

Bacterial Cellulose/Poly(vinyl alcohol)/Chitosan Films: Tuning Mechanical and Functional Properties for Sustainable Food Packaging

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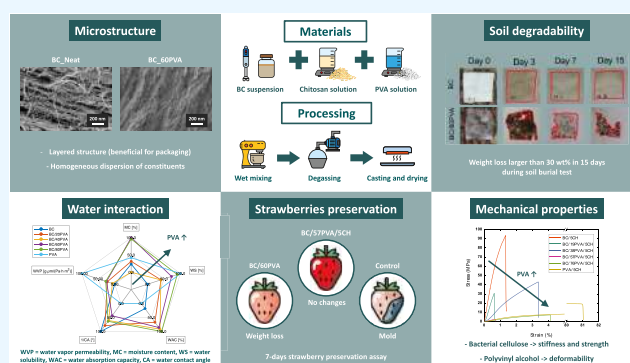
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ABSTRACT: This work reports a novel, green, and scalable wet mixing approach to prepare bacterial cellulose (BC)/poly(vinyl alcohol) (PVA)/chitosan (CH) composite films with precise control over barrier, mechanical, and antimicrobial properties, suitable for active food packaging. The resulting composites exhibit tunable water interaction, ultraviolet (UV) barrier, and mechanical properties dictated by the BC/PVA ratio. Increasing BC content reduces the water uptake, water solubility, water vapor permeability, and UV transmittance (<20%). Mechanical characterization reveals stiffness values up to 8.5 GPa and tensile strengths approaching 93 MPa, though excessive PVA content (>60 wt %) introduces interfacial defects limiting deformability. Soil burial tests demonstrated composition-dependent biodegradation, reaching a weight loss higher than 30 wt % after 15 days. Incorporation of CH (5 wt %) improves film homogeneity and imparts antimicrobial activity, effectively extending strawberry shelf life. This processing method eliminates harsh solvents while enabling precise compositional control, yielding renewable films with composition-dependent performance for sustainable packaging applications.



1. INTRODUCTION

Currently, the packaging industry predominantly relies on petroleum-based plastics due to low density, good processability, durability, mechanical and barrier properties, and low cost.¹ The main issue with this type of material is the lack of effective postuse treatments and the consequent accumulation of plastics in different ecosystems.² Specifically, the packaging sector is a primary contributor to the pollution problem,³ with plastic packaging accounting for 39% of the total plastic production by weight in Europe in 2022.⁴ A promising alternative to petroleum-based plastics is bioplastics, defined as biodegradable and/or renewable resource-derived polymers. Biodegradable bioplastics allow alternative disposal routes that reduce environmental accumulation, while bioderived bioplastics help decrease the carbon footprint at the resource extraction phase.⁵ Nevertheless, their performance still falls short of commercial plastics, limiting application.⁶ New research is exploring bioplastic blends that mimic the characteristics of synthetic materials without sacrificing biodegradability.^{6,7}

Among biopolymers, cellulose is a strong candidate to replace conventional plastics. Cellulose, the most common natural polymer on Earth, is a linear polysaccharide composed of glucose monomers linked by β -1,4-glycosidic bonds.⁸ It is the most abundant renewable bioresource, being the primary end product of photosynthetic activity in both aquatic and terrestrial ecosystems.⁹ The primary industrial origin is multicellular plants

like hardwood and softwood trees, cotton, flax, jute, ramie, and hemp, but also comes from bacteria, algae, and molds.¹⁰ Bacterial cellulose (BC), compared to plant cellulose, is purer and shows superior structural, mechanical, and physicochemical properties. Its fibers are about 100 times thinner, giving increased surface area and higher availability of surface hydroxyl groups and enhancing hydrophilicity, biodegradability, and chemical-modification capacity.⁹ Moreover, BC exhibits high molecular weight, crystallinity, strength, water retention, and solvent resistance, making it a versatile, pure, biocompatible biomaterial.^{11,12} In 1886, A.J. Brown created the first BC product from glucose; since then, many microorganisms and feedstocks have been studied.¹³ One of the most commonly studied bacteria strains used for BC production is the Gram-negative bacterium *Komagataeibacter* (formerly *Gluconacetobacter xylinus*) thanks to its capacity to metabolize a wide range of carbon and nitrogen sources and to the high cellulose productivity.^{9,11,14,15} BC synthesis is a self-assembly process forming a porous, hierarchical network of cellulose fibers

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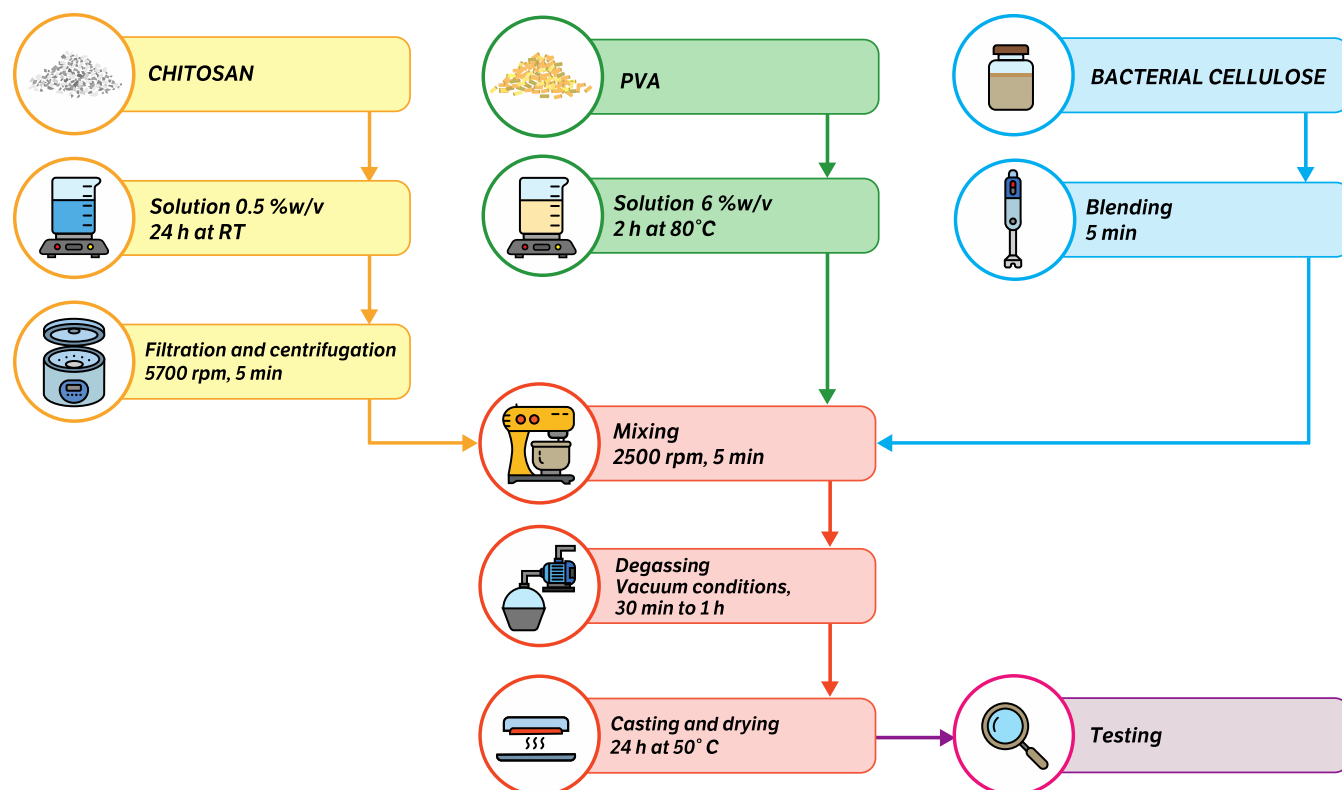


Figure 1. Overview of the production process of films from BC, PVA, and chitosan.

through polymerization of cellulose Ia α , secretion of protofibrils, and assembly into ribbons (20–100 nm).¹⁶ In static fermentation conditions, this process occurs at the liquid–air interface, producing a layered structure of isotropic fiber networks.¹⁷ Under stirring, BC forms spheres or pellets with lower strength but higher scalability.¹² After culture, BC is usually purified with alkaline treatment.^{15,18}

Thanks to its qualities, BC is used in biomedical, food supplement, electrical and energy, packaging, textiles, filtration, painting and coatings, and cosmetics industries.^{9,11,15,19} Its outstanding mechanical resistance, thermal stability, and shielding against water vapor, oxygen, and UV radiation make it attractive as a substitute for synthetic materials.²⁰ In 1992, the United States Food and Drug Administration (FDA) categorized BC as generally recognized as safe (GRAS),²¹ and many studies have since validated its use in food packaging, with different food products such as strawberries,²² tomatoes,^{23,24} and even fresh fish.²⁵

Despite its promising properties, BC suffers from inhomogeneity, low elasticity, and low transparency, limiting its application in the packaging field. The modification and mixing of BC with other compounds could enhance its properties while introducing new important characteristics, specifically improving mechanical and barrier properties.^{12,26} BC composites are prepared through in situ (additives added during synthesis) or ex situ (immersion in additive solutions) modifications.¹² Previous studies report in situ modifications with water-soluble natural polymers, such as starch,²⁷ xanthan gum,²⁸ chitosan,²⁹ pectin,³⁰ and cellulose derivatives, such as carboxymethyl cellulose, hydroxyethyl cellulose,³¹ and hydroxypropyl methylcellulose,³² with water-soluble synthetic polymers, such as poly(ethylene oxide)³³ and poly(vinyl alcohol) (PVA)³⁴ or water-insoluble polymers, such as poly-3-hydroxybutyrate³⁵ and

polycaprolactone.³⁶ This technique presents certain limitations concerning the type of additive and its concentration, with possible interferences with the synthesis process.¹²

Ex situ modifications are obtained through immersion of BC into a solution, whether in its wet or dry state. Water-soluble plasticizers were incorporated, such as glycerol^{37–39} or poly(ethylene glycol),⁴⁰ enhancing the flexibility of BC. Furthermore, combining BC with film-forming polymers, such as PVA³⁸ or chitosan,⁴¹ leads to both plasticizing and reinforcing effects. Alternatively, combinations of chitosan and PVA^{42–44} have been utilized. Materials like gelatin⁴⁵ modify the rehydration capacity of BC, while hydrophobic polymers such as polycaprolactone (PCL)⁴⁶ or poly(3-hydroxybutyrate) (P3HB)⁴⁷ produce optically transparent composite films. Despite the ease of the process, ex situ immersion is limited by incorporation capacity, process discontinuity, and long immersion times.¹²

To overcome these issues, other techniques have been developed, including wet spinning, twisting, tape peeling, and, especially, wet mixing. The wet mixing method is a simple, innovative, and effective technique that enables large-scale manufacturing with low energy consumption. It involves the physical treatment of BC using a blender or homogenizer to obtain a cellulose suspension, allowing a wider additive concentration range, improved dispersion, reduced time, and diverse product forms.⁴⁸ The advantages related to this technique open the opportunity to realize functional and smart packaging materials for food preservation. However, very little research focuses on the effect of the wet mixing technique on the properties of neat BC and on the possibility of blending this material with other polymers targeting food packaging applications.

In this work, BC-based films for active food packaging applications have been developed employing for the first time a

wet mixing technique to blend BC, PVA, and chitosan. The rationale behind the addition of PVA and chitosan to BC relies on the possibility of improving the potential applications of BC-based films. Specifically, the addition of a material possessing film-forming properties like PVA has been proven to enhance the flexibility of BC, facilitating its handling and processing,^{44,49} while the addition of chitosan serves as an antimicrobial agent to make active food packaging and improve antimicrobial activity, prolonging the food shelf life.^{50,51} Various concentrations of PVA, ranging from 0 to 80 wt %, were added to neat BC to tune its mechanical, optical, and water interaction properties. A comprehensive characterization was conducted, encompassing thickness and density measurements and microstructural, thermal, and mechanical analysis. Water interaction properties were assessed through the analysis of moisture content, water solubility, water absorption capacity, water vapor permeability, and contact angle. Additionally, UV-light barrier properties, biodegradability, and strawberry preservation were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Bacterial cellulose (BC) was provided by Bioniks (Verona, Italy) as wet disks (solid content approximately 1 wt %). Bacteria and yeasts were removed with a purification step with a 0.5 M sodium hydroxide (NaOH), followed by daily washings until the pH reached a neutral value of approximately 7.

Poly(vinyl alcohol) (PVA) was provided by Kuraray Specialties Europe GmbH (Frankfurt, Germany) with the trade name Mowiflex TC232. It presented a melt-flow index (MFI) at 2.16 kg and 190 °C of 39 g/(10 min), a glass transition temperature of 34 °C, a melting temperature of 178 °C, a density of 1.27 g/cm³, and it was soluble in water.

Chitosan was provided by Sigma-Aldrich (St. Louis, MO) under product number C3646, with a deacetylation degree higher than 75% and an average molecular weight (M_n) of 190 kDa. The chitosan solution was prepared in an acid medium, obtained with glacial-grade acetic acid supplied by J. T. Baker (Deventer, Holland) under the trade name Acetic Acid 99–100% glacial (average molecular weight of 60.05 g/mol).

A low-density polyethylene (LDPE) food-grade film was used as a reference. This material, provided in the form of a 41 μ m thickness film, presented a density of 0.926 g/cm³, an elastic modulus of 260 MPa, a stress at break of 20 MPa, and a strain at break larger than 500%, according to the datasheet.

2.2. Sample Preparation

Film samples based on BC/PVA/CH were prepared by means of wet mixing in an aqueous medium, followed by casting, as schematized in Figure 1.

The PVA solution was made by dissolving PVA in deionized water (concentration of 6%w/w) under vigorous magnetic stirring at 360 rpm and a temperature of 80 °C for 2 h. The chitosan solution (concentration of 0.5%w/v) was prepared from an aqueous solution of 1%v/v of glacial acetic acid (pH < 6), under vigorous agitation for 24 h at room temperature. Then, the solution was filtered to remove undissolved residues, in a two-step process. First, filtration was made using a water pump, a Büchner funnel No. 3 equipped with a Büchner flask, and a Perfecte 2 filter paper by the Cordeons group (Milan, Italy). The chitosan solution was then centrifuged using a Remi XS R8-C+ centrifuge (Mumbai, India), at 5700 rpm for 5 min.

Neat BC was subjected to a milling pretreatment using a Braun MQ7045X immersion blender (Kronberg im Taunus, Germany) for 5 min. All of the components were mixed with a VMA-GETZMANN Dispermat F1 (Germany), at a speed of 2500 rpm for 5 min, and then homogenized and degassed for a period of 30 min to 1 h. Then, the mixtures were cast in 12.5 $\times$ 12.5 cm² square silicon molds, dried for 24 h at 50 °C, and stored in a controlled humidity environment.

Thirteen BC/PVA/chitosan combinations were defined, as reported in Table 1; five films were produced for each composition. The amount

Table 1. List of the Prepared Samples with Nominal Dry-Weight Compositions

label	BC [wt %]	PVA [wt %]	chitosan [wt %]
BC	100	0	0
BC/20PVA	80	20	0
BC/40PVA	60	40	0
BC/60PVA	40	60	0
BC/80PVA	20	80	0
PVA	0	100	0
BC/5CH	95	0	5
BC/19PVA/5CH	76	19	5
BC/38PVA/5CH	57	38	5
BC/57PVA/5CH	38	57	5
BC/76PVA/5CH	19	76	5
PVA/5CH	0	95	5
CH	0	0	100

of wet BC, PVA solution, and chitosan solution was calculated with respect to the weight percentage of each component in the dry film. The BC/PVA combinations were selected to cover the entire compositional range, following a design of experiment (DoE) approach. The selected chitosan concentration was fixed at 5 wt % on the final dry film after a preliminary evaluation of its influence on the properties of the BC/PVA composites. Moreover, a 5 wt % of CH has been previously proven to have antimicrobial properties with other systems.^{29,52}

In this work, the samples without chitosan are referred to as BC/PVA samples, while those containing 5 wt % of chitosan are referred to as BC/PVA/CH.

2.3. Characterization Techniques

2.3.1. Thickness and Density Measurement. The geometrical density of the prepared films was calculated as the ratio between the mass and volume of the prepared specimens. Thickness and weight measurements were performed with a high-precision Benslants micrometer and a Gibertini E42 balance three times per specimen (on triplicate samples) on disc specimens with a diameter of 4 cm.

2.3.2. Microstructural and Spectroscopic Analysis. Fourier transform infrared (FT IR) spectroscopy was performed in attenuated total reflectance (ATR) mode with a PerkinElmer Spectrum One IR spectrometer (PerkinElmer GmbH, Waltham, MA), as described in Section S1. The microstructural features of the prepared films were analyzed with a field-emission scanning electron microscope (FE-SEM) Zeiss Supra 40 (Carl Zeiss AG, Oberkochen, Germany) at an accelerating voltage of 2.5 kV and a magnification level between 1.0 kX and 200.0 kX. Both the external and the cross-sectional surface, obtained through cryogenic fracture, were investigated after Pt–Pd sputtering. The width of cellulose ribbons was measured with the aid of ImageJ v. 1.53 software, and the width distribution fitting was performed through a normal distribution using Minitab19.0. Anderson–Darling normality test was performed to check the normal distribution of data.

2.3.3. Molecular Weight. The molecular weight (MW) of BC was evaluated following the procedure described in ref 53 by measuring its intrinsic viscosity using a tube viscometer with a single-point determination approach, as reported in Section S3.

2.3.4. Thermal Analysis. Differential scanning calorimetry (DSC) was carried out with a Mettler DSC30 calorimeter (Mettler Toledo, Columbus, OH) on all prepared films, as described in Section S4. Thermogravimetric analysis (TGA) was carried out using a Mettler TG50 thermobalance (Mettler Toledo, Columbus, OH). The specimens (approximately 20–50 mg) were subjected to a thermal ramp of 10 °C/min from 30 to 700 °C, under a nitrogen flow of 100 mL/min. One specimen per composition was tested.

2.3.5. Tensile Test. The mechanical properties were evaluated using an Instron 5969 electromechanical testing machine (Norwood, MA) equipped with a 100 N load cell. The films were cut in 5×0.5 cm² stripes and stored under controlled temperature and relative humidity conditions (25 °C and 50%, respectively). The width and thickness of the stripes were measured at three different points by using a vernier caliper and a micrometer. The test was performed following standard ASTM D882, at a rate of 10 mm/min and with a gauge length of 5 cm. The Young modulus (E) was determined as the maximum value of the derivative of the stress–strain curve, bypassing the initial toe region typical of BC.⁵⁴ The ultimate tensile strength (UTS) and elongation at break (ϵ_b) were determined in correspondence to the last data point prior to the film failure.²²

2.3.6. Water Vapor Permeability (WVP) and Water Interaction. Water vapor permeability (WVP), assessed following the standard ASTM E96, measures the amount of water vapor that passes through a film sample under certain conditions, such as pressure, temperature, and humidity. Glass cups with a 38 mm external diameter, 32 mm internal diameter, and 50 mm height were used. The lid of the cups was punched with a die cutter to produce a hole with a diameter of 35 mm, which allowed gas exchange. The glass cups were filled with 10 g of distilled water, and disks with a diameter of 4 cm were cut out of the films and fixed on top of the cups with the aid of the punched lid. Then, the cup–lid assembly was sealed on the contour with parafilm, ensuring that the only interaction with the environment was through the film on the top of the cup. Then, everything was placed in a Discovery My by ACS (Massa Martana, Perugia, Italy) climatic chamber for 28 h, at 30 °C, and 50 rh %. The weight was monitored every hour for the first 10 h of the first day of test and every 2 h the day after, until a constant value was measured.

The water vapor transmission rate (WVTR) [$\text{g}/(\text{h}\cdot\text{m}^2)$] and the water vapor permeability (WVP) [$\text{g}\cdot\mu\text{m}/(\text{Pa}\cdot\text{h}\cdot\text{m}^2)$] were calculated following eqs 1 and 2, respectively

$$\text{WVTR} = \frac{G}{t \cdot A} \quad (1)$$

$$\text{WVP} = \frac{\text{WVTR} \cdot L}{S \cdot (R_1 - R_2)} \quad (2)$$

where G is the weight change [g], t is the time [h], A is the test area (lid hole with a diameter of 35 mm),⁵⁵ L is the film thickness [m], S is the saturation vapor pressure at test temperature (equal to 4246 Pa), R_1 is the relative air humidity inside the glass cup, and R_2 is the relative humidity in the environment, respectively, equal to 1 r.h.% and 0.5 r.h.%. All measurements were taken in duplicate. Moreover, an additional sample of LDPE was used as a comparison of WVP values for the produced films.

Other water interaction properties were evaluated in terms of moisture content (MC),²² water solubility (WS),²⁵ water absorption capacity (WAC),²⁹ and water contact angle (CA). The experimental procedure, equations employed for the calculation of water interaction properties, and corresponding experimental results are reported in Section S5.

2.3.7. Optical Properties. The optical transparency of films was studied with a Spectrophotometer Jasco V-570 by Jasco International Co., Ltd. (Cremella, Italy). Each film was cut into 2 cm square specimens and clamped in a film holder. The measurement was performed over the wavelength range of 250–850 nm using a step scanning speed of 400 nm/min, in air, recording the intensity of light passing through the sample. Transmittance ($T\%$) and transparency were calculated according to eqs 3 and 4, respectively.^{42,56}

$$T(\%) = \frac{I_t}{I_0} \cdot 100 \quad (3)$$

$$\text{transparency value} = \frac{\log(T_t)}{L} \quad (4)$$

where I_0 and I_t are the light intensity before and after the beam of light passes through the sample, respectively, T_λ is the transmittance percentage at the wavelength λ , and L is the thickness in millimeters.

2.3.8. Strawberry Preservation Assay. The antimicrobial capacity of the films was qualitatively evaluated on fresh strawberries obtained from a local grocery store (Trento, Italy). A single strawberry was placed into a glass cup, and the cups were covered with film disks with a diameter of 4 cm cut out from the produced films, closed with a punched lid, and sealed with parafilm as reported in the description of the WVP test. The strawberries were maintained at room temperature, and their freshness was evaluated qualitatively each day for a period of 7 days. The film behavior was compared with a control sample (without coverage film), and a commercial LDPE film used in food packaging applications. The test was performed in triplicate.

2.3.9. Soil Burial Test. The soil burial test for the determination of the degradability of prepared films was carried out following a procedure adapted from that presented by Zahan,⁵⁷ as reported in Section S7.

2.3.10. Statistical Analysis. One-factor analysis of variance (ANOVA) was performed. The validation or rejection of the null hypothesis was done by looking at the F -value and p -value of the ANOVA test results.⁵⁸ The null hypothesis was rejected if the F -value exceeded the critical F -value or if the p -value was smaller than the chosen α level (0.05). Additionally, a Tukey test was performed to identify which groups of results were significantly different from others.⁵⁹ Data were analyzed using Minitab19.0 software.

3. RESULTS AND DISCUSSION

3.1. Thickness and Density Measurements

Thickness measurements were performed to calculate the density of the prepared films and to assess the possible changes in geometry due to the changes in the relative amounts of the different components. Thickness and density values of samples BC/PVA and BC/PVA/CH are reported in Table 2.

Table 2. Thickness and Geometrical Density of BC/PVA and BC/PVA/CH Samples along with Tukey Analysis Results in Brackets^a

sample	thickness [mm]	density [g/cm^3]
BC	0.077 ± 0.013 (b)	1.034 ± 0.040 (a, b)
BC/20PVA	0.114 ± 0.029 (a, b)	0.606 ± 0.141 (b)
BC/40PVA	0.072 ± 0.020 (b)	0.783 ± 0.211 (b)
BC/60PVA	0.094 ± 0.013 (b)	0.714 ± 0.137 (b)
BC/80PVA	0.098 ± 0.021 (b)	0.636 ± 0.038 (b)
PVA	0.157 ± 0.013 (a)	1.237 ± 0.061 (a)
BC/SCH	0.051 ± 0.013 (b)	0.961 ± 0.138 (a, b)
BC/19PVA/SCH	0.044 ± 0.014 (b)	1.136 ± 0.127 (a)
BC/38PVA/SCH	0.066 ± 0.011 (b)	0.969 ± 0.075 (a, b)
BC/57PVA/SCH	0.078 ± 0.015 (a, b)	0.883 ± 0.022 (a, b)
BC/76PVA/SCH	0.068 ± 0.018 (a, b)	0.536 ± 0.080 (b)
PVA/SCH	0.148 ± 0.009 (a)	1.029 ± 0.086 (a)
CH	0.020 ± 0.001 (/)	0.326 ± 0.053 (/)

^a(a, b) = Tukey analysis results. Different letters mean that the difference is significant.

Measured thickness values are in the range of commercial films for food packaging.⁶⁰ Generally, an upward trend of the thickness is observed with the increasing content of PVA, while chitosan addition decreases the thickness by 15 to 30% concerning BC/PVA films, for all compositions.

The lowest geometrical density was measured for the CH film with a value equal to $0.326 \text{ g}/\text{cm}^3$, the highest value is that of the PVA film, reaching $1.236 \text{ g}/\text{cm}^3$, and neat BC film presents an intermediate value of $1.034 \text{ g}/\text{cm}^3$. Hence, an increase in density

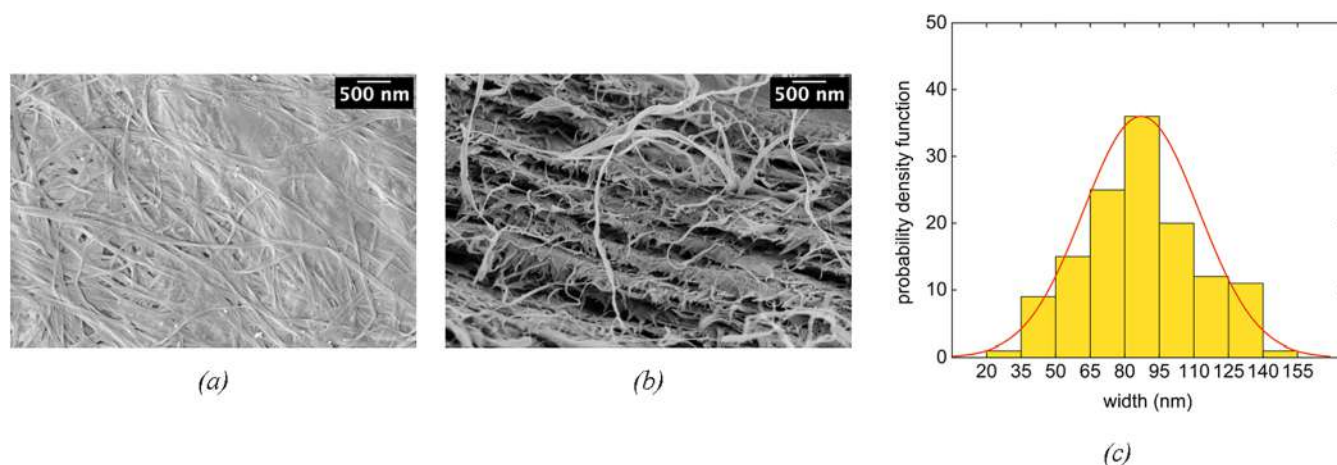


Figure 2. SEM micrograph of (a) top view of the BC film, (b) cross-section of the BC film, and (c) probability density function and normal distribution fitting of BC cellulose ribbon diameter.

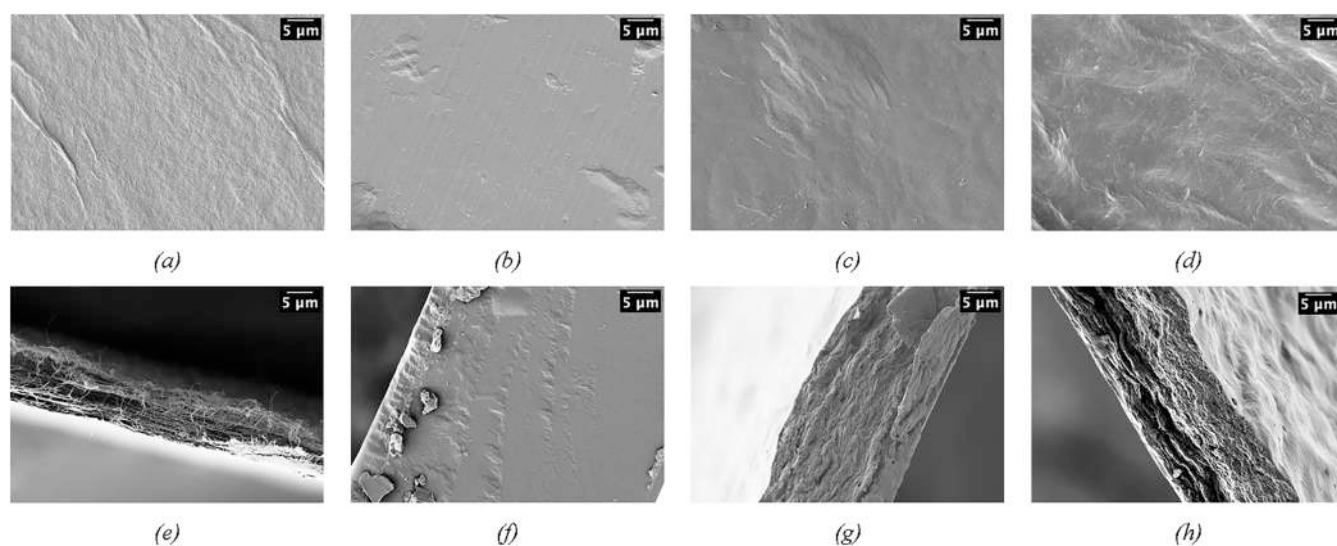


Figure 3. SEM micrographs of the film top view of samples (a) BC, (b) PVA, (c) BC/60PVA, and (d) BC/57PVA/5CH and cross-section of samples (e) BC, (f) PVA, (g) BC/60PVA, and (h) BC/57PVA/5CH.

is expected when the weight concentration of PVA increases, and the opposite with CH. Contrary to what could be expected, as the PVA content increases, the density of BC/PVA films decreases of approximately 40% compared to BC films. This is probably due to the increase in porosity, as confirmed by the enhancement in film thickness with increasing PVA content. On the other hand, the addition of chitosan seems to homogenize the different components, leading to higher values of density, and less variable thickness (approximately 15%) with the increasing PVA content for BC/PVA/CH samples. In general, considering the statistical approach, the density values are significantly different from one another, being the p -value lower than 0.05 for both BC/PVA and BC/PVA/CH films. Thickness and density results suggest that the inclusion of chitosan increases density, attributed to reduced porosity. This development is advantageous for packaging purposes, as high porosity in packaging materials is undesirable.

3.2. Microstructural and Spectroscopic Analysis

The effect of the processing route on the morphology of BC was evaluated by observing SEM micrographs of the BC film and

measuring the dimensions of the cellulose fiber network, as shown in Figure 2(a–c).

The typical BC structure is preserved after wet mixing:¹⁷ the interconnected ribbons and the layered structure are visible, respectively, from the SEM images of the top view (Figure 2(a)) and the cross-section (Figure 2(b)) of the BC film. This suggests that BC properties may remain unaltered to a certain degree after wet mixing, and the layered structure could be beneficial for the mechanical and gas barrier properties, fundamental for packaging applications.

The mean cellulose ribbon diameter is 86 ± 25 nm, in accordance with previous studies.^{15,17,61} Due to the difficulties in distinguishing between cellulose bundles and defibrillated cellulose fibrils and the consequent variability in the measured diameter, a normality test was performed. The Anderson–Darling test results present an A^2 value of 0.55, lower than the critical value of 0.79, and a p -value of the data set equal to 0.15, higher than the significance α level of 0.05. These results indicate that the cellulose ribbon diameter values follow a normal distribution.

The SEM analysis also allowed a qualitative evaluation of the interaction among the constituents present at different

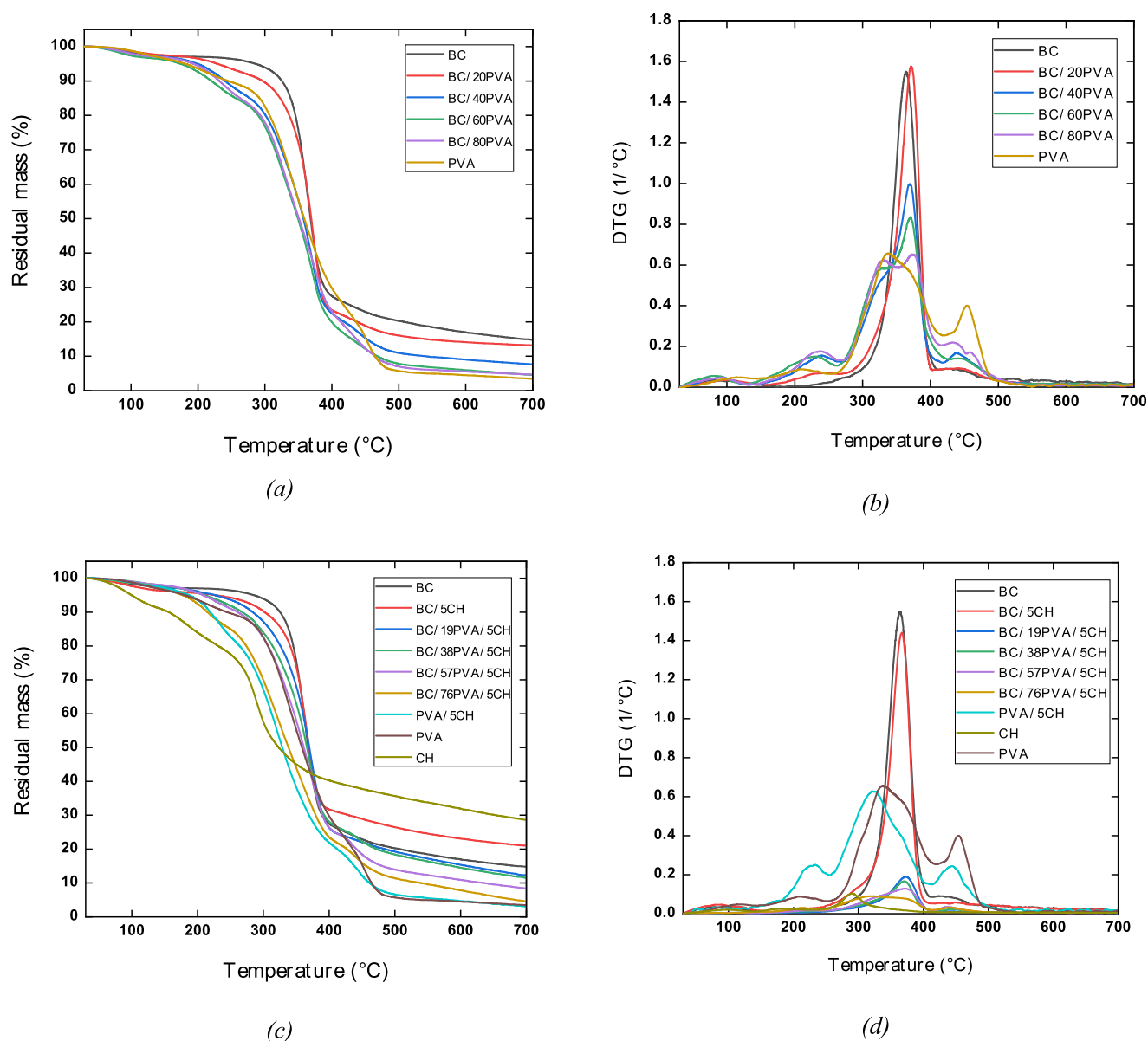


Figure 4. TGA tests on (a, b) BC/PVA films and (c, d) BC/PVA/CH films; (a, c) residual mass and (b, d) mass loss derivative curves as a function of temperature.

concentrations, observing both the surface and the cross-sectional surfaces of prepared samples. A visual comparison between BC, PVA, BC/60PVA, and BC/57PVA/5CH can be performed by looking at Figure 3(a–h).

Neat BC exhibits a rough surface where cellulose ribbons are clearly visible, while PVA film presents a smooth surface, although minor imperfections are observed. The addition of PVA to BC clearly decreases the surface roughness, thanks to PVA filling the interstitial spaces between cellulose ribbons. This interaction, progressively evident with increasing content of PVA, was also depicted in previous studies by Cazon et al.^{38,43} Nevertheless, there are two other phenomena competing with the densification of the material that could explain the thickness and density results (Section 3.1). First, mixing causes the trapping of air bubbles within PVA and at the PVA/BC interface that do not fully escape from the material during degassing. Specifically, the porosity is mainly located in the upper part of the film in contact with air during drying, as observed in Figure

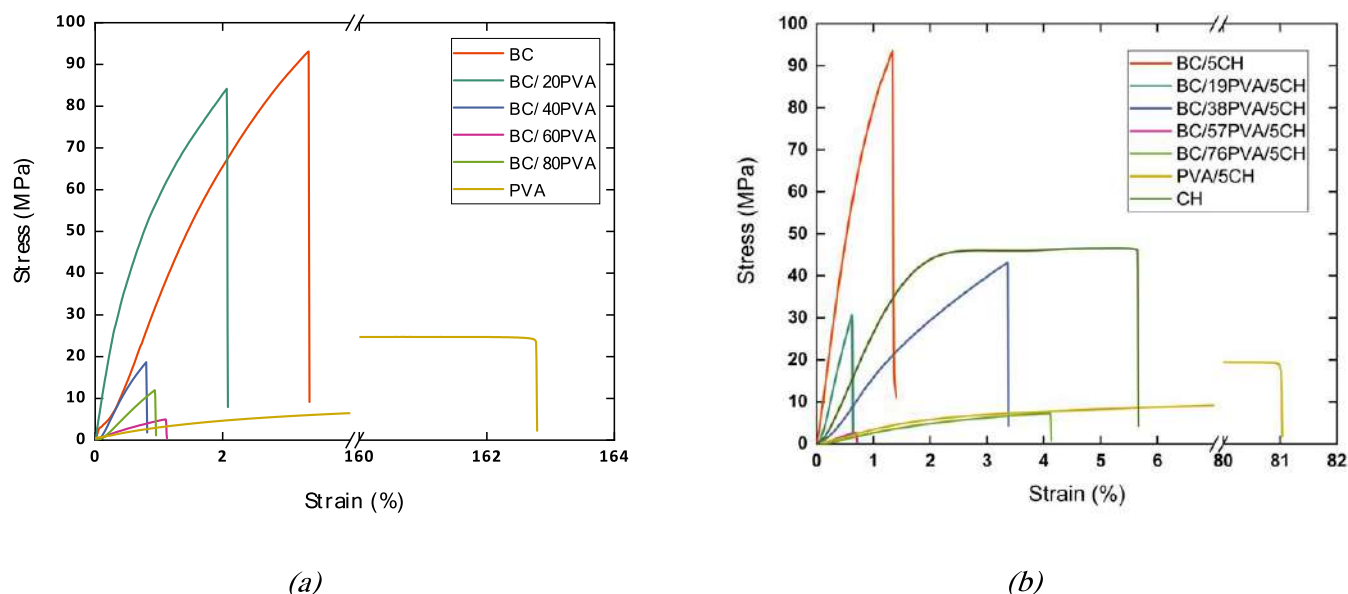
S2. Second, the increase in the concentration of PVA, which is a hydrophilic plasticizer, leads to an increase in water uptake in BC/PVA films (Section 3.4), resulting in slight rehydration of the cellulose fibers and thickening of the film. These results are consistent with previous studies on BC/PVA mixtures³⁸ and could explain the increase in thickness and decrease in density with the increasing PVA content.

On the other hand, the inclusion of chitosan alters the structure of the film by permeating the spaces between cellulose ribbons, enhancing component homogenization. This aligns with literature findings attributing material compatibility to hydrogen bond formation between BC and chitosan^{25,29} and PVA and chitosan⁶² and explain the observed increase in density, as described in Section 3.1. Specifically, in the study conducted by Cazon et al., which involved the immersion-based production of BC/PVA/CH films, the combination of PVA and chitosan resulted in improved densification.⁴⁴ Nevertheless,

Table 3. Main Results of TGA Tests Performed on BC, PVA, Chitosan, BC/PVA, and BC/PVA/CH Films^a

sample	$T_{1\%}$ [°C]	$T_{3\%}$ [°C]	$T_{5\%}$ [°C]	1st mass loss		2nd mass loss		3rd mass loss		4th mass loss		R_m [%]
				T_d [°C]	m [%]	T_d [°C]	m [%]	T_d [°C]	m [%]	T_d [°C]	m [%]	
BC	79.8	209.1	285.9	105.3	3.6	-	-	363.5	81.6	-	-	14.8
PVA	94.4	139.3	181.1	116.7	3.7	208.5	8.5	339.8	64.4	453.9	19.9	3.4
CH	59.9	82.8	99.7	112.1	7.5	180.7	13.9	289.5	50.1	-	-	28.6
BC/20PVA	91.3	183.7	228.9	106.0	3.2	238.5	6.5	371.8	69.4	442.4	7.7	13.2
BC/40PVA	78.0	149.6	199.3	91.0	3.2	239.7	13.5	370.0	64.0	438.5	11.6	7.7
BC/60PVA	68.5	112.2	173.9	82.0	4.9	227.8	14.7	369.5	64.5	444.4	11.3	4.6
BC/80PVA	76.4	143.1	193.0	90.2	3.5	236.7	14.3	374.0	60.6	439.2	16.9	4.7
BC/5CH	70.7	118.9	228.0	145.8	4.5	-	-	367.1	74.6	-	-	21.0
BC/19PVA/5CH	91.2	174.9	226.8	132.2	5.3	-	-	364.3	74.8	434.3	7.6	12.3
BC/38PVA/5CH	103.1	174.6	210.2	147.3	5.2	-	-	370.2	67.9	442.3	15.4	11.5
BC/57PVA/5CH	100.2	177.4	208.6	132.2	3.9	232.8	10.7	373.1	59.4	436.6	17.6	8.4
BC/76PVA/5CH	86.1	145.9	177.4	160.2	5.5	209.9	15.2	328.3	55.3	440.9	19.5	4.5
PVA/5CH	88.8	155.1	187.1	167.8	5.1	237.1	20.4	322.5	49.6	444.8	21.9	3.0

^a $T_{1\%}$, $T_{3\%}$, and $T_{5\%}$: temperatures associated with a mass loss of 1%, 3%, and 5%, respectively; T_d : peak degradation temperature; m : mass loss; R_m : residual mass at 700 °C.

**Figure 5.** Representative stress–strain curves of (a) BC/PVA and (b) BC/PVA/CH films.

chitosan seems to increase the surface roughness, as observed in Figure 3(d–h).

3.3. Thermal Analysis

Thermogravimetric curves along with the corresponding derivative curves (DTG), useful to evaluate the peak degradation temperatures (T_d), of all the prepared films are reported in Figure 4(a–d), and the most significant TGA results are presented in Table 3.

All samples present a first mass loss between 50 and 150 °C which is attributed to water evaporation and low-molecular-weight molecules degradation,^{38,63} corresponding to a weight loss of 3.6%, 3.7%, and 7.5 for BC, PVA, and chitosan, respectively. Considering neat BC, the main degradation peak corresponds to the thermal decomposition of the cellulose structure (363.5 °C, 81.6% mass loss), and the last degradation zone (above 400 °C) is associated with the degradation of the polymeric chains and the six-member cyclic structure, pyran.⁶⁴ The residual mass at 700 °C (R_m) of BC is about 14.8%, consistent with previous studies.^{25,64}

For PVA films, the second degradation step, attributed to the onset of the degradation of PVA, presents a relatively small weight loss (208.5 °C, 8.5%), indicating that PVA fully degrades in two further degradation steps at higher temperatures.^{38,42} The first is associated with the decomposition of the lateral hydroxyl groups (339.8 °C, approximately 64.4% mass loss), while the second (between 400 and 500 °C) is due to the degradation of the main PVA polymeric chains (approximately 19.9% mass loss).^{65,66} The residual mass at 700 °C corresponds to the carbonaceous residue products (less than 4%). These results resemble that obtained for PVA at a degree of hydrolysis of 87% found in the previous work.⁶⁵

Lastly, for the chitosan film, a low-temperature mass loss step is observed at 211.9 °C, due to the binding water loss from hydrophilic groups in chitosan molecules.⁶⁷ The thermal degradation of chitosan main chain, attributed to depolymerization, saccharide ring dehydration, and the decomposition of acetyl and amine groups of chitosan,^{50,67,68} is observed in one step (289.5 °C, approximately 50% mass loss), reaching a residual mass of 28.6% at 700 °C.

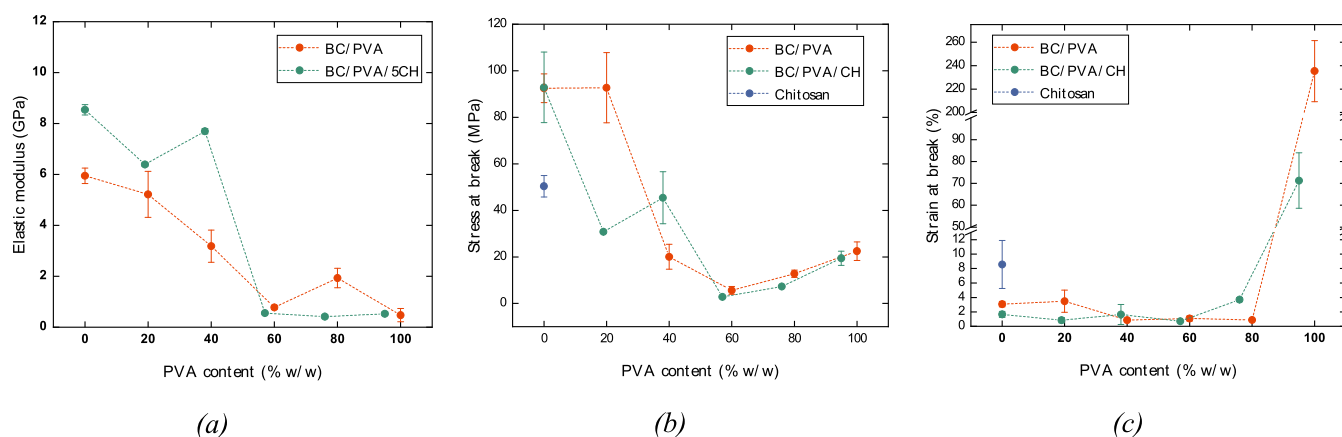


Figure 6. Main results of the quasi-static tensile tests on BC/PVA and BC/PVA/CH films: (a) elastic modulus, (b) tensile strength, and (c) strain at break as a function of the PVA content.

Table 4. Main Results Obtained from the Tensile Tests on the Prepared BC/PVA and BC/PVA/CH Films along with Tukey Analysis Results in Brackets^a

sample	E [GPa]	UTS [MPa]	ϵ_b [%]
BC	6.0 ± 0.3 (a)	92.5 ± 6.1 (a)	3.1 ± 0.4 (b)
PVA	0.5 ± 0.3 (c)	22.5 ± 4.0 (b)	235.4 ± 26.1 (a)
CH	3.4 ± 0.1 (/)	50.4 ± 4.6 (/)	8.6 ± 3.3 (/)
BC/20PVA	5.2 ± 0.9 (a)	92.7 ± 15.1 (a)	3.5 ± 1.5 (b)
BC/40PVA	3.2 ± 0.6 (b)	20.0 ± 5.4 (b)	0.9 ± 0.1 (b)
BC/60PVA	0.8 ± 0.1 (c)	5.6 ± 1.7 (b)	1.1 ± 0.4 (b)
BC/80PVA	1.9 ± 0.4 (b, c)	12.8 ± 1.6 (b)	0.9 ± 0.1 (b)
BC/5CH	8.5 ± 0.2 (a)	92.9 ± 15.2 (a)	1.7 ± 0.4 (b)
BC/19PVA/5CH	6.4 ± 0.1 (a)	30.8 ± 0.6 (b, c)	0.9 ± 0.4 (b)
BC/38PVA/5CH	7.7 ± 0.1 (a)	45.4 ± 11.2 (b)	1.7 ± 1.4 (b)
BC/57PVA/5CH	0.6 ± 0.1 (b)	2.8 ± 0.2 (d)	0.7 ± 0.1 (b)
BC/76PVA/5CH	0.4 ± 0.1 (b)	7.3 ± 0.3 (d)	3.7 ± 0.3 (b)
PVA/5CH	0.5 ± 0.1 (b)	19.4 ± 3.1 (c, d)	71.3 ± 12.7 (a)

^aE: elastic modulus; UTS: ultimate tensile strength; ϵ_b : strain at break.

The thermograms of the BC/PVA films (Figure 4(a,b)) present the characteristic degradation peaks of the single components. The weight loss attributed to the dehydration of moisture is in the range of 3–5%, and a decreasing trend of the corresponding peak temperature is observed with the increasing PVA content. Then, the first degradation peak temperature of PVA (208.5 °C for neat PVA) is increased by 30 °C, indicating the stabilizing effect of BC, becoming more intense and narrow with increasing PVA concentration.⁴³ The third weight loss step, corresponding to BC degradation, is shifted to higher temperatures, confirming the enhancement of the thermal stability of the two components, as observed in previous works.^{37,38} In the case of BC/40PVA, BC/60PVA, and BC/80PVA, this peak presents a shoulder on the left side associated with the main degradation step of PVA, which is more evident with its increasing content. Finally, as the percentage of PVA is increased, the fourth degradation peak is sharper and the corresponding mass loss increases. The remaining mass at 700 °C generally decreases with PVA concentration and may be indicative of the effective content of PVA.

After the addition of chitosan (Figure 4(c,d)) an increase in moisture content is observed, possibly attributed to the hydrophilic nature of chitosan. Moreover, the degradation of both BC and PVA occurs at higher temperatures for sample BC/5CH and PVA/5CH, suggesting their strong interactions, as confirmed by the FTIR analysis (Section S1).^{25,42}

Comparing BC/PVA/CH and BC/PVA samples, temperatures corresponding to a mass loss of 1, 3, and 5% are increased, as well as the residual mass at 700 °C, confirming the enhancement of the thermal stability by chitosan addition. The increasing PVA content, as for samples BC/PVA, slightly decreases the thermal stability of the films.

The interaction among the different constituents was demonstrated through TGA analysis given the improved thermal stability of the blends compared to the neat constituents. The prepared films are suitable for packaging applications, since the thermal decomposition temperature is higher than the expected service temperature.

3.4. Tensile Tests

The representative stress–strain curves from uniaxial tensile tests performed at 10 mm/min on BC/PVA and BC/PVA/CH films are reported in Figure 5(a,b). All samples exhibit brittle behavior without any sign of plasticization.

BC cellulose exhibits a characteristic initial small toe region (up to a strain of 0.5%), after which cellulose fibers tend to align and the material stiffness steadily rises,⁵⁴ resulting in an elastic modulus of 6.0 GPa. Neat BC reaches tensile stress above 90 MPa, exceeding the values reported in previous studies on BC produced through static fermentation.^{39,44,64,69} This implies that wet mixing is a viable method for producing films of different sizes and thicknesses without compromising the BC mechanical

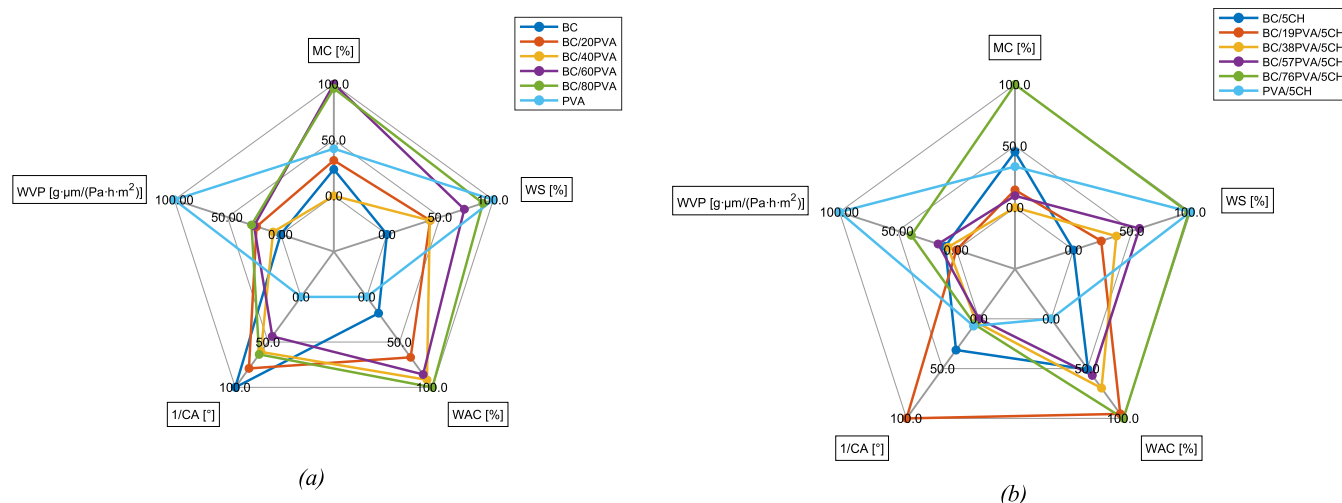


Figure 7. Spider plot of the water-related properties normalized between 0 and 100 of (a) BC/PVA samples and (b) BC/PVA/CH samples. The contact angle is represented as the complementary of the measured contact angle.

characteristics. The strain at break measured on neat BC is approximately 3%, in line with the literature.⁶⁴ On the other hand, the neat PVA film presents the highest elongation at break, with a value of 235% and a corresponding strength approximately equal to 22 MPa. However, when PVA is added to BC, no improvement in the deformability of the material can be appreciated. Lastly, chitosan presented an elastic modulus of 3.4 GPa, a deformation at break of 8.6%, and a corresponding strength of 50.5 MPa, higher than the values reported by Melro et al. for chitosan films produced with an acetic acid solution.⁵⁰

The comparison between the mechanical properties of BC/PVA films and BC/PVA/CH films as a function of the PVA content is presented in Figure 6 and Table 4. The addition of PVA to BC reflects in a marked decrease of the stiffness of the material, causing the elastic modulus to fall below 2 GPa, resembling the flexibility of PVA for concentrations higher than 40 wt %. On the opposite, the addition of CH leads to an enhancement of stiffness, confirming the interaction of chitosan with BC and PVA. According to Phisalaphong et al., the addition of chitosan generally improves the mechanical performance of BC, thanks to the strong binding by hydrogen bonds between their functional groups.²⁹ This is evident only at low PVA concentrations, probably due to the presence of defects and voids related to the processing route at higher PVA contents, as already depicted in Sections 3.1 and 3.2. The highest elastic modulus is shown by the BC/5CH film, reaching 8.5 GPa, followed by BC/38PVA/CH, BC/19PVA/CH, and neat BC films.

A decreasing tendency of the tensile strength is observed with the increasing PVA content, reaching a minimum of about 3–5 MPa at 60 wt % of PVA. A possible explanation for the decrease in stress at break with the addition of PVA is the large number of functional groups that can interact with water molecules, allowing an increase in water uptake. The correlation between mechanical properties and moisture content for BC/PVA blends was studied by Cazon, et. al, showing that as the relative humidity increases, the tensile strength of the material decreases.³⁹ The addition of chitosan does not evidently influence the strength of the composites.

Moreover, when PVA is added to BC a decrease in elongation at break is exhibited, presenting the minimum value of 0.9% for BC/40PVA. In general, all films show an elongation at break

between 0.5 to 4%, except PVA and PVA/5CH films which have an elongation of 235.4% and 71.3% respectively. No trend is observed as the amount of PVA is increased, while the addition of chitosan slightly reduces the maximum elongation. This can be attributed to the interaction of chitosan with BC and PVA, as depicted in FTIR analysis (Section S1), as well as to the highly interconnected structure of BC presenting low flexibility, further stiffened upon drying due to the formation of hydrogen bonds between cellulose fibers.

ANOVA statistical analysis highlights significant differences in elastic modulus, tensile strength, and ultimate tensile strain of BC/PVA and BC/PVA/CH films. According to Tukey analysis, all of the films can be grouped into two or three categories, resembling the behavior of BC, PVA, or a combination of the two, for all measured parameters.

In general, the influence of PVA and CH content on the mechanical properties of BC/PVA and BC/PVA/CH films underscores the complex interplay between polymer components and processing methods in determining film properties.

3.5. Water Vapor Permeability (WVP) and Water Interaction Properties

The WVP and water-related properties of BC/PVA and BC/PVA/CH films, normalized between 0 and 100, are reported in a spider plot in Figure 7. Numerical values of water-related properties are reported in Section S5.

For BC/PVA films, the WVP values are in the range of 0.7 and 1.6 g·μm/Pa·h·m²; the lowest value can be obtained for the neat BC film, while the PVA film presents the highest. As the amount of PVA is increased, an increase in WVP is observed, mainly attributed to the stronger interaction of PVA with ambient humidity. Water molecules interact with the amorphous part of the film rehydrating the cellulose ribbons. This interaction increases the size of the cellulose ribbons and generates higher free volume, allowing a larger flow of water vapor, as reported by Cazon et al. for BC/glycerol/PVA and BC/PVA/CH films.^{39,44} The interaction of water molecules and the increase in film thickness are confirmed by the aforementioned results. Moreover, as the geometrical density decreases due to the presence of defects and embedded porosity, the WVP increases by the presence of free space between the BC layers. On the opposite, WVP values of the BC/5CH film are lower than those of the BC film, which can be attributed to the reduction of empty spaces in

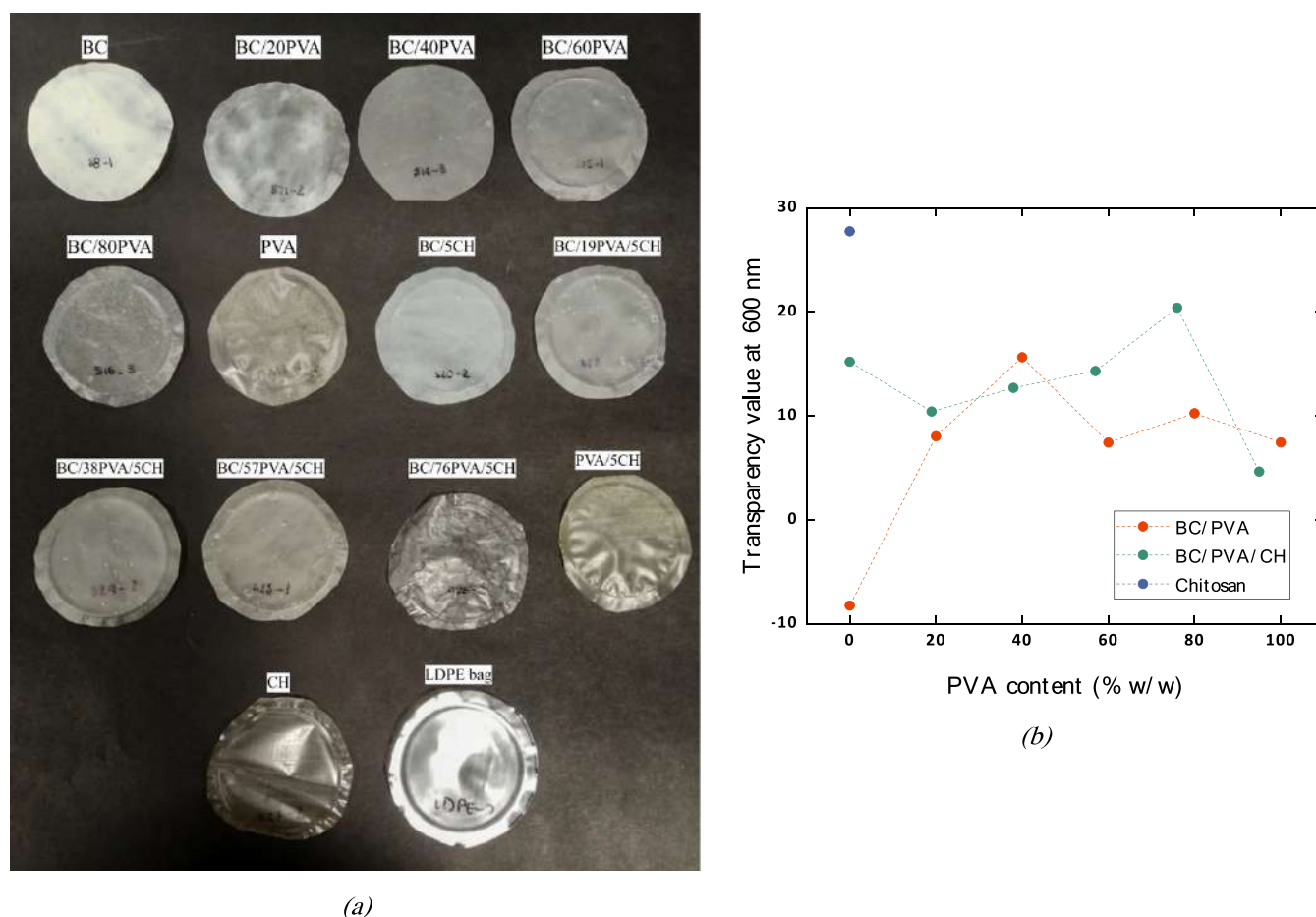


Figure 8. (a) Representative image of the prepared films (diameter approximately 5 cm) with different transparency values. (b) Transparency values at a wavelength of 600 nm for BC/PVA and BC/PVA/CH films.

the layered BC structure by the infiltration of chitosan. Moreover, the interaction between CH and PVA, demonstrated in the FTIR analysis (Section S1 and Table S1), could limit the possibility of interaction with water, as confirmed by the reduction of WVP by the addition of CH was already observed, for BC/PVA/CH films, by Kibédi-Szabó et al.⁷⁰ According to the statistical analysis, within BC/PVA and BC/PVA/CH sample groups, there is no significant difference in the mean values of WVP, with the exception of the PVA/5CH sample.

Finally, the LDPE film used as a reference does not show a dependence on ambient humidity conditions, and no change in the permeability value is evident during the measurement time, immediately reaching a steady state of the WVP. The WVP value of the LDPE film is $1.8 \times 10^{-3} \pm 0.5 \times 10^{-3} \text{ g} \cdot \mu\text{m} / \text{Pa} \cdot \text{h} \cdot \text{m}^2$, two orders of magnitude lower than the values of the produced films. A low WVP value could be advantageous for the storage of foods with low moisture content; however, it can be detrimental for fresh foods such as fruits and vegetables, accelerating ripening and decreasing their quality.⁷¹

3.6. Optical Properties

The transparency of the prepared samples is shown in Figure 8(a). A notable difference between the BC and PVA spectra is evident. The BC film exhibits transmittance below 1%, confirming its opacity, while the PVA film shows moderate transparency with transmittance values below 30%. The opacity of BC/PVA films is influenced by factors such as plasticizer

concentration, film homogeneity, surface properties, humidity, and water absorption.⁴⁴ The transmittance spectra of the prepared samples between 250 and 850 nm are reported in Figure S5.

The incorporation of PVA into BC enhances transmittance, consistent with the previous findings on BC/PVA/glycerol films.³⁹ In the UV range (100–400 nm), BC acts as an excellent UV barrier, making it highly suitable for food packaging applications to slow UV-sensitive food degradation. Specifically, composite films exhibit the lowest transparency, with transmittance below 10%, in the UV–C range (200–280 nm). Across all investigated wavelengths, chitosan demonstrates transmittance higher than that of PVA, reaching approximately 30%. However, when added to PVA, transmittance decreases due to surface imperfections, as seen in Section 3.2, increasing light scattering and enhancing UV protection in PVA-based films. Transparency values at 600 nm (visible range) are presented in Figure 8(b) (full spectra in Figure S5(a,b)). For BC/PVA films, transparency increases with PVA content up to 40 wt % but decreases at higher plasticizer levels due to surface imperfections. Similarly, BC/PVA/CH films exhibit increasing transparency with a higher PVA content. These results align with Cazon et al.⁴³ and confirm the possible application of prepared materials to prevent UV-light-induced oxidation. Overall, BC enhances UV protection, while PVA and chitosan improve film transmittance, although deviations may arise from surface defects and interactions with ambient humidity.

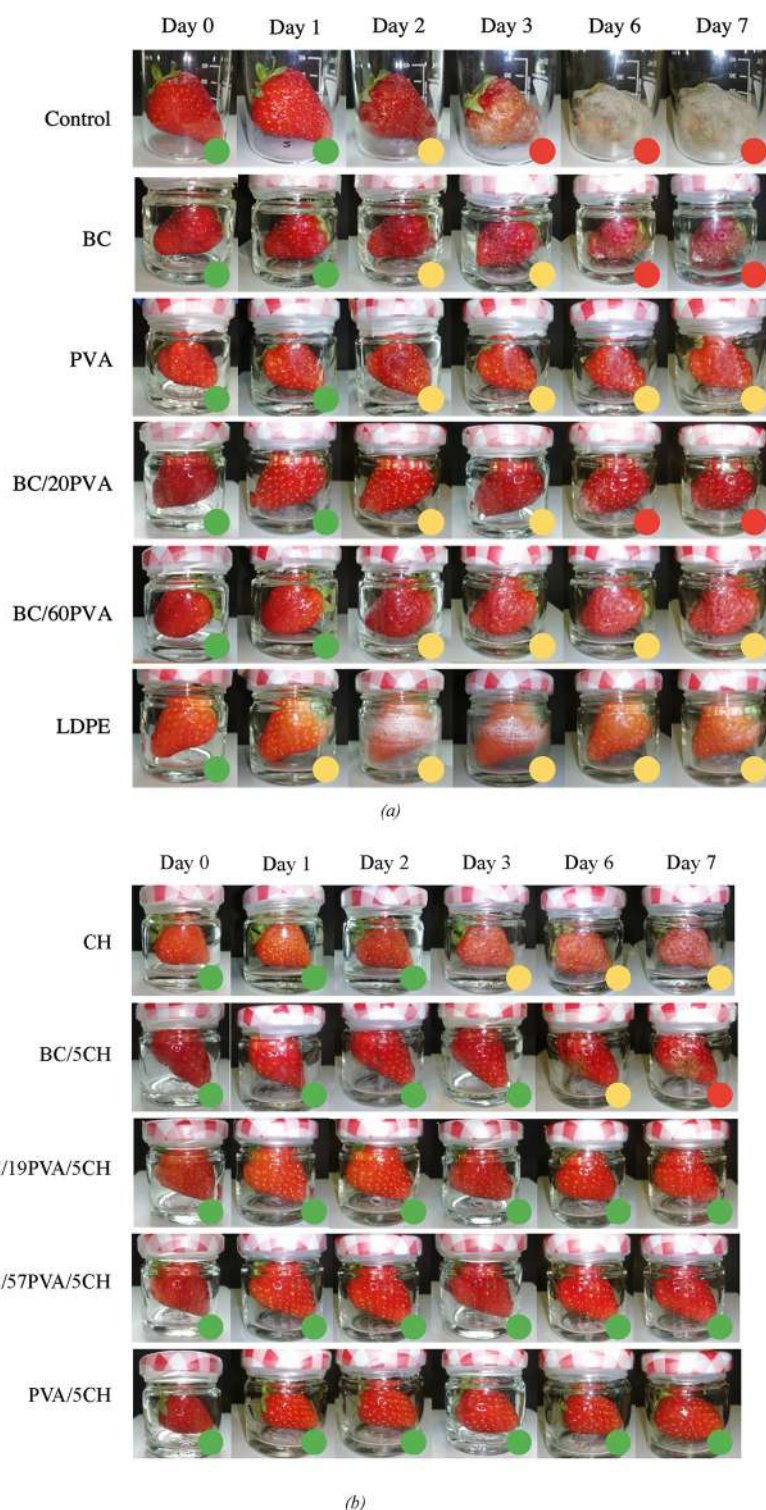


Figure 9. (a) Results of preservation of strawberries assay for control, BC, PVA, BC/20PVA, BC/60PVA, and LDPE samples. (b) Results of preservation of strawberries assay for CH, BC/5CH, BC/19PVA/5CH, BC/57PVA/5CH, and PVA/5CH samples. Markers of different colors to describe the deterioration process of the strawberry (green = no changes, yellow = change in color and/or texture, and red = fungus growth).

3.7. Strawberry Preservation Assay

During postharvest storage, strawberries are highly perishable and prone to dehydration, mechanical damage, and rapid deterioration. Under refrigeration with controlled humidity, they last 7–10 days, but this period significantly shortens at room temperature.⁷² This is evident in control samples exposed to air (Figure 9(a)), where color changes appear as early as Day

1 due to water loss. By Day 2, white fungal spots emerge on the seeds, progressing to brown rot by Day 3 as the fungus spreads. By Day 6, the fruit is entirely covered in a gray fungal layer (conidium) and the leaves wilt.

In contrast, BC films effectively act as a barrier against fungal growth, delaying their onset until Day 6. For the PVA film, due to the high WVP, mainly water desorption with slight color

changes is observed. No fungal presence is detected, but the film inflates, suggesting that PVA allows water vapor diffusion, while it limits the escape of decomposition gases (i.e., carbon dioxide and ethylene). For the chitosan film, despite its lower WVP than PVA, the amount of water desorbed by the strawberries is higher. This is due to its capability to absorb a significant amount of moisture from the environment, as proven by the high MC and reflected in film undulations. However, no fungal growth or decay signs are observed.

Commercial LDPE films, presenting poor water vapor permeability, trap moisture inside the container, leading to changes in texture and color in the strawberries. While no fungal growth is seen, fruit degradation occurs due to an increased temperature, moisture, and UV exposure.

The increasing PVA content in BC/PVA films delays fungal proliferation and decay, with the BC/60PVA film preventing fungal symptoms while avoiding inflation, indicating improved gas permeability with respect to neat BC. The addition of chitosan to BC postpones the fruit decay to Day 6 and the proliferation of the fungus to Day 7. The best performance can be observed for BC/19PVA/5CH and BC/57PVA/5CH films, demonstrating the positive action of the BC, PVA, and chitosan mixture (Figure 9(b)).

4. CONCLUSIONS

The objective of this study was to produce and analyze bacterial cellulose (BC) composite films for potential use in food packaging as substitutes for petroleum-derived plastics. Specifically, a wet mixing method was employed to blend BC, PVA, and chitosan. The films were divided into two groups: one with varying BC/PVA ratios and another with the same BC/PVA ratios but with the addition of 5 wt % chitosan.

SEM images reveal that the morphology of the BC film and the size of the cellulose ribbon characteristic of this material are not affected by the wet mixing process. For composite films, the presence of PVA and CH in the BC interlayer spaces can be observed, although porosity and inhomogeneities are present. The addition of chitosan increases the densification at PVA concentrations lower than 60 wt %, enhancing the film structure by reducing the porosity. Moreover, chitosan has a positive effect on the thermal stability of BC/PVA/CH films as it increases the onset of the degradation temperature. The interaction among the constituents is confirmed by ATR-FTIR analysis.

The water interaction of the prepared materials can be tuned by varying the PVA content: MC, WAC, WS, and WVP increased as the PVA content increases. Also, the addition of chitosan has a positive effect, stabilizing the MC, WAC, WS, and WVP at lower values. Neat BC shows a hydrophilic behavior with a CA lower than 65°, while the addition of PVA in BC/PVA films increases the contact angle up to 90°. The increase in the hydrophobic character of the material can be associated with both the chemical nature of PVA and the change in the roughness of the surface.

According to the tensile test results, the films present a brittle fracture and a decreasing tendency in the stress at break when increasing the concentration of PVA, with a minimum at 60 wt % of PVA. At higher concentrations, the stress at break increases. Additionally, a negative trend is evidenced for the elastic modulus as the amount of PVA increases. The addition of chitosan does not show a relevant change in elongation at break and strength, in correspondence with the values obtained in the

BC/PVA films. However, the presence of chitosan generally increases the stiffness of the prepared films.

The films seem to be suitable for food protection because of the low transmittance in the UV range, especially at higher BC concentrations. The study of the antimicrobial effect of films, evaluated by the capability of preservation of strawberries, provides evidence that the addition of both PVA and chitosan has a positive effect on the preservation of food, and the biodegradability test reveals that the durability of the film in soil can be tuned by varying the PVA content.

The present study showcases the potential of developing BC composite films with tunable properties for food packaging applications. Specifically, considering the different tests performed and the intended application of the films, it is possible to conclude that the films that present a better combination of properties are the samples with a PVA content lower than 60 wt % and containing 5 wt % of chitosan. Further analysis will be performed on the optimization of the casting and drying of the films, a critical process that influences the presence of defects and porosity. Moreover, a microbial growth analysis was performed to quantify the antimicrobial effect of the prepared packaging films and the contribution of chitosan.

■ ASSOCIATED CONTENT

Data Availability Statement

Data availability: data will be made available upon request.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.5c10875>.

Experimental results obtained from Fourier transform infrared (FTIR) spectroscopy, scanning electron microscope (SEM), molecular weight measurements, differential scanning calorimetry (DSC), moisture content (MC), water solubility (WS), water absorption capacity (WAC), contact angle (CA) measurements, UV–Vis spectroscopy, and soil burial tests (PDF)

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Notes

The authors declare no competing financial interest.

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Supplementary material

Bacterial cellulose/poly(vinyl alcohol)/chitosan films: tuning mechanical and functional properties for sustainable food packaging

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1 Fourier-transform infrared (FT-IR) spectroscopy

Fourier-transform infrared (FT-IR) spectroscopy was performed in attenuated total reflectance (ATR) mode on the surface of the prepared films with a Perkin-Elmer Spectrum One IR spectrometer (Perkin Elmer GmbH, Waltham, MA, USA), equipped with a ZnSe crystal. For each spectrum, a total of 100 scans were superimposed, in a wavenumber range of 650–4000 cm^{-1} (resolution = 4 cm^{-1}). Due to the rigidity of some films, the samples were slightly moistened to better adhere to the ZnSe crystal and consequently obtain clearer spectra. The ATR-FTIR baseline-corrected, and vertically shifted spectra of BC/PVA and BC/PVA/CH films are presented in *Figure S1*.

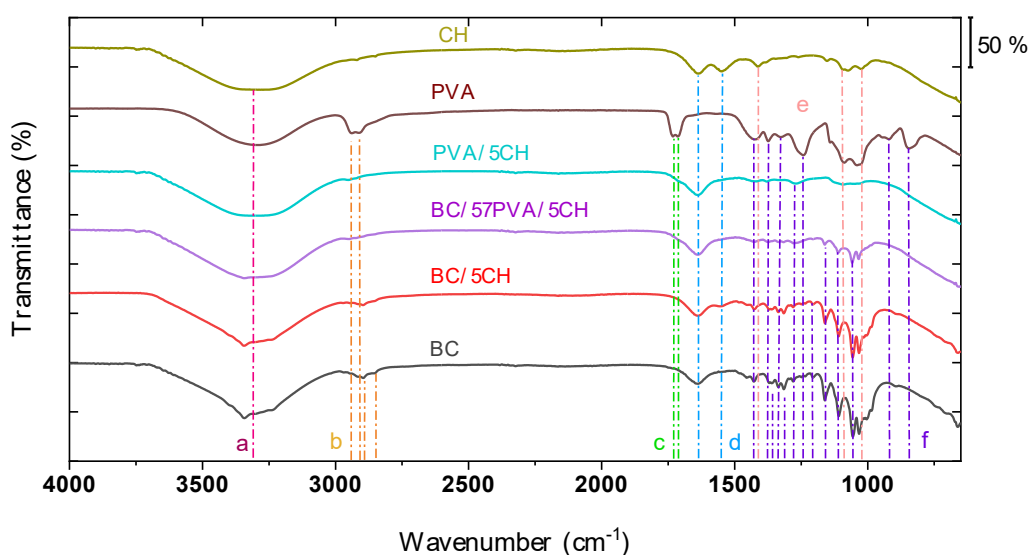


Figure S1: ATR-FTIR spectra of BC, BC/5CH, BC/57PVA/5CH, PVA/5CH, PVA, and CH films, where the main peaks are grouped into categories from “a” to “e”.

At first, a broad band is present at a wavenumber between 3000 and 3600 cm^{-1} corresponding to hydroxyl (-OH) stretching vibrations of water molecules contained in the samples. For BC film two narrow bands at approximately 2899 and 2848 cm^{-1} can be ascribed to vibrations resulting from symmetric and asymmetric -CH stretching respectively ¹. The addition of PVA to BC results in a slight displacement of these bands to higher wavenumbers with respect to BC film, probably related to the formation of hydrogen bonds ². At a wavenumber between 1730 and 1750 cm^{-1} , two peaks are observed in the PVA spectra related to the stretching of C=O and C-O. This bond is present in the PVA spectra due to the non-complete hydrolyzation process of acetate groups to the -OH bond. The intensity of these peaks is weak, indicating that only a few acetate groups are present in the polymeric chain ³. At 1650 cm^{-1} a lower intensity peak is observed in BC spectra related to -NH bending vibrations, probably due to the presence of residues from the culture medium and fermentation process ^{2, 4}. The absorption bands between 1000 and 1500 cm^{-1} are associated with the C-O-C pyranose ring stretching vibrations and C-H ring deformation for PVA and BC ^{2, 3}. Finally, by comparing all the samples, as the PVA content of the film increases, the signals resemble the peaks present in PVA spectra; the band visible of BC spectra at 1650 cm^{-1} is diminished, as well as the bands presented between 1000 and 1500 cm^{-1} .

For chitosan film a wide band at 3295 cm^{-1} is evidenced, corresponding to the -OH stretching that is also present in the other films. Two peaks at 1637 and 1549 cm^{-1} are indicative of -CH stretching and -NH bending respectively ⁵. In addition, three peaks between 1000 and 1500 cm^{-1} are observed. The first peak at 1412 cm^{-1} may be associated with CH₂ bending, the second one at 1096 cm^{-1} is ascribed to the asymmetric stretch of C-O-C and C-N, and the third peak at 1018 cm^{-1} corresponds to the skeletal vibration of CO stretching. When PVA and chitosan are combined (PVA/5CH film), the intensity of the peak at 3301 cm^{-1} is higher with respect to PVA and chitosan spectra, indicating the interaction of the components through strong hydrogen bonding between the -OH group of PVA and the -OH and NH₂ groups of chitosan ⁶. Likewise, for the BC/5CH film, the peak at 1549 cm^{-1} present in the chitosan spectrum is slightly visible, indicating the interaction of these two compounds ⁷. Finally, with BC/PVA/CH films, the intensity of the peak at a wavenumber range of 1710 to 1730 cm^{-1} , which is present in PVA spectra, decreases as the amount of PVA decrease in the film. Similarly, it can be shown that as PVA concentration rises, the number of C-C, and C-H associated peaks decreases, and their intensities shift in accordance with the PVA spectra.

The main peaks assignment is summarized in *Table SI*, grouped in sets of peaks following *Figure SI*.

Table S1: Main peak assignment (cm^{-1}) for FTIR analysis on BC, BC/5CH, BC/57PVA/5CH, PVA/5CH, PVA, and CH films.

Category	Assignment	BC	BC/5CH	BC/57PVA/5CH	PVA/5CH	PVA	CH
a	OH stretching	3346	3343	3342	3301	3292	3295
b	CH stretching	2899	2897	-	-	2939	-
		2488	-	-	-	2910	-
c	C=O and C-O stretching	-	-	-	-	1730	-
		-	-	-	-	1750	-
d	NH bending	1650	1636	1639	1638	-	1637
e	C-O-C and C-H stretching	1457	1455	1426	1426	1426	1412
		1428	1426	1375	1378	1372	1381
		1373	1370	1336	1270	1325	1261
		1358	1359	1317	1125	1241	1152
		1337	1335	1275	1096	1142	1096
		1314	1314	1206	1056	1088	1071
		1280	1281	1162	1030	1041	1024
		1251	1249	1111		1026	
		1235	1234	1058			
		1207	1206	1033			
		1160	1160	1015			
		1056	1108	1003			
		1031	1057				
		1015	1032				
		1003	1012				
			1003				

2 Scanning electron microscope (SEM)

A SEM image of the cross section of sample BC/57PVA/5CH is reported in *Figure S2*.

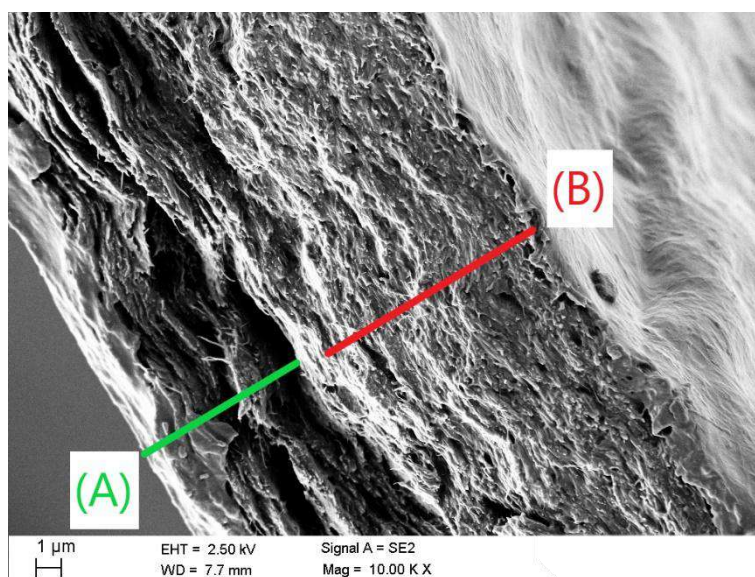


Figure S2: SEM micrograph of the cryofractured surface of BC/57PVA/5CH film. (A) Green segment indicating the upper section of the film in contact with air, (B) red segment indicating the lower section of the film in contact with mold.

In this SEM micrograph, two possible sections can be identified: section (A), which is the part in contact with the air exhibits larger pores, higher roughness, and the characteristic layered structure of BC, and section (B), the section in contact with the mold which shows a denser and more compact structure as a result of the material precipitating during drying.

3 Molecular weight

The molecular weight (MW) of BC was evaluated by measuring its intrinsic viscosity using a Tube viscometer with a single point determination approach, as described in ⁸. Specifically, the MW was calculated through Mark–Houwink–Sakurada equation, employing the constants derived in ⁹ for BC in a 1 M copper (II) ethylenediamine (CED) solution. The BC hydrogel was subjected to a mechanical treatment to produce a BC suspension, which was freeze-dried to obtain an aerogel. The aerogel was dissolved in the CED solution at a concentration of 0.005 g/mL. The average flow time of the reference CED solution (t_0) and the CED solutions of BC (t_{BC}) were measured using a Ubbelohde viscometer equipped with a 531-13 capillary and a thermostatic bath at 25 °C. The relative viscosity (η_r) and specific viscosity (η_{sp}) of the CED solutions of BC were calculated through *Equation S1* and *S2*, respectively ¹⁰.

$$\eta_r = \frac{t_{BC}}{t_0} \quad S1$$

$$\eta_{sp} = \eta_r - 1 \quad S2$$

Then, the intrinsic viscosity (η , [mL/g]) of the CED solution of BC was obtained through Solomon-Ciuta equation, as reported in *Equation S3* ¹¹.

$$\eta = \frac{\sqrt{2 \cdot (\eta_{sp} - \ln(\eta_r))}}{c} \quad S3$$

Where c is the concentration of BC (0.005 g/mL). The Mark–Houwink–Sakurada equation was used to calculate the MW, as reported in *Equation S4* ¹².

$$\eta = K \cdot MW^\alpha \quad S4$$

Where K [mL/g] and α are Mark–Houwink–Sakurada constants investigated in ⁹, respectively equal to 0.3752 mL/g and 1.4532. Finally, the degree of polymerization (DP) was calculated via *Equation S5*, considering a molecular weight of one unit of cellulose equal to 0.162 kDa ¹³.

$$DP = MW/0.162 \quad S5$$

The measured flow time of the reference CED solution (t_0) and the CED solutions of BC (t_{BC}) were equal to 65 ± 0 s and 1060 ± 45 s, respectively. The intrinsic viscosity of the CED solution of BC was calculated, yielding a MW of 228 ± 4 kDa and a corresponding DP of 1408 ± 25 , in agreement with values reported in the literature for purified BC ¹³.

4 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was carried out with a Mettler DSC30 calorimeter (Mettler Toledo, Columbus, OH, USA) on specimens of approx. 10 to 15 mg cut from the prepared films. The

tests were performed through a heating–cooling–heating cycle at 10 °C/min between 0 °C and 250 °C, under a nitrogen flow of 100 mL/min. One specimen per composition was measured. The test allowed the determination of the glass transition temperature (T_g) and the melting, crystallization, and cold crystallization temperatures, and the respective enthalpies (T_m , T_c , T_{cc} , H_m , H_c , H_{cc}) of the different components. The crystallinity degree of the PVA fraction (X_c) was calculated following *Equation S6*.

$$X_c (\%) = \frac{\Delta H_m - \Delta H_c}{\Delta H_0 * W_{PVA}} * 100 \quad S6$$

where ΔH_0 refers to the theoretical melting enthalpy of PVA, which is equal to 138.6 J/g¹⁴, and W_{PVA} is the nominal weight fraction of PVA in the sample. Moreover, the water percentage [wt%] was calculated according to *Equation S7*.

$$Water \% = \frac{\Delta H_e}{\Delta H_{et}} * 100 \quad S7$$

where ΔH_e is the enthalpy values of the peaks related to dehydration of water in the first heating scan and ΔH_{et} refers to theoretical evaporation enthalpy of water, which is equal to 2260 J/g¹⁵.

BC, PVA, and chitosan DSC thermograms for the first heating, cooling, and second heating scans are reported in *Figure S3*.

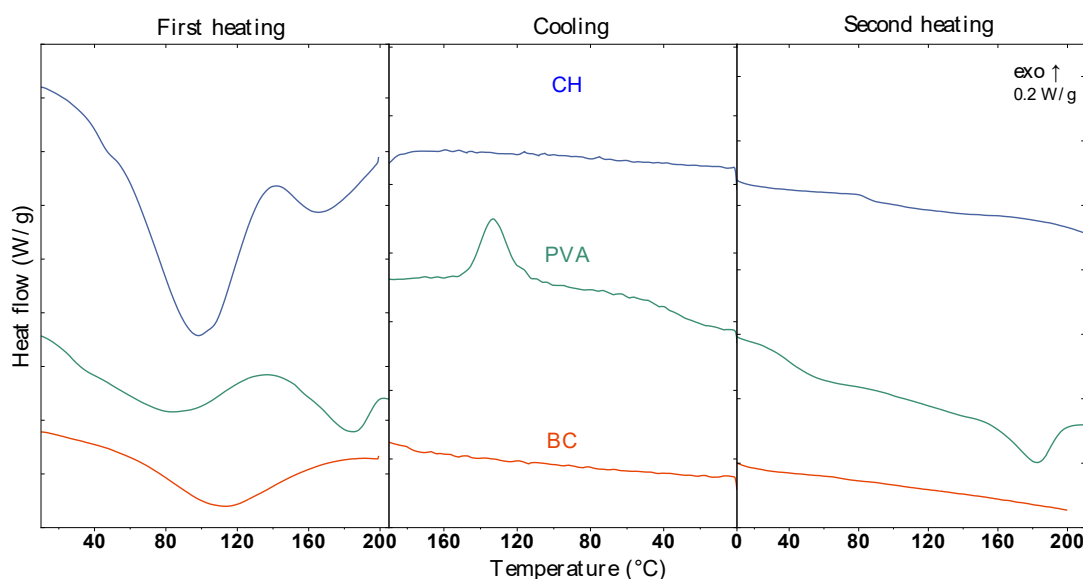


Figure S3: DSC thermograms of BC, PVA, and chitosan films (first heating scan, cooling scan, and second heating).

In the first heating scan, an endothermic peak is observed for BC, PVA, and CH, attributed to the evaporation of water. Neat BC presents a water content of around 0.8 %. No cold crystallization peak or melting peak is observed in the cooling and second heating scans but a glass transition temperature (T_g) is present for neat BC at 66 °C, only visible in the second heating scan. This temperature is higher with respect to the values found in the literature, which are in the range of 41 to 44 °C^{16,17}, probably due to the different bacterial strain cultures and to the percentage of amorphous phase in the material, related to changes in molecular mobility after chemical treatment¹⁷. The PVA film presents a T_g at 26.0 °C during the first heating scan and at 38.7 °C during the second heating scan related to the amorphous part of the material, similar to the temperatures reported in literature^{14,18}. The variation between first heating and second heating scans is attributed to the influence of absorbed water, which increases the mobility of individual polymer chains¹⁹. Then, two endothermic peaks are present in the first heating part. The first peak is related to the dehydration process of water, with a water percentage of around 2.0 %. The second peak with a T_m of 181.3 °C and a melting enthalpy (ΔH_m) of 26.7 J/g is assigned to the melting process of the crystalline part of PVA, in accordance with previous studies¹⁸.

During the cooling scan, a cold crystallization peak is present with a crystallization temperature (T_c) equal to 134.2 °C, and a crystallization enthalpy (ΔH_c) of 28.7 J/g. Then, in the second heating scan, the T_m is observed at 181.1 °C with a ΔH_m of 28.7 J/g. The crystallinity of PVA, evaluated through the measured melting and crystallization enthalpies, is equal to 19.2 % and 20.7 % respectively in the first and in the second heating scan. These values are lower than the values reported in literature (30

- 33 %) ²⁰. The explanation behind this discrepancy is related to the decrease in crystallinity after a long time of storage and the influence of water uptake.

The chitosan thermogram presents two endothermic peaks in the first heating scan, respectively related to the dehydration of water filling the free volume and the evaporation of bonded water ²¹. No cold crystallization peak or melting peak is observed in the cooling and second heating scans. The glass transition temperature of chitosan is equal to 43.2 °C for the first heating, and 81.6 °C for the second heating scan. The variation between these values is related to the presence of water that acts as a plasticizer in chitosan. Moreover, the T_g values are lower compared with the values found in the literature (110 - 140 °C) ²². The discrepancy in the values is likely related to the degree of acetylation and moisture content of the samples.

The main results of DSC analysis performed on BC, PVA, and CH are reported in *Table S2*.

Table S2: Main results of DSC tests performed on BC, PVA, and chitosan films.

Sample	BC			PVA			CH		
	1H	C	2H	1H	C	2H	1H	C	2H
T_g (°C)	-	-	66.1	26.0	-	38.37	43.2	-	81.6
T_{endo} (°C)	113.0	-	-	82.6	-	-	97.6	-	-
ΔH_{endo} (J/g)	17.8	-	-	44.9	-	-	166.6	-	-
T_m (°C)	-	-	-	181.3	-	181.1	165.5	-	-
ΔH_m (J/g)	-	-	-	26.7	-	28.7	15.4	-	-
T_c (°C)	-	-	-	-	134.2	-	-	-	-
ΔH_c (J/g)	-	-	-	-	28.7	-	-	-	-
X_c (%)	-	-	-	19.2	-	20.7	-	-	-
Water (%)	0.8	-	-	2.0	-	-	8.1	-	-

1H: first heating scan; C: cooling scan; 2H: second heating scan; T_g : glass transition temperature; T_{endo} and ΔH_{endo} : temperature and enthalpy for the first endothermic peak respectively; T_m and ΔH_m : temperature and enthalpy for the second endothermic peak respectively; T_c : crystallization temperature; ΔH_c : crystallization enthalpy; X_c : crystallinity degree; Water %: percentage of water in the sample.

DSC thermograms for the first heating, cooling, and second heating scans of BC/PVA and BC/PVA/CH samples are reported in *Figure S4*.

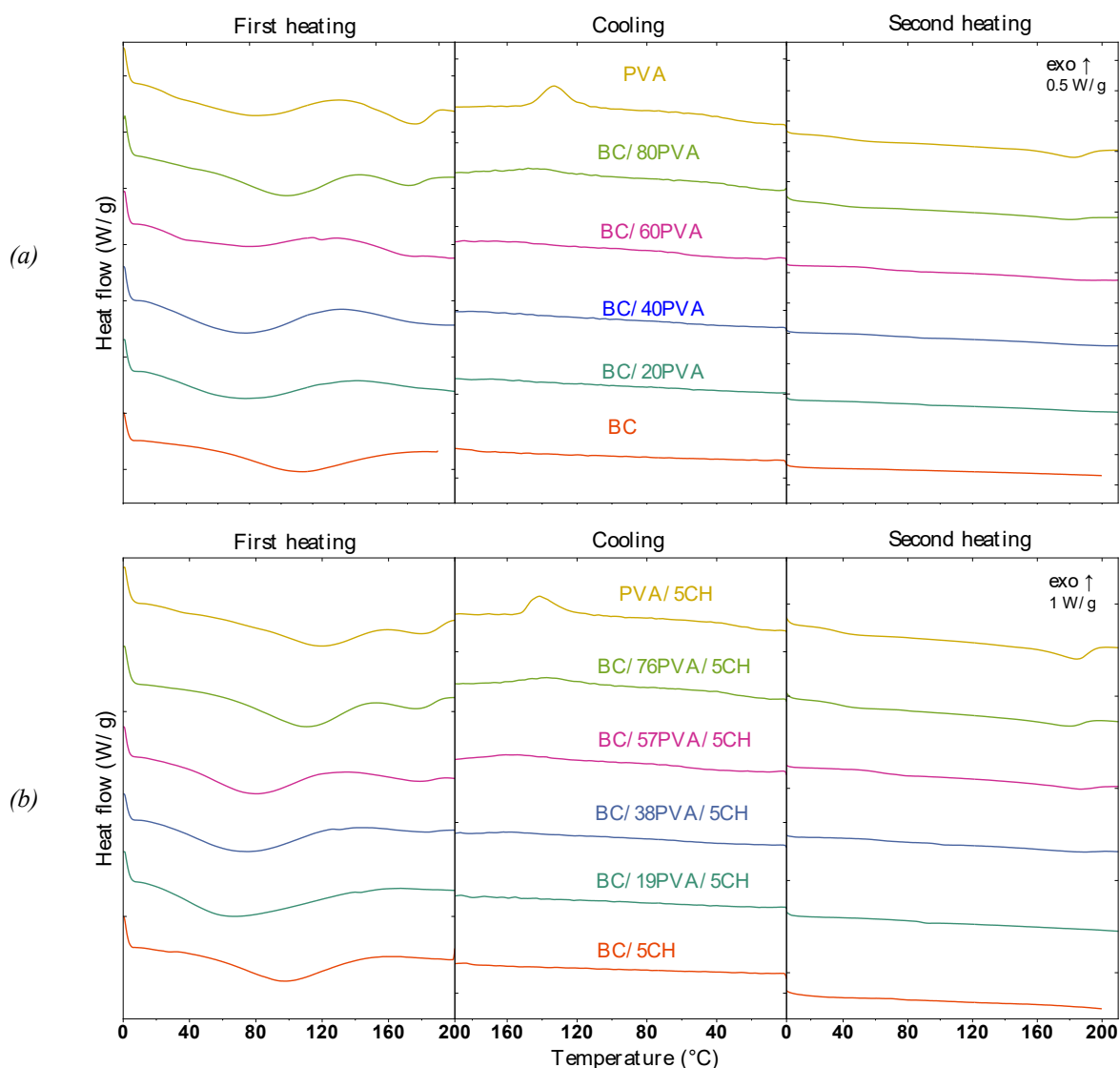


Figure S4: DSC thermograms of (a) BC/PVA films and (b) BC/PVA/CH films.

For BC/PVA films, all samples exhibit two endothermic peaks during the first heating scan; the first peak is ascribed to the dehydration of absorbed water, while the second peak is attributed to the melting of the crystalline phase of PVA. Compared to the neat BC and PVA films, the endothermic peak related to the evaporation of water is shifted to lower temperatures, while the enthalpy for this thermal change is increased suggesting that a higher water content is present in the samples. The T_m values in the second heating scan are in the range of 177 to 184 °C and the ΔH_m is increased as the content of PVA is increased in the film. The crystallinity degree of BC/PVA blends in the first heating scan are lower than the values obtained for neat PVA film; nevertheless, no trend is observed concerning PVA concentration. The highest crystallinity degrees are obtained for BC/40PVA and BC/80PVA films, with values of 11.2 and 11.6 % respectively. These results can be attributed to the BC interaction with the hydroxyl radical of PVA and the chain entanglement which reduces the

crystallinity degree. Other studies confirm that when BC content is increased, the PVA crystallinity degree is decreased²³. In the cooling scan, one exothermic peak related to the crystallization of PVA is observed for all BC/PVA blends, the corresponding T_c are shifted to higher values, but the ΔH_c is decreased for all samples in comparison with neat PVA film. During the second heating, the T_m values of PVA are shifted to higher values compared to neat PVA film, indicating strong intermolecular forces between BC and PVA, mainly related to the possible formation of hydrogen bonds between functional groups. The crystallinity degree presents an upward trend as the PVA percentage is increased in the film. For blended films, further analysis can be made considering the miscibility of the components in the blend since the difference between their T_g is high. The glass transition temperatures of all samples during the first heating scan are hidden by the presence of the first endothermic peak, but the changes in T_g during the second heating scan enable the evaluation of the miscibility of the components. Two T_g can be identified, the first corresponding to PVA and the second to BC, suggesting the immiscibility of the components. However, both T_g are shifted to higher temperatures with respect to the neat constituents, indicating a certain interaction between BC and PVA which generates strong bond formation, decreasing the molecular chains mobility, which leads to a higher glass transition temperature. Lastly, a trend is present for both glass transition temperatures of all samples; as the percentage of PVA is increased in the blend, the glass transition temperatures tend to decrease.

The addition of CH leads to slight variations of the DSC thermograms, suggesting a certain interaction between the different constituents. During the first heating scan, the evaporation enthalpy of water and the corresponding temperature in BC/PVA/CH films is found to be higher than that of BC/PVA films, suggesting a higher moisture content ascribed to the water uptake capacity of chitosan².

For the second endothermic peak, related to the melting of the crystalline phase, no relevant variation in the melting temperatures is observed; however, a reduction in the crystallinity degree is observed both for neat PVA film and for BC/PVA/CH films. Specifically, the crystallinity of PVA film decrease from 19.2 % to 10.9 % for PVA/5CH film while an outstanding behavior can be seen in BC/19PVA/5CH film, where no crystallization peak is observed during the first and second heating scans. The highest crystallinity degrees are obtained for BC/57PVA/5CH and BC/76PVA/5CH films, with a value of 10.6 and 10.1 % respectively. These results can be related to the plasticizing effect of chitosan, which interferes with the ordered structure of PVA chains, decreasing the crystalline degree. In the cooling scan, a single exothermic peak correlated to the crystallization of PVA is observed for all the samples with the exception of BC/19PVA/5CH. The crystallization temperatures are shifted to lower temperatures, but the enthalpy of crystallization is increased compared with the correspondent BC/PVA film counterparts.

During the second heating, all the films except BC/19PVA/5CH present two T_g , which primarily suggests the partial miscibility of the three compounds in the blend. The first T_g corresponds to the glass transition temperature value of PVA and exhibits a decreasing tendency as the PVA content is increased in BC/PVA/CH films, resembling the temperature obtained for neat PVA. The second glass transition temperature related to BC also exhibits a reducing trend as the PVA content is increased. For neat BC films, instead the T_g is approximately 7 °C higher compared to neat BC film; this increase indicates that the reduced mobility of the polymeric chains due to the formation of hydrogen bonds between CH and BC. Moreover, a single endothermic peak is seen regarding the melting of the crystalline part of PVA with no discernible variation in the melting temperature with respect to BC/PVA films, showing that the crystalline morphology could still be maintained ²⁴. Considering PVA/5CH film, PVA crystallinity is reduced in the second heating scan, due to the plasticizing effect of CH. This behavior was also observed for Abdeen Z., et al. ²⁵, where a lower value of melting enthalpy was recorded for PVA upon the addition of chitosan. In the case of BC/PVA/CH samples, a different trend is observed: in general, the addition of chitosan causes an increase in the crystallinity of PVA for with respect to BC/PVA samples, and a rising tendency is observed in the crystallinity with the increasing PVA content. The highest degrees of crystallinity are observed in BC/57PVA/5CH and BC/76PVA/5CH films with a value of 15.4 % and 15.3 % respectively, higher than the results of BC/60PVA and BC/80PVA films. Main results of DSC analysis performed on BC/PVA and BC/PVA/CH are reported in *Table S3*.

Table S3: Main results of DSC tests performed on all BC/PVA and BC/PVA/CH samples.

Sample		T_g 1 (°C)	T_g 2 (°C)	T_{endo} (°C)	ΔH_{endo} (J/g)	T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)	X_c (%)	Water (%)
BC/20PVA	1H	-	-	73.5	87.8	184.8	0.9	-	-	3.4	3.9
	C	-	-	-	-	-	-	168.4	1.0	-	-
	2H	66.1	88.9	-	-	187.3	1.0	-	-	3.6	-
BC/40PVA	1H	-	-	73.5	100.2	181.3	6.2	-	-	11.2	4.4
	C	-	-	-	-	-	-	180.7	4.5	-	-
	2H	66.1	85.9	-	-	186.0	4.3	-	-	7.7	-
BC/60PVA	1H	-	-	75.1	51.3	177.1	4.5	-	-	5.4	2.3
	C	-	-	-	-	-	-	184.5	6.0	-	-
	2H	58.7	84.7	-	-	183.3	4.4	-	-	5.3	-
BC/80PVA	1H	-	-	78.1	72.1	182.9	12.9	-	-	11.6	3.2
	C	-	-	-	-	-	-	157.8	16.2	-	-
	2H	57.3	80.0	-	-	178.4	15.6	-	-	14.1	-
BC/5CH	1H	-	-	96.5	88.7	-	-	-	-	-	3.9
	C	-	-	-	-	-	-	-	-	-	-
	2H	73.8	-	-	-	-	-	-	-	-	-
	1H	-	-	64.9	144.0	-	-	-	-	-	6.4

BC/19PVA/ 5CH	C	-	-	-	-	-	-	-	-	-	-
	2H	88.2	-	-	-	-	-	-	-	-	-
BC/38PVA/ 5CH	1H	-	-	74.3	101.1	182.0	4.0	-	-	7.6	4.5
	C	-	-	-	-	-	-	162.0	4.5	-	-
BC/57PVA/ 5CH	2H	63.1	99.2	-	-	182.2	4.2	-	-	8.1	-
	1H	-	-	78.0	93.2	175.1	4.5	-	-	10.6	4.1
BC/76PVA/ 5CH	C	-	-	-	-	-	-	154.5	13.2	-	-
	2H	59.8	75.1	-	-	185.6	12.2	-	-	15.4	-
PVA/5CH	1H	-	-	107.5	87.6	177.3	10.6	-	-	10.1	3.9
	C	-	-	-	-	-	-	137.9	16.9	-	-
PVA/5CH	2H	59.8	75.1	-	-	185.6	12.2	-	-	15.3	-
	1H	31.1	-	116.8	49.3	181.5	10.4	-	-	10.9	2.2
PVA/5CH	C	-	-	-	-	-	-	142.4	24.4	-	-
	2H	35.3	-	-	-	183.2	23.5	-	-	17.9	-

1H: first heating scan; C: cooling scan; 2H: second heating scan; T_g : glass transition temperature; T_{endo} and ΔH_{endo} : temperature and enthalpy for the first endothermic peak respectively; T_m and ΔH_m : temperature and enthalpy for the second endothermic peak respectively; T_c : crystallization temperature; ΔH_c : crystallization enthalpy; X_c : crystallinity degree; Water %: percentage of water in the sample.

5 Water interaction properties

The water interaction properties of the prepared films, namely moisture content (MC)¹³, water solubility (WS)¹³, water absorption capacity (WAC)¹³, and contact angle (CA) [°], along with water vapor permeability (WVP)¹³, are reported in *Table S4*.

5.1 Moisture content (MC)

Moisture content (MC) was determined considering the mass change of the sample, stored at ambient conditions (23 °C and 56 r.h. %), after all the contained moisture was dried out. Square samples with 2-cm sides were weighed in a Gibertini E42 balance and then dried at 80 °C for 24 h in a vacuum oven. The MC was calculated according to *Equation S8*:

$$MC(\%) = \frac{W_0 - W_1}{W_0} \cdot 100 \quad S8$$

where W_0 is the initial mass before drying and W_1 is the mass after drying. All measurements were made in triplicate.

The moisture content, closely linked to the existence of polar groups capable of interacting with water, could affect several characteristics of the material, particularly the mechanical properties. Among the neat constituents, BC film presents the lowest MC, equal to $2.5 \pm 0.8 \%$, followed by PVA and chitosan films, which values are around $4.0 \pm 0.8 \%$ and $15.1 \pm 2.2 \%$ respectively. The high MC of chitosan is related to its chemical nature, as previously observed in other studies^{26, 27}.

For BC/PVA films, an increasing tendency is present with the PVA content, reaching the maximum value for BC/60PVA sample at 8.4% , increased approx. by 300% with respect to neat BC. The higher MC of the prepared composite films concerning the neat constituents may be related to the presence of porosity, increasing the available surface for water absorbance, as discussed in Section 3.1 and corroborated by the DSC analysis (Section S3). The high moisture content of samples containing 60 wt% and 80 wt% of PVA, is reflected in the poor strength measured during the tensile tests, confirming the plasticizing effect of water, already discussed in Section 3.4 and previously observed by Cazon et. al²⁸.

When chitosan is added, a more constant value of the moisture content is evidenced. For certain samples, higher values of MC are obtained mainly due to the large amount of hydrophilic amino and hydroxyl groups present in chitosan²⁶. Nevertheless, for samples containing 60 wt% and 80 wt% of PVA, chitosan reduces the moisture content probably due to the reduction of macroscopic defects and air bubbles and to the reciprocal interaction between BC, PVA, and CH, reducing the possibility of binding with water^{6, 29}. The FTIR analysis (Chapter S1, Supplementary material), confirms the reduced availability of OH groups due to the interaction between BC, PVA, and CH. According to the statistical analysis, for BC/PVA samples at least one film mean is significantly different from the others while for BC/PVA/CH films there are no significant differences between the mean MC values of different samples.

5.2 Water solubility (WS)

Water solubility (WS) was measured on 2-cm side square specimens. Dry samples (vacuum, $80 \text{ }^{\circ}\text{C}$, 24 h) were weighed and placed in 100 mL distilled water at $25 \text{ }^{\circ}\text{C}$ for 24 h, then dried again at $80 \text{ }^{\circ}\text{C}$ for 24 h and weighed. The WS was calculated using *Equation S9*.

$$WS(\%) = \frac{W_1 - W_2}{W_1} \cdot 100 \quad \text{S9}$$

where W_1 is the dry weight before immersion and W_2 is the dry weight after final drying. All measurements were made in triplicate.

The water solubility test revealed that BC can be regarded as insoluble, corroborating findings from other studies where BC solubility values in water were below 10%^{26, 30}, while for PVA film, a complete dissolution is observed. As the PVA concentration is increased, an upward trend is observed in water solubility for both types of films, reaching higher values than the PVA content in the sample. These results suggest that BC and PVA interactions promote the dispersion of BC.

For chitosan film, a water solubility value of around 8.6 ± 0.5 % is observed. When chitosan is added, a decrease in water solubility is evidenced in comparison with BC/PVA films at all PVA concentrations. This suggests that CH interacts with both BC and PVA, decreasing the water solubility and increasing the preservation capability of the film. Tukey's analysis corroborates that almost all BC/PVA samples exhibit a significant difference in WS mean values from the others, as confirmed by the statistical analysis (F-value > F-critical value and p-value < p-critical).

5.3 Water absorption capacity (WAC)

Water absorption capacity (WAC) was calculated as described in³¹. Dry samples (vacuum, 80 °C, 24 h) with 2-cm sides were weighed and immersed in 100 mL of distilled water. The wet samples were gently dried with tissue paper and weighed every 20 min of immersion until the difference in subsequent measurements was less than 2%. The WAC was calculated via *Equation S10*:

$$WAC(\%) = \frac{W_h - W_d}{W_d} \cdot 100 \quad S10$$

where W_d is the dry weight before the water immersion and W_h is the hydrated weight after water immersion. All measurements were made in triplicate.

Since PVA film is completely dissolved before the first WAC measurement (20 min), no measurements could be made. For BC/PVA films, an increase in water absorption is observed as the PVA content increased due to its hygroscopic properties. The addition of only 20 wt% of PVA to BC leads to an increase in WAC of approx. 300 %, in accordance with the MC measurements. The maximum value can be obtained by BC/80PVA film with a value of approx. 683 ± 4 %, but no

significant changes in film dimensions or swelling is observed. Likewise, the high deviation presented for some of the films is related to the loss of weight due to the solubilization of the material during the measurement. This is reflected in the statistical analysis since at least one film mean is significantly different from the others.

Neat CH film presents a WAC of 500 ± 5 %, 40 times higher than BC film, due to its high degree of swelling related to a large number of available hydrophilic groups³². The addition of CH to neat BC causes an increase in WAC up to approx. 190 %, consistent with previous works³¹. Additionally, comparing the BC/PVA and BC/PVA/CH films, chitosan has a stabilizing effect on water absorption in a range between 180 % and 240 %. This is advantageous for controlling water absorption-related features at various PVA concentrations. Nevertheless, this result is unexpected since CH presents a higher MC than PVA and BC and was previously demonstrated to enhance the WAC and swelling of BC/PVA films³³; a possible explanation could rely on the improved homogeneity of the films after the addition of CH and the consequent reduction of imperfections and porosity where water could be easily absorbed. The statistical analysis evidences that there are no significant differences between WAC values of the samples.

5.4 Contact angle (CA)

Contact angle (CA) is a quantitative measure that enables the determination of the wettability of a solid by a liquid: CA greater than 90° is evidence of hydrophobic surfaces, while CA less than 90° indicates hydrophilic surfaces. The CA evaluation was performed using a specific setup composed of a Dino-Lite Digital Microscope by AnMO Electronics Corporation (Sanchong Dist., Taiwan), a VL49 RGB lamp by Shenzhen Ulanzi Technology (Sheung Wan, Hong Kong), and a mobile platform. A sessile drop of deionized water (5 μ L) was placed on the target samples (2 x 1 cm²) using a micropipette, and an image was captured, focused on the water drop. Five measurements from five different random regions of each sample were made. Then, the measurement of CA was performed with ImageJ[®] software (v. 1.53) and Dropsnake plugin.

The contact angle measurement enables to determine the hydrophobic properties of the material surface, which is influenced by the chemical nature of the material and the surface roughness. For BC/PVA films, as the PVA concentration is increased, the surface presents a more hydrophobic behavior with an increase in the CA, as evidenced in *Table S4*. The PVA, CH, and BC/60PVA films exhibit contact angles greater than 90° , suggesting that these films present hydrophobic surfaces; this behavior can be a consequence of the change of surface roughness at a micro and nano level produced

during casting and drying. Tukey's analysis results evidences that the contact angle values for BC, and PVA films are significantly different from the other films, as confirmed by the statistical analysis ($F\text{-value} > F\text{-critical value}$ and $p\text{-value} < p\text{-critical}$).

As for MC and WAC, chitosan seems to perform a stabilizing effect on water-interaction properties moving the values of the CA approx. at 85° for BC/PVA/CH samples. In any case, the hydrophilic behavior of the BC-chitosan mixture was also evidenced by Liu et.al¹. An outstanding point is observed for BC/19PVA/5CH film, value significantly different from the others according to the statistical analysis and Tukey's analysis.

Table S4: Main properties related to the interaction of water with the produced films along with Tukey's analysis results in brackets.

Sample	MC (%)	WS (%)	WAC (%)	CA ($^\circ$)	WVP ($\text{g}\cdot\mu\text{m}/\text{Pa}\cdot\text{h}\cdot\text{m}^2$)
BC	2.6 ± 0.8 (b, c)	0.2 ± 0.4 (e)	124.2 ± 6.2 (b)	64.5 ± 3.4 (d)	0.73 ± 0.05 (a)
PVA	4.0 ± 0.8 (b)	100.0 ± 0.0 (a)	/	110.5 ± 5.8 (a)	1.57 ± 0.43 (a)
CH	15.1 ± 2.2 (/)	8.6 ± 0.5 (/)	500.2 ± 5.2 (/)	121.6 ± 6.1 (/)	0.99 ± 0.31 (/)
BC/20PVA	3.2 ± 1.2 (b, c)	40.7 ± 6.1 (d)	457.3 ± 36.1 (a)	74.1 ± 6.0 (c, d)	0.92 ± 0.03 (a)
BC/40PVA	0.8 ± 0.8 (c)	40.5 ± 1.7 (d)	627.8 ± 3.6 (a)	82.5 ± 5.9 (b, c)	0.79 ± 0.22 (a)
BC/60PVA	8.4 ± 1.0 (a)	72.9 ± 4.9 (c)	587.6 ± 86.9 (a)	90.4 ± 4.8 (b, c)	0.94 ± 0.08 (a)
BC/80PVA	8.1 ± 0.1 (b)	90.2 ± 2.8 (b)	683.8 ± 4.0 (a)	81.2 ± 4.5 (b, c)	0.96 ± 0.08 (a)
BC/5CH	5.0 ± 0.5 (a)	1.7 ± 0.8 (d)	189.5 ± 12.4 (a)	78.3 ± 4.7 (a)	0.64 ± 0.10 (b)
BC/19PVA/5CH	3.7 ± 0.8 (a)	21.5 ± 1.9 (c)	226.3 ± 95.6 (a)	60.4 ± 4.8 (b)	0.56 ± 0.22 (b)
BC/38PVA/5CH	3.1 ± 0.6 (a)	32.4 ± 3.7 (c)	204.9 ± 0.5 (a)	85.6 ± 6.9 (a)	0.62 ± 0.05 (b)
BC/57PVA/5CH	3.5 ± 1.3 (a)	49.0 ± 4.2 (b)	194.4 ± 1.3 (a)	86.5 ± 3.4 (a)	0.69 ± 0.06 (b)
BC/76PVA/5CH	7.3 ± 0.5 (a)	84.2 ± 8.4 (a)	230.0 ± 0.7 (a)	85.0 ± 7.0 (a)	0.88 ± 0.24 (b)
PVA/5CH	4.5 ± 0.5 (a)	85.5 ± 0.7 (a)	147.4 ± 5.0 (a)	84.6 ± 10.9 (a)	1.39 ± 0.23 (a)
LDPE	/	/	/	/	0.00179 ± 0.0005

MC: moisture content; WS: water solubility; WAC: water absorption capacity; CA: contact angle; WVP: water vapor permeability.

6 UV-Vis spectroscopy

The transmittance spectra of the prepared samples between 250 and 850 nm are reported in *Figure S5(a,b)*.

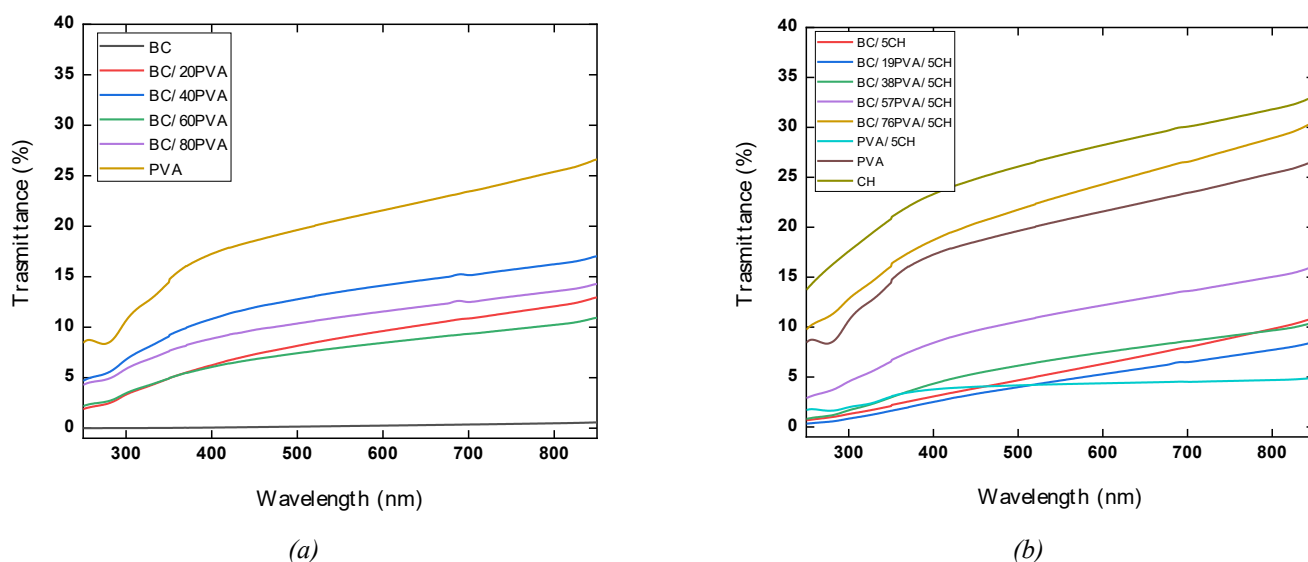


Figure S5: Transmittance spectra between 250 and 850 nm of (a) BC/PVA films and (b) BC/PVA/CH films.

7 Soil burial tests

Biodegradability, crucial for reducing the environmental impact of packaging materials, involves material breakdown by biological agents like animals, plants, fungi, and microorganisms, influenced by factors such as chemical composition and environmental conditions. The biodegradability assay was carried out following a procedure adapted from that presented by K. A. Zahan³⁴. Squares of 2 cm side were cut out from the film and weighed in a Gibertini E42 balance. Aluminum trays (one per time point) were filled with soil from a local urban garden with an initial moisture content of 3.5 wt%. The soil was poured into the tray forming a uniform layer of 2 cm of thickness, then the squares specimens were positioned in the trays, and buried with an additional compost soil layer of 2 cm. The trays were maintained at 23 °C and 56 r.h.% during all the assays. Then, the films were collected from the soil and slightly cleaned on days 3, 7, and 15. The harvested films were weighed, dried in a ventilated oven at 50 °C for 24 h to remove the absorbed water, and weighed again. The disintegration percentage was calculated through *Equation S11*, reported also in the standard ISO 20200:

$$\text{Degradation (\%)} = \frac{m_i - m_r}{m_i} * 100 \quad \text{S11}$$

where m_i is the initial dry mass of the test material and m_r is the dry mass of the residual test material recovered after the buried period.

The results of soil burial test for BC/PVA and BC/PVA/CH films after 3, 7, and 15 days are reported in *Figure S6 (a, b)*. The contour of the film and its defects are highlighted in red.

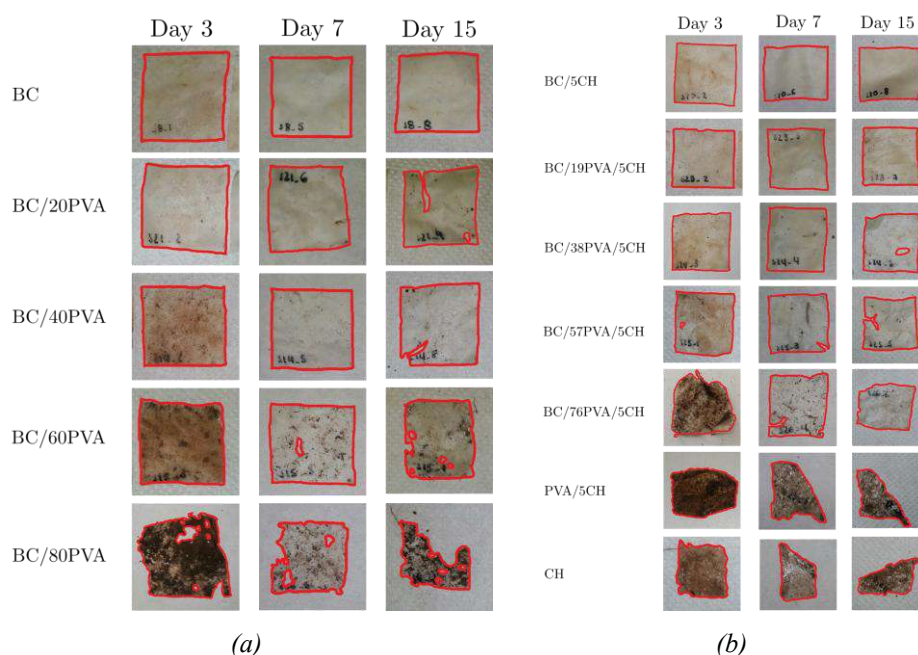


Figure S6: Results of biodegradability test on Day 3, 7, and 15 for samples: (a) BC, BC/20PVA, BC/40PVA, BC/60PVA, and BC/80PVA, and (b) BC, BC/19PVA/5CH, BC/38PVA/5CH, BC/57PVA/5CH, BC/76PVA/5CH, PVA/5CH, and CH.

BC/PVA films exhibit increased degradation kinetics with higher PVA concentrations, evidenced by early defects appearing. In contrast, pure BC films show no change, remaining intact after 15 days. This is in contrast with previous studies where the degradation of BC was evident from Day 1³⁴, or was completed in 5 days at a relative humidity of 90 % and a temperature of 30 °C³⁵. Hence, the discrepancy can be attributed to the temperature and moisture conditions and/or soil composition. PVA films degrade primarily due to humidity, resulting in soil impregnation, making degradation assessment challenging. For BC/20PVA and BC/40PVA films, the defects become visible on Day 15, while for BC/60PVA and BC/80PVA films, the flaws on the film surface occur on Day 7 and Day 3, respectively. The increase in degradation can be ascribed to the joint action of cellulose degradation and PVA dissolution due to its interaction with soil moisture. However, literature studies report that the degree of degradation of PVA is lower than the degree of cellulose, being less than 10 % at an incubation period of more than 60 days³⁶.

Chitosan addition seems to slow film degradation, reducing the presence of defects and the brownish coloring due to soil impregnation. However, comparing the percentage of disintegration on Day 15, presented in *Figure S7*, it is evident that BC/PVA/CH films have a faster degradation. According to the literature, chitosan films exposed to industrial and garden soil exhibit complete degradation after

seven days ³⁷, thanks to the action of chitosan-hydrolyzed enzymes (chitosanases) present in the soil ³⁸. Thus, chitosan generally favors interaction with moisture and the attack of microorganisms ³⁹, improving the biodegradability both in aerobic and anaerobic conditions, as demonstrated by Kibédi-Szabó et al. for BC/PVA/CH composite ³³.

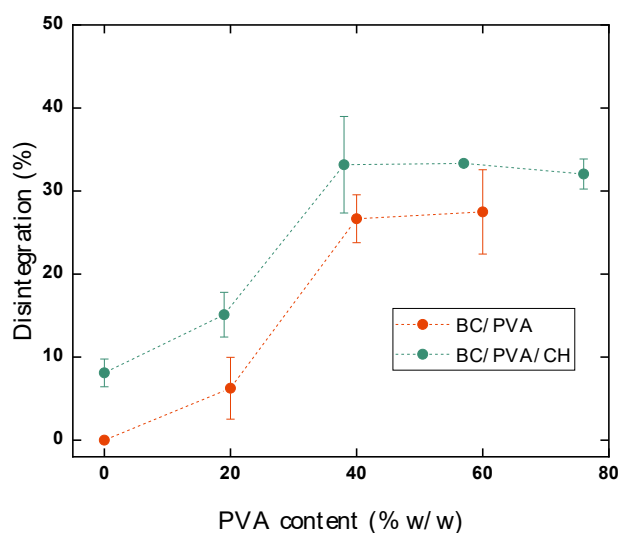


Figure S7: Disintegration percentage on day 15 for BC/PVA and BC/PVA/CH films.

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