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Article

Antimicrobial Effect of Cellulose Nanofibrils (CNFs) and Biobased Additives in Polyvinyl Alcohol Nanocomposite Materials for Sustainable Food Packaging Application

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Abstract

This study evaluates the antimicrobial properties of nanocomposite materials based on polyvinyl alcohol (PVA) reinforced with cellulose nanofibrils (CNFs) and/or supplemented with biobased additives derived from blueberry pruning wastes, with the objective of developing biodegradable food packaging systems with antimicrobial properties. The nanocomposites were prepared using a solvent-casting processing approach, and their thermal, physicochemical, and antimicrobial properties were assessed. All the nanocomposites exhibited thermal stability up to 200 °C, confirming their suitability for conventional food packaging processing conditions. Antimicrobial activity tests revealed inhibitory effects against both Gram-positive and Gram-negative bacteria. Bleached PVA/CNFs films showed complete growth inhibition (100%) against *E. coli* and *S. aureus*. In contrast, unbleached PVA/CNFs and PVA/CNFsB supplemented with blueberry-derived additives exhibited selective inhibition against *E. coli*, highlighting the influence of nanofibril composition and additive incorporation on antimicrobial performance. Zeta potential measurements revealed values of −35.3 mV for the CNFs, confirming their negatively charged surface, which may contribute to interactions with bacterial membranes. Additionally, scanning electron microscopy (SEM) showed that the incorporation of CNFs generates nanostructured surfaces with exposed fibrillar domains, where bacterial cells become adhered and immobilized. These topographical features suggest that the antimicrobial behavior of the nanocomposites is associated with direct bacteria–surface interactions, supporting a contact-active antimicrobial behavior associated with the CNFs.

Keywords: nanocomposite; PVA/CNFs; antimicrobial properties; packaging food

1. Introduction

Today, food packaging materials are one of the main challenges in the food industry. There is a growing consumer demand for high-quality fresh food packaged in biobased and preservative-free biodegradable materials [1]. Food packaging systems with antimicrobial properties represent an emerging technology that can enhance both food shelf life and safety. Therefore, the production of packaging with antimicrobial characteristics based on biodegradable materials is an area of growing

interest [2–4]. Biobased packaging can improve food quality and increase shelf life, functioning as a barrier system against oxygen, environmental odors, humidity, and microorganisms [5,6].

The polymers commonly used for food packaging are those derived from petroleum: mainly polypropylene, polyethylene and polystyrene. Their low cost of obtaining, its availability, its good mechanical, and barrier properties make them the most competitive alternative. However, in view of both the global problem of the accumulation of non-biodegradable plastics and the need to develop materials for the packaging of fresh foods with antimicrobial characteristics, it is essential to advance in processes that are more respectful of the environment and to develop new packaging materials [7].

In this search, biodegradable polymers have been widely studied as substitutes for petroleum-derived plastics. Biopolymers are used in many applications: in the biomedical area (implants), biomimetic materials, material coatings, insulating materials, as additives in paints, some food packaging, additives in adhesives, as thickeners in lubricants and plasticizers [5,8,9]. The main limitation of biodegradable polymers for fresh food packaging lies in their low processing efficiency and suboptimal mechanical or barrier properties (e.g., oxygen/moisture permeability and thermal resistance) [7,8]. To overcome these limitations presented by biopolymers, various materials have been studied to be used as reinforcement or fillers to obtain a bio-nanocomposite and thus improve the properties of the biopolymer [10–16]. Bio-nanocomposites are composite materials made by mixing a biodegradable polymer (natural or synthetic) with a nanoscale reinforcement material, which typically does not exceed 5% by weight of the mixture. These bio-nanocomposites exhibit notable improvements in mechanical and barrier performance, driven by the synergistic effects of their high surface area at the nanoscale.

Among the biodegradable polymers used for food packaging, polyvinyl alcohol (PVA) stands out. PVA is a low-cost, non-toxic, water-soluble synthetic polymer [17] that is obtained commercially from the hydrolysis of polyvinyl acetate. Despite its synthetic character, this polymer is biodegradable and shows high tensile strength, adequate barrier properties, and resistance to acid and alkaline media [18]. The safety of PVA has been extensively evaluated in various studies by entities in the food and health area (JECFA, FAO, WHO) [19]. This biopolymer, thanks to its hydroxyl groups (OH), forms hydrogen bonds with the hydrophilic surfaces of biopolymers or biomaterials to produce bio-based compounds [17]. Due to this capacity, PVA has been combined with other reinforcements or fillers to improve its performance and barrier properties, for example with nanoparticles of silver [20], zinc and titanium oxide [21], etc. Heavy metal nanoparticles such as silver, copper and zinc possess effective antimicrobial activities against a wide variety of bacteria [22]. Their high surface area, thermal stability, and low volatility have made metallic nanoparticles attractive candidates for incorporation into active packaging systems [22,23]. However, these elements are inorganic in nature and therefore have limited biocompatibility and biodegradability. On the other hand, the reinforcement of PVA with nanocelluloses has been studied, obtaining a significant improvement in the mechanical and barrier properties of PVA [24–28].

In the last decade, the use of cellulose nanofibrils (CNFs) as a reinforcement or filler agent has been investigated, mainly in biodegradable polymers. CNFs are obtained from cellulose, the most abundant biopolymer in nature. CNFs are commonly obtained by chemical or enzymatic pretreatment, followed by mechanical treatment to achieve a nanometric size of their fibrils. CNFs have been used as a reinforcing agent (due to their large aspect ratio and high specific surface area) in various polymeric matrices to mainly improve their mechanical and barrier properties [29,30]. CNFs can be obtained from any source of lignocellulosic biomass (mainly wood) including agro-industrial waste [31–35], allowing the comprehensive use of natural resources, favoring the circular economy [7].

The use of CNFs in food packaging with antimicrobial properties is a little explored area to date. The available studies focus on the elaboration of CNFs films with antimicrobial properties, mainly through the physical adsorption of metallic nanoparticles [36–38] which are gradually released into the matrix, leading to the inhibition of microbial growth. Other studies are based on the incorporation of protein grafts [4,39] or polycationic compounds [40] on the surface of CNFs. Although metals are

effective in inhibiting a wide range of microorganisms, they are toxic and their continuous use has generated resistance in microorganisms [41,42]. Polycations are poorly biocompatible, while enzymes suffer from chemical instability. In the reviewed literature, the most studied antimicrobial agents in food packaging applications were Ag nanoparticles [43], ethyl lauryl arginate (LAE) [44], TiO₂ [45], Nisin [46], Citric acid and chitosan [47], Gallic acid grafted chitosan [48], Dehydroabietylamine [3], Porphyrin [49], Lysozyme and nisin [39], and Aminosilanes [40].

Most studies on obtaining CNFs have focused on bleached chemical pulps, however, CNFs from unbleached kraft pulps have also been reported to have good barrier properties [7,32,35]. CNFs from unbleached pulps contains residual lignin, which allows the production of pulps with higher yields and lower production costs [50]. It has been investigated that the presence of residual lignin in CNFs has several advantages, such as improved compatibility [51,52] and higher hydrophobicity [53].

Espinosa et al., (2019) obtained CNFs with and without residual lignin from wheat straw. These nanocelluloses were used as a reinforcing agent in PVA films to improve their barrier properties, specifically their antioxidant characteristics. The results showed that this capacity increased from 10 to 50% using unbleached nanocellulose, especially in small amounts of reinforcement of CNFs (1-3% wt.) [7].

Patiño et al. (2022) prepared cellulose nanocrystals nanocomposites incorporated with an antimicrobial agent, ethyl lauroyl arginate (LAE). These LAE-CNC nanocomposites films demonstrated antimicrobial activity against the Gram (+) and (-) bacteria, *L. monocytogenes* and *S. typhimurium*, even with just a 1% addition of LAE [44].

Within biopolymers, lignin has been extensively studied mainly due to its antioxidant and antimicrobial properties. The main function of lignin is to provide rigidity to the plant to strengthen the structure of cell walls [54]. Among its various functions in plants, its behavior as an antimicrobial agent stands out [54]. Lignin's antimicrobial activity is attributed to its phenolic structure, particularly the α , β -unsaturated side chains and γ -methyl substitutions, which enhance the potency of its phenolic fragments [55]. Many investigations have suggested that lignin's could be applied to stabilize foods due to their antioxidant, antifungal and antimicrobial properties [56–62]. Its biocidal activity makes it an alternative and more attractive compound than metallic nanoparticles due to its reduced environmental impact.

Considering the above, the use of unbleached cellulose nanofibrils (L-CNFs) as reinforcing agents in PVA-based bio-nanocomposites is proposed. The presence of residual lignin in L-CNFs is expected to contribute to inherent antimicrobial properties, enabling the development of active, biodegradable food packaging materials. Figure 1 presents the conceptual framework of the proposed system.



Figure 1. Conceptual diagram of proposed work to develop a green-packaging food with antimicrobial properties.

2. Materials and Methods

Raw materials and Chemicals

The unbleached nanocellulose hydrogel (with lignin) was supplied by Bioforest (Arauco cellulose). The blueberry pruning and corn husks waste were provided by the agricultural company FISTUR (Yumbel, Biobío region, Chile). The Polyvinyl alcohol, PVA (M.W.: 146,000–186,000; and degree of hydrolysis +99%), (2,2,6,6-tetramethylpiperidiny-1-oxyl) TEMPO, NaBr, NaClO, ethanol, which were purchased from Merck/Sigma Aldrich, Santiago de Chile.

2.1. Removable Extraction from Blueberry Pruning and Corn Husks (Modified TAPPI T204 cm-97)

For its determination, 4.0 ± 0.1 g of sample (dry weight) at equilibrium moisture content, prepared according to TAPPI standard 257 cm-85, were used. The raw material was placed in a pre-weighed filter paper cartridge. On top of the cartridge with the sample inside, another cartridge was placed as a lid, also pre-weighed, to prevent sample losses during extraction. The assembly was placed in a Soxhlet, and 150 mL of analytical-grade acetone was added, approximately up to one and a half siphons. The Soxhlet was connected to a flask with a condenser and heated to reflux under a hood, bringing the mixture to a boil. After 6 hours, during which no fewer than 24 extractions must occur, and once cooled, the Soxhlet was disassembled, and the solvent in the Erlenmeyer flask was partially evaporated at room temperature under a hood. The cartridge with the sample was collected for subsequent analyses, dried in an oven at 105 °C for 24 hours. Finally, it was weighed, and the extracted mass (mf) was obtained [31].

2.2. CNFs Obtention

The method described by Zuluaga et al. (2009), with some modifications, was applied for obtaining cellulose pulp from corn husks [63].

TEMPO-mediated oxidation of corn husk-cellulose pulp and nanocellulose hydrogels was carried out according to the protocol described by Saíto et al., 2006 with some modifications. A total of 10 g of 3 wt% sample was suspended in 300 mL of deionized water containing 0.050 g TEMPO and

0.50 g NaBr. TEMPO-mediated oxidation began with the addition of 30 mL of a commercial NaClO solution (10% wt.) at room temperature with gentle stirring. The pH was maintained at 10.5 by adding 0.5 M NaOH. The reaction was considered complete once the pH remained stable at 10.5. The oxidized product was filtered, washed with deionized water, and stored at 4 °C [67]. Each experiment was conducted in triplicate. The oxidized fibers were dispersed in deionized water with a pulp concentration of 1 wt.% and then homogenized with a high-pressure homogenizer (NS1001L PANDA 2K-GEA) 8 times at a pressure drop of 800 bar to produce nanocellulose hydrogels [64]. Films (20 g/m²) were prepared in plastic petri dishes, based on dispersions of 0.25% CNFs. The drying temperature was 24-26 °C [31].

2.3. Preparation of PVA/CNFs Nanocomposites

A 5 wt% PVA solution was prepared by dissolving the polymer in distilled water under stirring at 90 °C for 4 h. After cooling to room temperature, a 2 wt% CNFs suspension (bleached, unbleached, or with blueberry extractives) was added and stirred for an additional 4 h. The final films were cast in Petri dishes and dried under ambient conditions (20–25 °C, 40–60% RH) for approximately 7 days until complete water evaporation. Each experiment was performed in triplicate. The final CNFs content in the nanocomposites was approximately 28 wt% relative to dry PVA [7]. Each experiment was conducted in triplicate. Table 1 provides the nomenclature of the nanocomposite samples manufactured for this study.

Table 1. Nanocomposite nomenclature.

Nomenclature	Description
CNFs	Krat CNFs film
CNFs-L	Lignin-CNFs film
CNFs-B	CNFs film additivity with blueberry pruning extractable.
PVA	Polyvinyl alcohol film
PVA/CNFs	PVA/CNFs nanocomposite
PVA/CNFs-L	PVA/lignin-CNFs nanocomposite
PVA/CNFs-T	PVA/TEMPO-CNFs nanocomposite
PVA/CNFs-LT	PVA/lignin-CNFs-TEMPO nanocomposite
PVA/CNFs-B	PVA/CNFs additivity with blueberry pruning extractable nanocomposite.

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

The IR measurements were performed with an Agilent Tensor 27 instrument (Malaysia) in Fourier transform mode (FTIR). The Agilent MicroLab PC software (version IQ/OQ, 21 CFR Part 11 compliant) was used for data acquisition. A total of 40 scans were collected across a spectral range of 400 to 4000 cm⁻¹ [64,66].

2.5. Thermo-Gravimetric Analysis (TGA)

Thermal stability and decomposition profiles were determined using a NETZSCH TG 209 F3 Tarsus® thermal analyzer (MB & Cia, Selb, Germany). Approximately 5 mg of each sample was placed in an alumina crucible and heated from room temperature (~25 °C) up to 600 °C under an inert nitrogen atmosphere, at a constant flow rate of 50 mL/min. The heating rate used was 10 °C/min [64,66].

2.6. Zeta Potential

Zeta potential measurements were performed using a Malvern Zetasizer Ultra (Malvern Panalytical, UK). A total of 1 mL of the sample was dispersed in deionized water and used as the

measurement medium. All measurements were conducted at room temperature with a fixed scattering angle of 90°.

Prior to measurement, the zeta potential cell was carefully filled with the sample, ensuring the absence of air bubbles. External electrodes were cleaned thoroughly to avoid contamination, and the refractive indices of both the solvent (water) and the sample were correctly set in the instrument software. The temperature was maintained at 25 °C throughout the analysis. Each measurement was performed in triplicate to ensure reproducibility.

The Zetasizer software provided the average zeta potential value (in mV), the standard deviation, and the corresponding distribution graph for each set of measurements.

2.7. PVA/CNFs Antimicrobial Activity Nanocomposites

The antimicrobial activity of PVA/CNFs nanocomposites was analyzed by modifying the Japanese Industrial Standard (JIS Z 2801:2000) to test antibacterial activity on plastic surfaces. The JIS test is based on a comparison of bacteria counts (*S. aureus*, and *E. coli*) in saline solution on reference (PVA) and sample materials (PVA + CNFs) after a defined incubation temperature and time (37 °C, 24 h) [68,69]. The material that shows a calculated log10-reduction ≥ 2.0 log10 units after 24 h is considered as an effective antimicrobial agent [68]. Each experiment was performed in triplicate for each nanocomposite.

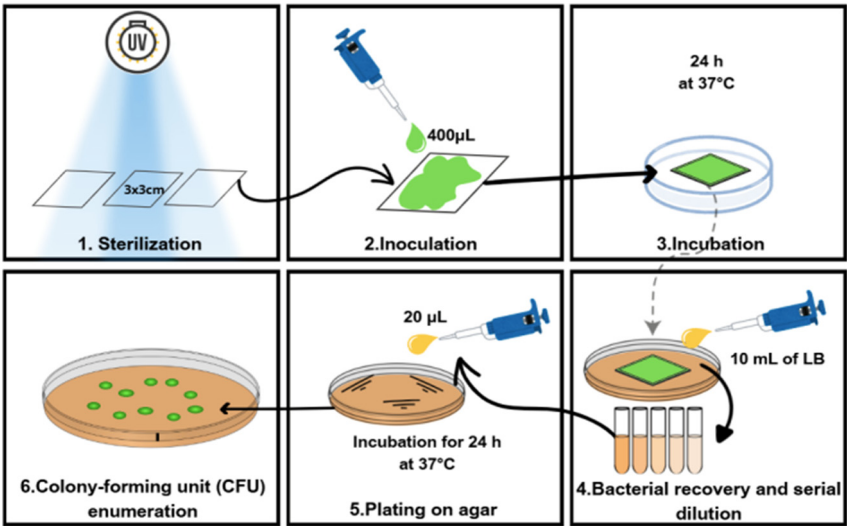


Figure 2. Scheme of the antimicrobial activity JIS test for PVA/CNFs nanocomposites.

2.8. Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Spectroscopy (EDS)

After the bacterial contact period with *S. aureus*, the films were removed and cut into 0.5 × 0.5 cm fragments. The samples were transferred to 50 mL Falcon tubes containing 1 mL of 4% paraformaldehyde (PFA) as a fixative agent and incubated for 4 h at room temperature to preserve bacterial morphology and bacteria–surface interactions. Subsequently, the samples were washed three times with phosphate-buffered saline (PBS, pH 7.2) and subjected to gradual dehydration using a graded ethanol series (30, 50, 70, 90, and 100%), with 10 min incubations at each concentration; the absolute ethanol step was repeated. After dehydration, the samples were dried by critical point drying [65].

The dried film fragments were mounted on SEM stubs and coated with a thin gold layer using a sputter coater (SPI-MODULE, USA) to ensure surface conductivity. Morphological analysis was evaluated using a scanning electron microscope (JEOL JSM-6380LV, Japan) equipped with an energy-dispersive X-ray spectroscopy (EDS) detector, with image acquisition performed via a computer-connected system.

3. Results

3.1. Infrared Spectroscopy Analysis (FTIR)

Figure 3 shows the infrared spectra of CNFs films and its nanocomposites. The infrared spectra of CNFs films and PVA/CNFs nanocomposites showed typical bond vibrations of these materials. The broad peak around 3300 cm^{-1} in all nanocomposite films indicates the presence of hydroxyl groups corresponding to both PVA and CNFs. The C–H stretching vibrations of PVA, and CNFs was identified at the peak of 2900 cm^{-1} in all samples, while the peak around 1700 cm^{-1} in the PVA/CNFs and PVA/L-CNFs samples is attributed to the stretching vibration of the C=O bond resulting from TEMPO-mediated oxidation of CNFs. The peak around 1580 cm^{-1} in all films is due to the bending vibration of the C-H bond. The peak around 1100 cm^{-1} in all samples is attributed to the stretching vibration of the C-O bond in secondary alcoholic groups or to CH_2 bending vibrations [7].

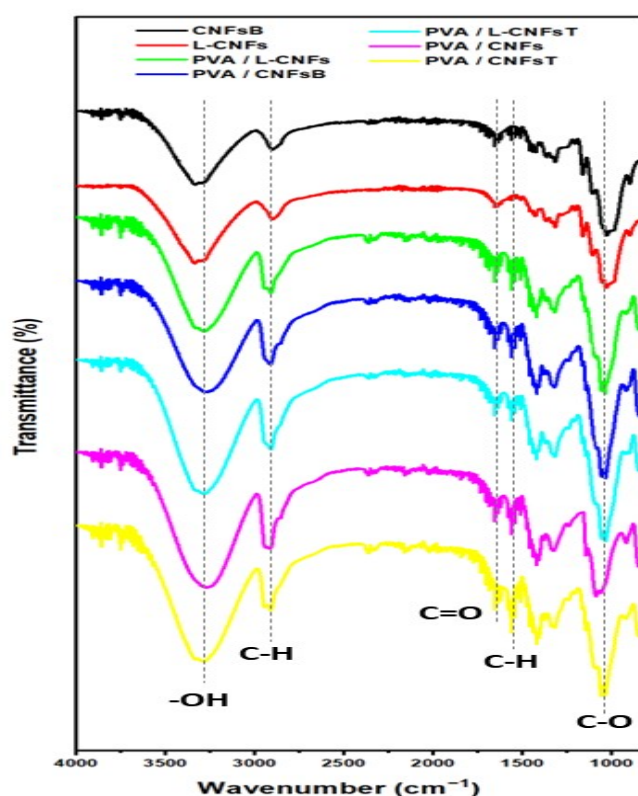


Figure 3. FTIR spectra of PVA/CNFs nanocomposite films.

3.2. PVA/CNFs Nanocomposites Thermo-Gravimetric Analysis (TGA)

In Figure 4, L-CNFs and CNFsB samples display a single major degradation step, with degradation starting at approximately $330\text{--}350\text{ }^{\circ}\text{C}$. The slightly improved thermal resistance of CNFsB may be attributed to the presence of extractable phenolic compounds from blueberry pruning waste, which may contribute to enhanced thermal shielding during decomposition.

The nanocomposite PVA/CNFs show a two-step degradation pattern. The first mass loss event, between $250\text{--}300\text{ }^{\circ}\text{C}$, is associated with the thermal degradation of the PVA matrix, while the second, occurring above $350\text{ }^{\circ}\text{C}$, corresponds to the decomposition of the nanocellulose component. TEMPO-oxidized samples showed a slightly lower degradation onset, likely due to the introduction of carboxylic groups. Overall, CNFs reinforcement led to a minor enhancement in thermal stability compared to pure PVA films.[7]. Sirviö et al., 2015, reported that pure PVA films begin to degrade at $262\text{ }^{\circ}\text{C}$. Therefore, due to the reinforcement of CNFs, the thermal stability of the PVA/CNFs nanocomposite was slightly increased.

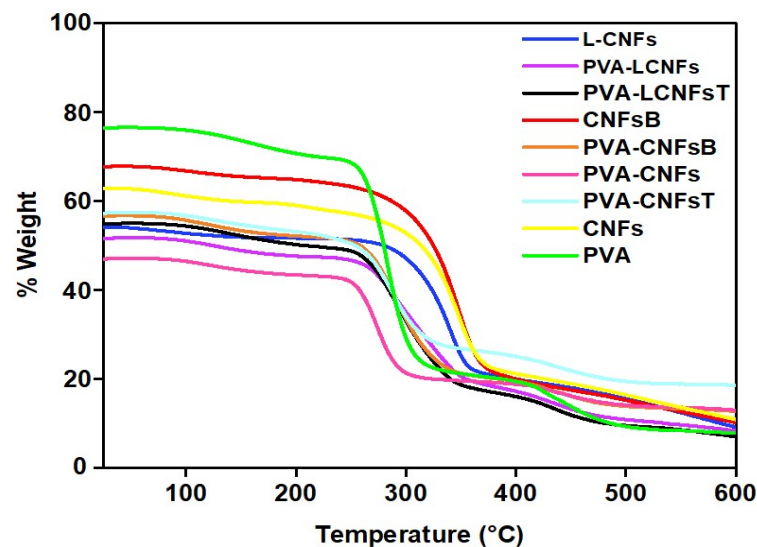


Figure 4. TGA curves of PVA/CNFs nanocomposite films.

3.3. PVA/CNFs Nanocomposites Zeta Potential (mV) Analysis

In Table 2, it can be observed that CNFs exhibit a strong negatively charged surface, attributed to the presence of hydroxyl and/or carboxylate groups [70], particularly when subjected to TEMPO-mediated oxidation pretreatment. This negative charge of CNFs increases the overall charge of the PVA/CNFs nanocomposite when reinforced with CNFs, considering that the PVA matrix is weakly charged and accounts for approximately 98% wt. of the nanocomposite by mass. Consequently, the charges induced by the small fraction of CNFs in the nanocomposite (2% wt.) can interact with bacterial membranes, particularly those of Gram-positive and Gram-negative bacteria, which differ in their cell membrane structure, contributing to destabilization.

Table 2. Zeta potential (mV) of PVA, CNFs and PVA/CNFs samples.

Sample	Zeta potential (mV)
PVA	-1.307
CNFs	-35.29
PVA/CNFs	-3.766

3.4. Antimicrobial Activity of PVA/CNFs Nanocomposites

As shown in Figure 5A all the nanocomposites studied demonstrated an inhibitory effect on Gram-negative bacteria (*E. coli*). Those showing a calculated log10-reduction ≥ 2.0 log10 units compared with PVA after 24 h of incubation are marked with a red asterisk in Figure 5. This means they have a validated antimicrobial activity, according to the JIS 2801 test. The bleached PVA/CNFs tested showed a 100% inhibition of *E. coli* and *S. aureus* growth (Figure 5). This is the same effect observed when using a nanocomposite of CNFs alone. PVA/CNFs-T showed a 100% inhibition of *E. coli* only. The unbleached CNFs (CNF-L), PVA/CNFs-L and PVA/CNFs with blueberries additives tested showed significant antibacterial activity for *E. coli* only.

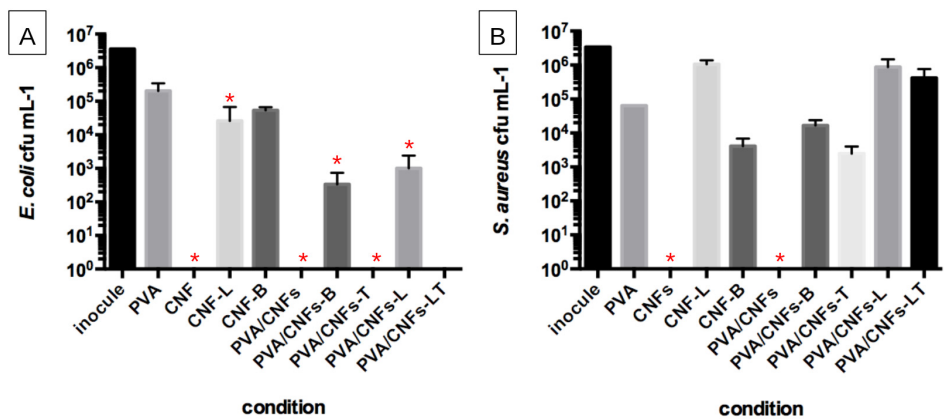


Figure 5. Antibacterial activity against *E. coli* (A) and *S. aureus* (B) of the PVA/CNFs tested. Those showing a calculated log10-reduction ≥ 2.0 log10 units compared with PVA after 24 h of incubation are marked with a red asterisk.

Although CNFs in general do not exhibit antimicrobial properties as strong as those of specific antimicrobial compounds, the nanocomposites with CNFs tested have demonstrated measurable activity, which can be attributed to their topo-chemical and supramolecular characteristics. One key factor is their negatively charged surface, resulting from naturally occurring hydroxyl groups or carboxyl groups introduced during pretreatment. This surface charge can interact with bacterial membranes, leading to membrane disruption. In addition, type II cellulose, which has lower crystallinity (approximately 60%), imparts greater flexibility to the nanofibrils. This flexibility may enhance their ability to interact with bacterial cell walls, contributing to their antimicrobial activity.

3.5. Surface Topography and Bacteria–Material Interactions in PVA/CNFs Nanocomposites

The surface morphology of the PVA/CNFs nanocomposites was examined by scanning electron microscopy (SEM) to elucidate the role of nanoscale topographical features on the antibacterial behaviour of the materials. As shown in Figure 6A, the nanocomposite surface exhibits a heterogeneous and rough morphology, characterized by an interconnected network of cellulose nanofibrils partially exposed at the surface of the PVA matrix. This fibrillar architecture, typical of TEMPO-oxidized and highly fibrillated CNFs, has been widely reported to generate high surface area and pronounced nanoscale roughness [71,74]. After incubation with *S. aureus* (Figure 6B), bacterial cells are observed adhered and immobilized on the PVA/CNFs surface. The bacteria appear preferentially located in regions with higher nanofibrillar density, indicating that surface topography plays a dominant role in governing bacteria–material interactions. Similar SEM observations have been reported for carboxylated and TEMPO-oxidized CNFs systems, where bacteria are physically entrapped within nanofibrillar networks rather than repelled from the surface [71,73].

The immobilization of *S. aureus* observed in Figure 6B is consistent with a contact-active antibacterial mechanism, previously described for nanocellulose-based materials. In these systems, the antibacterial effect arises from prolonged physical contact between the bacterial cell wall and the nanostructured surface, leading to restricted motility, impaired cell division, and inhibited biofilm formation [73]. SEM images reported by Knutsen et al. (2021) showed bacterial cells surrounded and confined by a dense CNFs network, a phenomenon that closely resembles the bacterial immobilization observed in the present PVA/CNFs nanocomposites.

For Gram-positive bacteria such as *S. aureus*, the thick peptidoglycan layer provides mechanical rigidity, which explains the absence of severe cell collapse or lysis in the SEM images. However, this structural robustness also promotes strong physical anchoring to rough and fibrillar surfaces, enhancing immobilization effects. Previous studies have demonstrated that nanocellulose surfaces with high aspect ratio fibrils induce mechanical stress at localized contact points, leading to

membrane fatigue and loss of bacterial viability over time, even in the absence of chemical biocides [71].

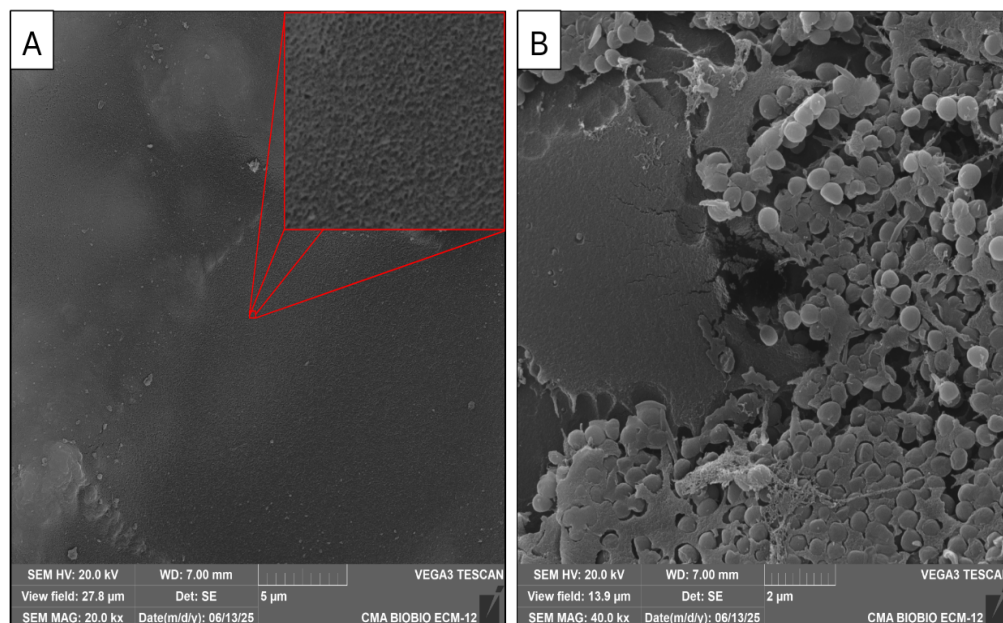


Figure 6. SEM images of PVA/CNFs nanocomposites alone (A) or after incubation with *S. aureus* (B).

The topographical contribution to antibacterial activity is further reinforced by studies on oxygenated and highly carboxylated CNFs, where SEM analyses revealed that increased fibrillation degree and surface charge result in denser nanofibrillar networks capable of surrounding bacterial cells and limiting nutrient and oxygen diffusion [73,74]. Although oxygenation was not applied in the present system, the morphological similarities observed by SEM suggest that the PVA/CNFs nanocomposites operate through a comparable physical confinement mechanism.

Importantly, the bacterial adhesion observed in Figure 6B does not indicate a loss of antimicrobial functionality. On the contrary, it supports a non-leaching, contact-based antibacterial mode of action, which is particularly advantageous for food packaging applications. Contact-active antimicrobial surfaces prevent bacterial proliferation directly at the material interface while minimizing the risk of antimicrobial migration into food matrices [4].

Overall, the SEM results confirm that the antibacterial performance of PVA/CNFs nanocomposites is strongly governed by their nanoscale surface topography. The combination of nanofibrillar architecture, surface roughness, and physical confinement of bacterial cells aligns well with previously reported antibacterial mechanisms of TEMPO-oxidized, carboxylated, and oxygenated CNFs [71–74]. These findings highlight the potential of PVA/CNFs nanocomposites as contact-active antimicrobial materials for sustainable food packaging applications.

4. Discussion

Antibacterial metal nanoparticles or antibacterial peptides grafted onto cellulosic materials tend to aggregate upon release, reducing their capture efficiency and shortening their effective lifespan. In contrast, contact-active antibacterial surfaces eliminate the release step, thereby offering greater durability. However, their main limitation lies in their passive mechanism of action, as they require direct contact with bacteria to be effective. Despite this, such materials are ideal candidates for applications where this contact naturally occurs, such as antibacterial filters, water purification systems, food packaging, wound dressings, and antimicrobial coatings.

Conclusions

PVA/CNFs nanocomposites were successfully developed as bio-based antimicrobial materials, and their antibacterial performance was shown to be strongly governed by nanoscale surface topography rather than by the release of antimicrobial agents. The incorporation of cellulose nanofibrils into the PVA matrix generated heterogeneous and rough nanostructured surfaces with exposed nanofibrillar networks, which played a decisive role in bacteria–material interactions.

SEM analysis provided direct evidence that *Staphylococcus aureus* cells become adhered and immobilized on the surface of the PVA/CNFs nanocomposites. This immobilization is attributed to a contact-active antibacterial mechanism, where nanoscale roughness and fibrillar architecture restrict bacterial mobility and proliferation at the interface. These findings are consistent with previously reported antibacterial behaviors of TEMPO-oxidized, carboxylated, and oxygenated cellulose nanofibrils, in which physical confinement within nanofibrillar networks is a dominant mode of action.

Importantly, the observed bacterial adhesion does not indicate a loss of antimicrobial efficacy but rather confirms a non-leaching and physically driven antibacterial mechanism. This characteristic is particularly advantageous for food packaging applications, as it minimizes the risk of antimicrobial migration into food products while ensuring localized and sustained inhibition of bacterial growth upon contact.

Overall, this study demonstrates that controlling nanoscale surface topography is a key strategy for designing effective antimicrobial polymer nanocomposites based on cellulose nanofibrils. The results highlight the potential of PVA/CNFs nanocomposites as sustainable, contact-active antimicrobial materials for advanced food packaging systems, contributing to improved food safety and extended shelf life.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: CNFs hydrogel; Figure S2: PVA/CNFs hydrogel; Figure S3: PVA/CNFs casting process; Table S1: *S. aureus* Colony count using JIS test; Figure S4: Colony count using JIS test; Table S2: *E. Coli* Colony count using JIS test; Figure S5: Zeta Potential of PVA hydrogel sample (5% wt.); Figure S6: Zeta Potential of CNFs hydrogel sample (2% wt.); Figure S7: Zeta Potential of PVA/CNFs hydrogel nanocomposite sample.

Author Contributions: Conceptualization, F.V and CP.Q.; methodology, F.V, D.P and V.R.; software, E.E and R.C.; validation, R.C., G.P and L.A.; formal analysis, F.V, CP.Q, R.C and C.F.; investigation, F.V and CP.Q; resources, F.V and CP.Q.; data curation, M.P.; writing—original draft preparation, F.V, CP.Q.; writing—review and editing, F.V and R.C.; visualization, V.R; supervision, F.V and R.C.; project administration, F.V and E.E.; funding acquisition, F.V and CP.Q. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study protocol involving pathogenic bacteria was approved by the Bioseguridad Committee of UCSC (protocol code 61/2025 and date of approval 04/09/2025).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding author.

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