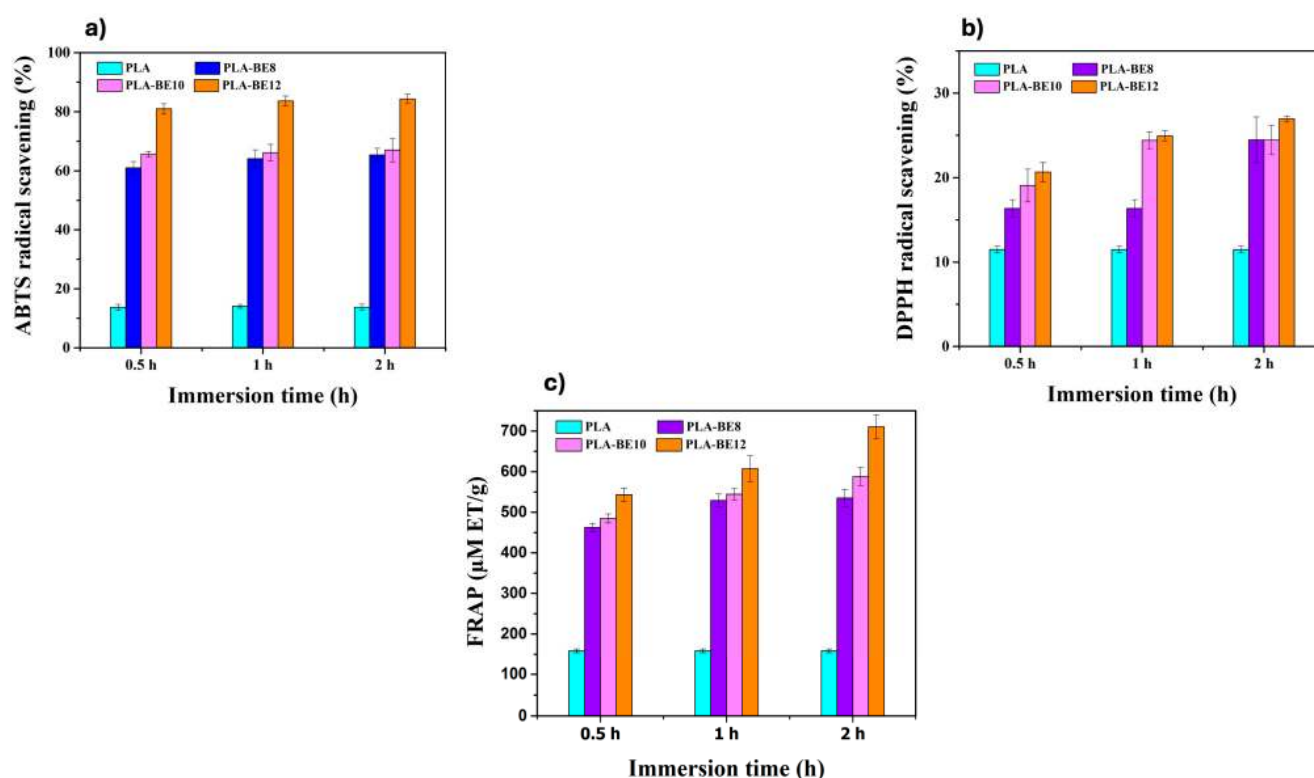


Figure 12. ABTS•+ radical inhibition (a), DPPH• radical inhibition (b), and FRAP $\mu\text{M TE/g}$ (c) of different fibrous films at various immersion times. Data (mean $\pm$ SD) are from three independent experiments.

These values are comparable to 6.2% by the ABTS•+ and 12% by the DPPH• assays, as reported by Xu et al. [85] and Aytac et al. [110], for PLA nanofiber films. As anticipated, PLA nanofibers exhibit relatively low antioxidant activity, which may be attributed to the adsorption of radicals due to the high surface area of the nanofibers, facilitating increased contact with the surrounding medium [111].

The antioxidant activity of PLA-BE increased with higher concentrations of BE across all assays ($p < 0.05$). At a BE concentration of 12% (w/v), the ABTS•+, DPPH•, and FRAP radical scavenging activities were at the highest values of $84.28 \pm 1.59\%$, $29.95 \pm 0.34\%$, and $710.57 \pm 28.90 \mu\text{M ET/g}$, respectively. These results demonstrate the successful encapsulation of BE through electrospinning and confirm that this process does not impair the antioxidant capacity of BE.

The high antioxidant activity observed in the PLA-BE12 fibrous films can be attributed to the potent antioxidant properties of the phenols and betalains present in the extract. These compounds exhibit antioxidant capacities due to their molecular structures, which enable them to participate in hydrogen atom and electron transfer reactions. The ABTS•+

assay primarily utilizes a Single Electron Transfer (SET) mechanism, similar to that of the FRAP assay, resulting in enhanced radical scavenging activity and higher $\mu\text{M TE/g}$ values. Furthermore, betalains are water-soluble compounds, which confer a greater affinity for the ABTS $\bullet$ + assay compared to the DPPH $\bullet$ assay, as the latter is more compatible with organic solvents and is therefore better suited for assessing lipophilic compounds, limiting its applicability in aqueous systems. Overall, the antioxidant activity of natural polymer films containing active ingredients is influenced by the release of these ingredients from the matrix and the degree of interaction between the active component and the polymer [8,33].

3.4.3. Reusability

Figure 13 shows that the color parameters of the fibrous films remain stable throughout the cycles. The color intensity is represented by the values a^* (red intensity) and b^* (yellow tones), while L^* , a^* , and b^* indicate measurements after exposure to extreme alkaline (ammonia) and acidic (acetic acid) gaseous systems. In the first cycle, under acidic conditions, the fibrous film exhibited a high a^* value of 11.27, which remained stable at 11.49 in the final cycle ($p > 0.05$), indicating that the red color was preserved. Conversely, the b^* value increased from 18.16 to 25.01 ($p < 0.05$) in the last cycle, signifying a gradual shift towards a yellow tone. This change is attributed to the alkaline degradation of betalains, involving the protonation and deprotonation of phenolic hydroxyl groups and leading to the formation of degradation products such as 4-methylpyridine-2,6-dicarboxylic acid, formic acid, and cyclo-DOPA-5-O-glucoside [112].

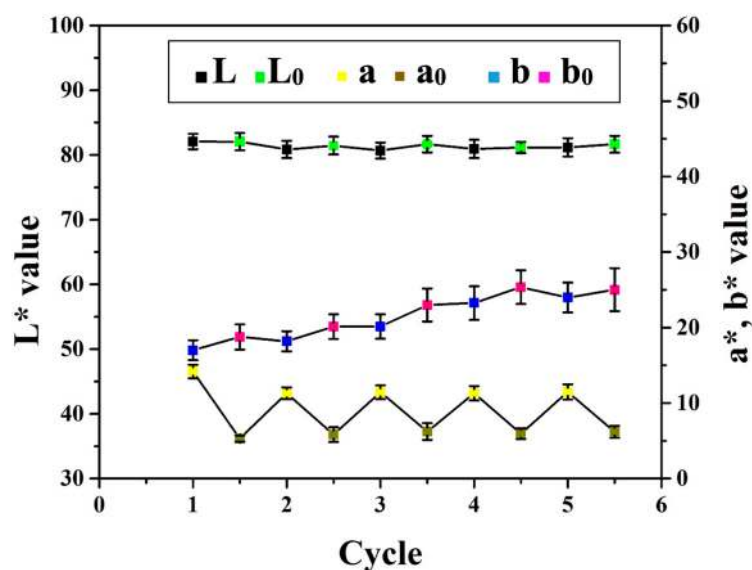


Figure 13. Color responses of PLA12-BE12 fibrous film after reaction during four cycles in extremely acidic and alkaline atmospheres.

Similarly, stability has been reported in fibrous films of PLLA containing anthocyanins from blueberry extract [17]. However, the PLA12-BE12 film demonstrated greater reusability than cellulose acetate films with curcumin extract, which exhibited good reversibility for at least three exposure cycles [95]. These findings suggest that betalains in fibrous films are chemically stable and highly reversible to pH changes, allowing for multiple reuses. This characteristic aligns with the principles of the circular economy [113].

3.4.4. Color Stability

The results in terms of ΔE values are presented in Figure 14, where it is shown that during storage at 25 °C, it started with a ΔE value of 1.22 ± 0.14 . The maximum value was 1.94 ± 0.12 within 30 days, demonstrating its stability at room temperature, which is due to the establishment of hydrogen bonding interactions between PLA and betalain,

according to FT-IR analyses in the present work. In addition, the high color stability of the electrospinning films was superior to some of the cast films, such as that of kaifirin-polyethylene oxide containing beet extract with an ΔE value of 7.77 after 30 days at the same temperature [114].

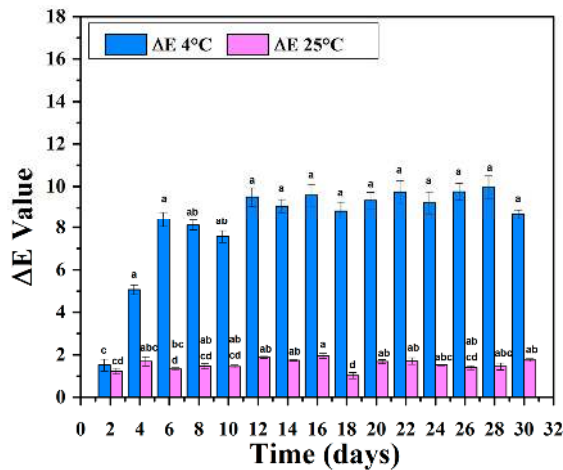


Figure 14. Total color change (ΔE) for PLA12-BE12 fibrous film during storage at 4 °C and 25 °C at 30 days. Data with different letters on the same column color refer to significant differences ($p < 0.05$). Data (mean $\pm$ SD) are from three independent experiments.

Regarding the nanofilms stored at 4 °C, the indicator color did not change visually during the first four days (ΔE value 5.06 ± 0.23) of storage until the sixth day, where the maximum ΔE value was 9.95 ± 0.54 . According to [62], if the ΔE value is higher than 5, the colors are distinguishable to the naked eye, while if the ΔE value is greater than 12, the color becomes attributed to a different space and indicates absolute difference in color. At 4 °C, the color difference is due to the reaction of the betalains with the humidity of the environment as the fibrous film color became redder, which is shown in Figure 15 and could be due to hydration of the dye. However, it is important to note that the fibrous film did not change towards yellow tones, which would be indicative of changes in the structural chemical stability of the betalains [112]. The color stability and efficacy of fibrous film are essential issues in its practical application, as they are critical parameters that determine the quality and reliability of intelligent fibrous film for assessing food quality.

During storage, at both temperatures, it is important to note that the film did not show noticeable fluctuations in Hue angle and Chroma according to Figures 16 and 17, respectively. Thus, the good compatibility of betalains and PLA could avoid the degradation reactions of betalains, which makes the PLA12-BE12 fibrous film have good color stability and makes the visual evaluation of pH changes promising.

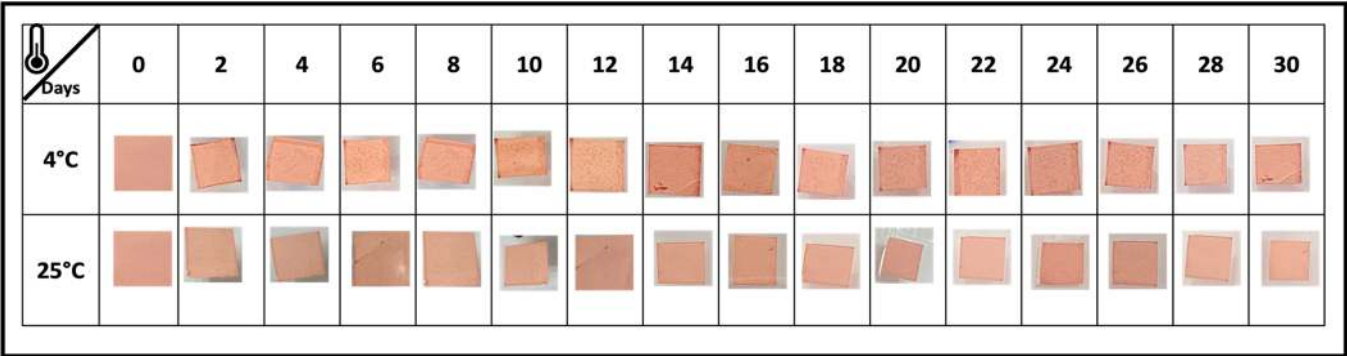


Figure 15. Photograph of PLA12-BE12 intelligent fibrous film indicator during storage at 4 °C and 25 °C at 30 days.

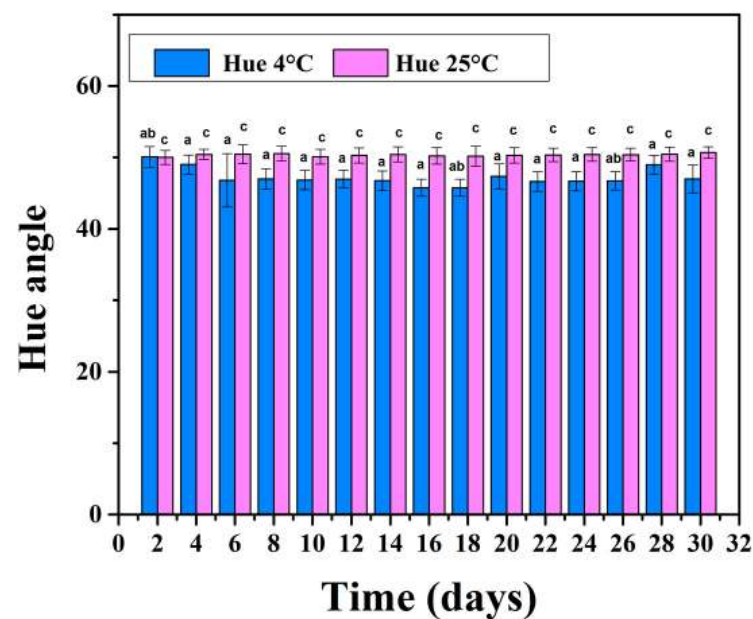


Figure 16. Hue angles of the intelligent fibrous film indicator during storage at 4 °C and 25 °C at 30 days. Data with different letters on the same column color refer to significant differences ($p < 0.05$). Data (mean $\pm$ SD) are from three independent experiments.

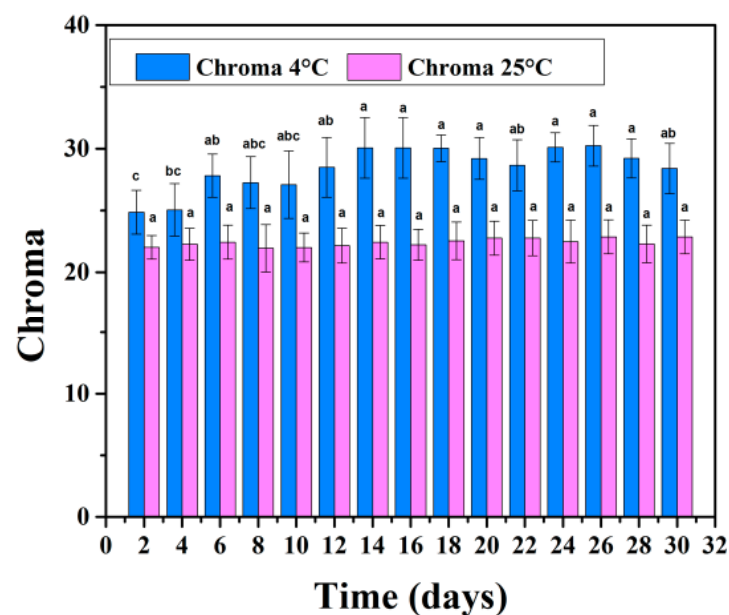


Figure 17. Chroma values of the indicator fibrous film during storage at 4 °C and 25 °C at 30 days. Data with different letters on the same column color refer to significant differences ($p < 0.05$). Data (mean $\pm$ SD) are from three independent experiments.

4. Conclusions

This research successfully designed a novel PLA12-BE12 nanofibrous film with potential functions in the food industry for quality control and color monitoring using the electrospinning technique. The results of SEM and FT-IR confirmed that the PLA12-BE12 film is characterized by smooth and defect-free morphology characteristics. PLA and BE successfully interacted through intermolecular hydrogen bonding. The nanofibrous film exhibited enhanced antioxidant capacity and pH color response properties at low ammonia concentrations, which could be easily identified by the naked eye based on the color change of the film from reddish to yellow. The film demonstrated excellent reversibility

and can be used several times without a decrease in efficiency. These findings highlight the film's potential as a smart packaging system for real time freshness assessment and food quality monitoring. Thus, it is recommended that further studies evaluate its sensitivity and selectivity in a food matrix.

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