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Active Bio-Based Packaging for Fresh-Cut Melon: Antimicrobial Efficacy and Zinc Migration of Nanocellulose/ZnO Nanocomposite Films

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Abstract

Growing environmental concerns, together with the need to extend shelf life and protect food from pathogens and mechanical damage, are driving the development of innovative active packaging materials. In this study, nanocellulose (NC) films incorporating zinc oxide nanoparticles (ZnO NPs) with three different morphologies (spherical, sheet and flower) were produced by solvent casting for food packaging applications. Films were characterised by scanning electron microscopy (SEM), and antimicrobial activity was addressed by agar diffusion assay against *Escherichia coli* and *Staphylococcus aureus.* NC/ZnO films were used as package for fresh-cut melon, which was stored at 4 °C for one week, and subjected to microbiological, pH and zinc migration analysis.

SEM revealed a porous, and nanofibrillar cellulose, suitable for NP retention, while cross sections showed the dispersion of NPs among the films. *In vitro* antimicrobial studies demonstrated the influence of morphology, with the sheet shape producing the highest inhibitory halos. In contact with melon, NC/ZnO films suppressed microbial proliferation relative to controls, keeping microbiological levels within acceptable limits up to day 7. Sheet shape showed the most significant effect. Total Zn migration plateaued at 7–8 mg kg^{-1} under a realistic area-to-mass scenario, with no significant differences among morphologies. Measurements reflect total Zn after acid digestion and ionic versus particulate species could not be distinguished. NC/ZnO films maintained slightly higher pH than controls.

Overall, these findings highlight the potential of bio-based NC/ZnO films to extend melon shelf life, with antimicrobial efficacy strongly influenced by nanoparticle morphology.

Keywords: zinc oxide nanoparticles; active food packaging; shelf life extension; fresh-cut melon; zinc migration; nanocellulose films; bio-based materials.

1. Introduction

Consumers are increasingly interested in minimally processed foods due to their health benefits. Fresh fruits and vegetables are a priority in many purchasing decisions, as they provide vitamins, minerals, dietary fibre, and antioxidants that support a balanced diet (Török et al., 2023). However, these foods are unstable after harvesting due to physical damage, biochemical changes, moisture loss, microbial proliferation, and oxidative deterioration, compromising quality and shelf life (Venkatesan & Muniyan, 2024). Packaging plays an important role in preserving product quality during storage, handling, and final use. In particular, active packaging has gained significant attention for its ability to extend shelf life, preserve freshness, and enhance safety by limiting the growth of potentially hazardous bacteria (Ahmed et al., 2022; Fadiji et al., 2023; Pascall, 2020). Additionally, by reducing spoilage, active packaging can also help in decreasing food waste and lowering corresponding environmental impacts. Growing global concern about sustainability is driving the replacement of petroleum-based plastics with bio-based, environmentally friendly materials (Basumatary et al., 2022; Dejene et al., 2024).

Within this context, nanocellulose (NC) is a natural polymer derived from various plants and agricultural products, increasingly used for paper and paperboard, biomedical products, food packaging, and textiles (Zhang et al., 2023). Nanofibrillated cellulose comprises long, flexible fibrils with at least one dimension in the nanometre range, containing abundant hydroxyl groups that enable surface modifications and strong bonding networks (Hansini et al., 2025; Soares Silva et al., 2020). The resulting porous matrix is an effective carrier for active compounds as it stabilises inorganic fillers and metal ions via hydrogen bonding through electrostatic interactions (Kumar et al., 2025; Roy et al., 2021). For food contact applications, cellulose is also used as reinforcing agent to improve physicochemical and functional properties (Maloufi et al., 2025; Singh et al., 2025).

Zinc oxide nanoparticles (ZnO NPs) stand out for their broad-spectrum of antimicrobial activity, UV blocking capability, and high surface-to-volume ratio, and are therefore of interest to be incorporated into bio-based polymers for food contact materials (FCM) (Lebaka et al., 2025; Dejene et al., 2025). Antimicrobial efficacy is strongly dependent on particle size and morphology, which modulate the reactive oxygen species (ROS) generation and Zn^{2+} release. Together, these pathways disrupt bacterial cells, inhibiting their proliferation (Kim et al., 2022;

Mendes et al., 2025). Despite these functional advantages, potential Zn migration raises concerns about toxicological risk against human health and regulatory compliance. Presently, ZnO (nano) is considered safe by the European Food Safety Authority (EFSA), as an ultraviolet light absorber for plastic FCM (EFSA, 2016). Although extensive literature has documented the antimicrobial role of ZnO and its migration into food simulants (Bumbudsanpharoke et al., 2019; Peter et al., 2022; Singh et al., 2024), studies addressing migration into real food models are scarce. Most available data relate to meat products, particularly poultry (Sasidharan et al., 2024; Soares Silva et al., 2023; Souza et al., 2020).

To address this gap, melon fruit was selected for shelf life studies because it has high water activity, which promotes rapid microbial growth even under refrigeration. In addition, melon is commonly commercialized and highly appreciated by consumers in a ready-to-eat cube format, which increases its exposed surface area and, consequently, accelerates microbial spoilage. Therefore, developing a packaging system that enables the assessment of antimicrobial efficacy and zinc migration is particularly relevant. Most previous studies on active packaging for melon have focused on chitosan- or alginate-based films incorporated with silver nanoparticles (NPs), mainly assessing quality attributes, microbial safety, and sensory evaluation (Danza et al., 2015; Ortiz-Duarte et al., 2019; Sun et al., 2022). Only one study has tested cellulose absorbent pads containing silver NPs (Fernández et al., 2010), and studies using ZnO NPs or investigating nanoparticle migration into melon are absent, underscoring the novelty of the present work. In fact, ZnO NPs offer advantages over silver, as they combine lower toxicity and lower production cost with a more sustainable production process (Karuppan Perumal et al., 2025).

Regarding the legislative framework, Regulation (EU) No. 2073/2005 lays down microbiological criteria for foodstuffs, providing a well-established control standard for microbial hazards (European Commission, 2005). In contrast, there is a concern and regulatory gap regarding safety of NPs if their migration occurs. According to Regulation (EU) No. 1935/2004, FCM must not release their constituents into food in quantities that could compromise human health, cause unacceptable changes in the food's composition, or deteriorate its organoleptic characteristics (European Commission, 2004). For plastic materials, Regulation (EU) No. 10/2011 sets a specific migration limit (SML) for ionic zinc of 5 mg kg^{-1} of food, while indicating that nanoparticles require a case-by-case risk assessment (European Commission, 2011). Additionally, EFSA reported that ZnO NPs do not migrate in nanoparticulate form when incorporated in an unplasticized polymer, so safety assessment should focus on soluble ionic zinc (Zn^{2+}) (EFSA, 2016). However, these assessments were performed only for plastic materials, where the NPs are strongly embedded in the polymeric matrix. In more porous systems, such as cellulose-based materials (ex, films or absorption pads), different migration profiles can occur,

requiring case-by-case risk assessment. In fact, a previous study concluded that both ionic Zn and nanoparticle ZnO migrated from nanocellulose films into food simulants (Mendes et al., 2026). Therefore, additional research using real food models is needed to address this regulatory gap and complement the existing regulatory framework (Poças & Franz, 2018).

In this context, the present work aims to develop NC nanocomposites incorporating ZnO NPs with three morphologies (spherical, sheet, and flower) and evaluate their performance in a fresh-cut melon model. The films were first characterised regarding their structure and nanoparticle distribution, and agar diffusion assays were performed to confirm *in vitro* antimicrobial efficacy. The films were then applied to melon slices and stored under refrigeration over one week. Microbiological counts and pH were monitored for shelf life assessment. Total Zn migration over time was quantified by atomic absorption spectrometry (AAS) after acid digestion, providing a realistic exposure scenario in a real-food matrix. By combining microbial shelf life studies with migration analysis, this study provides new data on the safety and functionality of bio-based NC/ZnO nanocomposite packaging. The results are expected to contribute to the current knowledge gap between studies using food simulants and those in real food systems, and to support regulatory assessment of nanomaterials in food contact applications.

2. Materials and Methods

2.1. Preparation of NC/ZnO NPs Nanocomposite Films

The ZnO NPs employed in this work were synthesized via sol-gel method in three different morphologies: spherical (ZnO-SP), flower (ZnO-FL), and sheet shape (ZnO-SH), represented in Figure S1. Their physicochemical and functional properties have already been extensively characterized in earlier studies, and a summary of these data is provided in Table S1 (Mendes et al., 2024, 2025).

NC/ZnO composite films were obtained through the solvent casting technique. The nanocellulose Valida S231C 3% (Sappi Biochemtech BV, Maastricht, Netherlands) was used. This material originates from wood pulp and presents fibrils with widths between 20 and 60 nm and lengths distribution mainly below 31 µm, as reported by the supplier's technical datasheet. Initially, 1% (w/v) of NC was dispersed in ultrapure water under magnetic stirring for 30 min at room temperature. Glycerol (Sigma-Aldrich, St. Louis, MO, USA) was then incorporated as a plasticizer at 5% (w/w relative to NC dry weight) under constant agitation. Afterwards, each ZnO NPs type was added at 10% (w/w relative to NC dry weight), and homogenized for a further 30 min. Subsequently, 32 mL of each formulation was cast into 10x10 cm trays and dried for 48 h under controlled conditions (23 °C, 50% relative humidity). The resulting films were designed

according to the incorporated nanoparticle morphology: NC/ZnO-SP, NC/ZnO-FL, and NC/ZnO-SH. Control films without nanoparticles (neat NC) were prepared under identical conditions. Films were cut into discs with a diameter of 13.5 mm for agar diffusion assay, and into 5x5 cm squares for antimicrobial, migration and pH assays using the model food.

All composite films were UV irradiated prior to use in antimicrobial assays to reduce surface microbial load. Films were exposed to a bank of four germicidal UV lamps (TUV G30T8, 16 W, Phillips, Amsterdam, Netherlands) with peak emission at 254 nm and an average intensity of 12.36 Wm^{-2}, for 10 min. To validate the efficacy of the decontamination process, UV-treated films were placed in direct contact with plate count agar (PCA) (Biokar Diagnostics, Allone, France) plates using sterile tweezers, ensuring full surface contact with the agar. The plates were incubated at 30 °C for 3 days. No microbial growth was observed during this preliminary test, confirming the effectiveness of the UV treatment in eliminating surface contamination (Figure S2). Therefore, this decontamination step was systematically applied to all films prior to microbiological assays.

2.2. Characterisation of NC/ZnO NPs Nanocomposite Films

Valida S231C 3% and NC/ZnO composite films were characterized using a FEI QUANTA 400 FEG ESEM (FEI, Hillsboro, OR, USA) microscope equipment, operating at an accelerating voltage of 15 kV. Prior to scanning electron microscopy (SEM) examination, the NC/ZnO films were treated with liquid nitrogen to obtain a clean cut cross-section. Additionally, films were also freeze-dried to observe the fibrillar structure and identify potential sites for ZnO NPs incorporation using a Phenom XL G2 SEM (Thermo Fisher Scientific—FEI, Eindhoven). Prior to testing, samples were frozen at -80 °C and subsequently lyophilized in a freeze dryer (LABCONCO, FreeZone, MO, USA) at -99 °C and 0.025 mbar until completely dry. The analysis was conducted at an accelerating voltage of 15 kV, and a secondary electron detector (SED) was used for micrographs capture.

The thickness of the NC/ZnO films was determined using a digital micrometer (Adamel Lhomargy, France) by randomly measuring five distinct points on each sample.

2.3. *In Vitro* Antimicrobial Activity of NC/ZnO Nanocomposite Films

The antimicrobial activity of the NC/ZnO NPs films was assessed by agar diffusion assay against *Escherichia coli* ATCC 8739 *and Staphylococcus aureus* ATCC 6538P. Test bacteria were aseptically inoculated into Tryptic Soy Agar (TSA) (Biokar Diagnostics, Allone, France) plates and incubated at 37 °C for 24 h. Colonies were then harvested with a sterile loop to prepare a cell suspension in brain–heart infusion (BHI) broth (Biokar Diagnostics, Allone, France), which was incubated overnight at 37 °C to obtain a bacterial population of approximately 10^8–10^9 CFU mL^{-1}.

Subsequently, sterile swabs were immersed in each inoculum suspension and uniformly spread the suspension onto TSA plates. Film discs were then placed directly onto the inoculated plates. After incubation at 37 °C for 24 h, inhibition zones were measured and expressed in mm. The discs were then removed, and the plates were re-incubated at 37 °C, with halos monitored over a 7-day period.

2.4. Fresh Melon Sample Preparation and Packaging

"Pele de Sapo" melons (*Cucumis melon L.*) were purchased from a local Portuguese supermarket and selected based on ripeness, uniform peel colour, and absence of physical damage (Figure 1a). The melons were washed with potable tap water and dried using absorbent paper. All the materials and surfaces were disinfected with ethanol, and the procedures were carried out under aseptic conditions using a Bunsen burner flame. The cleaned fruit was cut with a sterile knife into thin slices after removing the seeds and surrounding spongy tissue. Slices of approximately 10 g were prepared for microbiological analysis and approximately 2 g for migration assay. An extra set of melon slices was prepared for pH measurements. The samples were stored under refrigeration conditions (4 °C).

Previously decontaminated NC/ZnO films (5x5 cm) of different morphologies were used to package the melon samples, ensuring full contact between melon surface and the film at all sides of the small melon cube (Figure 1b). The wrapped slices were then placed inside sterile bags and stored under refrigeration conditions (Figure 1c). Microbial, migration, and pH analyses were conducted at five different time points over the course of one week. Triplicate analyses were performed for each nanocomposite type: NC/ZnO-SP, NC/ZnO-FL, NC/ZnO-SH and NC control.

2.4.1. Microbiological Analyses

Total mesophilic aerobic counts at 30 °C and psychrotrophic counts at 7 °C, *Enterobacteriaceae*, and yeast and mould populations were evaluated at days 0, 3, 5, 6, and 7 of the storage period. Under sterile conditions, the NC film was removed from the melon, and a 10 g portion of the slice was homogenized for 30 s with 90 mL of Ringer's solution (Biokar Diagnostics, Allone, France) using a Stomacher SMASHER® Sample Blender (bioMérieux, Marcy-l'Étoile, France). Serial dilutions were prepared in Ringer's solution as required for microbial enumeration. PCA was used for total mesophilic aerobic counts (incubated at 30 °C for 3 days), and for psychrotrophic counts (incubated at 7 °C for 10 days). *Enterobacteriaceae* were quantified using RAPID' *Enterobacteriaceae* Agar (Bio-Rad Laboratories, Marnes-la-Coquette, France) after incubation at 37 °C for 24 h. Yeasts and moulds were enumerated on Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Biokar Diagnostics, Allone, France) following

incubation at 25 °C for 5 days. A melon sample without film was used as a control. All microbial counts were expressed as log colony-forming units per gram of sample (log CFU g^{-1}).

2.4.2. Zinc Migration Analyses

The migration of Zn from NC/ZnO nanocomposite films into melon was assessed over a storage period of 8 days. Fresh-cut melon samples were collected after 0, 1, 3, 5, and 8 days for the Zn determination. The Zn concentration was quantified by flame AAS according to standard EN 14084, following microwave digestion of the whole melon cube. A realistic scenario was considered, based on the actual surface area-to-mass ratio (A/M) for half a melon (Figure S3).

Microwave digestion of 2 g of melon was performed in digestion vessel with the addition of 5 mL of suprapur nitric acid 65% (Panreac, Barcelona, Spain). The samples were then processed using a microwave digestion system (Speedwave MWS-3+, Berghof, Germany), according to the program detailed in Table 1. Upon completion of the microwave treatment, the digested samples were diluted with ultrapure water to a final volume of 50 mL and subsequently analysed by AAS.

Table 1 - Microwave digestion program.

Stage	1	2	3	4	5
T (°C)	130	170	200	100	100
Pressure (bar)	20	20	20	20	20
Time (min)	5	10	15	2	2
Ramp (min)	5	5	1	5	1
Power (watt)	30	40	50	30	20

Zinc quantification was performed using a flame AAS (Perkin Elmer Aanalyst 400, Waltham, MA, USA), equipped with a zinc hollow cathode lamp at a wavelength of 213.9 nm. Measurements were carried out using an air–acetylene flame under standard operating conditions recommended by the manufacturer. Zinc working standard solutions with 1 % (v/v) HNO_3, ranging from 0 to 0.50 mg L^{-1}, were prepared from a 1000 mg L^{-1} standard stock solution to obtain a calibration curve. Five replicate measurements were performed for each sample and working solution. System performance was assessed by determining the limit of detection (LOD: 0.008 mg L^{-1}), the limit of quantification (LOQ: 0.025 mg L^{-1}), and recovery (90 - 110%). When necessary, samples were further diluted with ultrapure water to ensure that the absorbance signal was within the specified Zn concentration range.

2.4.3 pH Determination

Melon samples were collected on days 0, 1, 3, 5, and 7 for pH determination. The samples were removed from the cold storage, the NC films carefully removed from the slices and the

measurements were taken after 3 min at room temperature. The pH of the melon slices was measured using a solid-state pH meter (Mettler Toledo, SevenDirect SD50, Greifensee, Switzerland).

2.5. Data Handling and Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 28), GraphPad Prism (version 10.6.1), and Microsoft Excel. Results were presented as mean ± standard deviations using tables and graphic representations.

One-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc tests was used to compare inhibition zone diameters among film groups for each microorganism. For the one week experiments (microbiological analysis, Zn migration and pH evaluation), two-factor ANOVA was applied, with storage time (days) and packaging condition (melon control, NC control, NC/ZnO-SP, NC/ZnO-SH, NC/ZnO-FL) as factors. Tuckey's and LSD post hoc tests were used to identify statistically significant differences between each factor. A two-sided $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Characterisation of NC/ZnO Films

SEM micrographs of the Valida S231C 3% cellulose are represented in Figure 2. Figures 2a,b reveal a relatively homogeneous structure, with fine and long interconnected fibres forming a dense, entangled network. SEM micrographs of the freeze-dried NC matrix show an open, highly porous network composed of interconnected cellulose fibres (Figure 2c,d). The voids and fibre intersections serve as potential sites for ZnO NPs incorporation, enabling their dispersion within the matrix.

After incorporating ZnO NPs into the NC matrix, cross-section SEM micrographs of the NC-based films were obtained to evaluate the distribution of ZnO NPs and their impact on the overall film structure (Figure 3). The neat NC film exhibits a compact and homogeneous structure, with no visible particles (Figure 3a,b). This control serves as a reference to assess the structural modifications induced by ZnO NPs incorporation. Regarding the NC/ZnO-SP composite film (Figure 3c,d), the presence of ZnO NPs is evident, but their distribution appears somewhat uneven, with regions showing higher particle concentration. This may indicate partial aggregation of the ZnO NPs within the cellulose matrix. In contrast, the NC/ZnO-FL film (Figure 3e,f) exhibits a more homogeneous dispersion of ZnO NPs, with the particles appearing to be better distributed throughout the matrix. The micrographs suggest that ZnO NPs are not only embedded within the NC fibres but also deposited on the surface. Regarding NC/ZnO-SH (Figure

3g,h), NPs appear to be well distributed across both the surface and internal regions of the film, with some small particle clusters observed within the layered NC structure. All the films present a torn, layered appearance as they were not rigid enough to fracture cleanly. Nevertheless, this did not hinder the observation of the internal structure and particle distribution.

ZnO NPs embedded in the matrix might be bound to the hydroxyl groups of cellulose fibres through hydrogen bonding (Ghule et al., 2006). Comparing the ZnO-loaded films with the neat NC film, it is evident that ZnO NPs incorporation influences the cellulose matrix: an increase in porosity can be observed within the NC network, potentially leading to greater inter-fibre spacing. Additionally, the formation of ZnO NPs clusters in certain regions may introduce structural irregularities that could influence the mechanical integrity of the films. Abbas et al., 2019 reported that a uniform distribution of nanoparticles is crucial for enhancing mechanical properties, whereas aggregation can lead to decreased flexibility and potential defects in the composite structure.

The interaction between ZnO NPs and the cellulose matrix is also crucial for evaluating Zn migration. Uniform dispersion of ZnO NPs with strong bonding to the matrix can lead to lower migration rates. In contrast, higher aggregation can alter the structural integrity of the composite, potentially enabling nanoparticle migration (Babakhani, 2019). Furthermore, in areas where ZnO NPs are more exposed to the film surface, a higher release of ZnO over time may be expected. Studies have reported that the extent of NPs exposure on the film's surface plays a significant role in Zn migration behaviour (Bumbudsanpharoke et al., 2019). Excellent compatibility and miscibility between the NC matrix and the filler materials were also observed in cellulose nanofiber-based composite films reinforced with zinc oxide nanorods and grapefruit seed extract (Roy et al., 2021).

The thickness of neat NC was 29.6 ± 1.1 µm, which slightly increased upon the incorporation of ZnO NPs. Among the samples, NC/ZnO-SH exhibited the highest thickness value (42.0 ± 4.0 µm), compared to the films with spherical (33.4 ± 4.2 µm) and flower-shaped (32.4 ± 2.5 µm) ZnO NPs incorporation. This increase confirms that the incorporation of fillers into the film matrix influences its thickness, as previously reported (Ahmadi et al., 2021). This trend aligns with prior findings, in which the incorporation of ZnO NPs into chitosan-based films resulted in a gradual increase in thickness from 42.0 µm to 59.3 µm, depending on the ZnO NPs concentration (Souza et al., 2021). Another study reported that incorporating Fe_3O_4 NPs into the cellulosic matrix increased the distance between cellulose fibres in the cross-section of the films, thereby increasing film thickness (Furlan et al., 2019).

3.2. Antimicrobial Activity of NC/ZnO Nanocomposite Films

The antimicrobial activity of the different NC/ZnO films was evaluated against gram-negative *E. coli* and gram-positive *S. aureus* using an agar diffusion assay. The results are presented in Table 2 and Figure 4. Incorporation of ZnO NPs into NC films enhanced antimicrobial efficacy compared to neat NC, particularly against *S. aureus*, although the magnitude of the effect depended on nanoparticle shape.

Among the tested morphologies, sheet-shaped ZnO NPs produced the strongest inhibitory effect against both bacteria, followed by spherical shape; flower-like particles showed only limited or negligible effect. For *S. aureus*, the NC/ZnO-SP and NC/ZnO-SH films produced inhibition zones of 20.5 ± 1.0 and 21.8 ± 1.2 mm, respectively, compared with 17.1 ± 4.2 mm for the NC control. In contrast, the NC/ZnO-FL film produced a smaller inhibition zone (16.1 ± 1.0 mm) than the control. Statistical analysis revealed overall differences among films (one-way ANOVA, $p < 0.05$). NC/ZnO–SH films showed significantly larger inhibition zones than neat NC (Fisher's LSD test, $p < 0.05$). Conversely, NC/ZnO–FL films produced significantly smaller zones than both NC/ZnO–SP and NC/ZnO–SH (Fisher's LSD test, $p < 0.05$), indicating lower antibacterial efficacy against *S. aureus*. Although neat NC films exhibited initial antimicrobial activity, bacterial regrowth was observed within the inhibition zone upon disc removal after 48 h, indicating that the effect was not maintained. In contrast, inhibition zone corresponding to the films containing ZnO NPs maintained clear and stable aspect for at least 7 days, confirming their long-term antimicrobial performance.

For *E. coli*, NC/ZnO-SH and NC/ZnO-SP films produced the largest inhibition zones (17.5 ± 0.3 and 16.9 ± 1.1 mm, respectively), while NC/ZnO-FL and neat NC film produced smaller zones (11.4 ± 5.6 and 10.5 ± 5.4 mm, respectively). However, differences among films were not statistically significant (one-way ANOVA, $p = 0.126$). After disc removal, no bacterial regrowth was observed in any inhibition zone, including those produced by the NC control, for at least 7 days. This apparent absence may be partially influenced by the hygroscopic and adhesive nature of NC, which can retain moisture and physically entrap bacterial cells, thereby potentially preventing the visualization of colonies (Solhi et al., 2023). Another possible explanation is a baseline antimicrobial activity of the neat NC film, attributed to the presence of the 1,2-Benzisothiazolin-3-one, a cellulose dispersion additive with well-documented antimicrobial properties against bacteria, fungi, and yeast (Liu et al., 2025; Viani et al., 2017). Incorporating ZnO NPs, especially those in the SH and SP morphologies, further enhanced the effect, resulting in larger and more uniform inhibition zones.

These findings are consistent with previous reports on ZnO NPs tested in suspension (Mendes et al., 2024, 2025), in which SP and SH morphologies demonstrated superior

antimicrobial activity compared with FL. When incorporated into NC films, however, the ZnO-FL antibacterial effect was almost completely suppressed, suggesting that the NC matrix plays a critical role in modulating ZnO antimicrobial activity. Notably, in some cases NC/ZnO-FL films even displayed smaller inhibition zones than neat NC, indicating that the presence of FL particles may also attenuate the intrinsic antimicrobial activity of the NC matrix. This effect could arise from the three-dimensional geometry of FL particles, which may become entrapped within the fibrillar cellulose network, thereby hindering the diffusion of antimicrobial agents. Such interference may affect not only the release of Zn^{2+} ions and ROS from the FL particles, but also the diffusion of compounds inherently present in neat NC (benzisothiazolinone). Indeed, our previous work demonstrated that ZnO-FL possesses a significantly lower specific surface area compared with ZnO-SP and ZnO-SH particles (Mendes et al., 2024), further supporting their reduced antimicrobial performance. In contrast, SP and SH nanoparticles offer great surface area and more efficient Zn^{2+}/ROS release, which explains the larger inhibition zones observed.

In this study, *S. aureus* (gram-positive) was more susceptible to ZnO NPs than *E. coli* (gram-negative). This difference is likely due to the structural differences in their cell walls, as the outer lipopolysaccharide membrane of gram-negative bacteria acts as an additional barrier that limits the penetration of antimicrobial agents, such as Zn^{2+} ions and ROS (Babayevska et al., 2022). This trend contrasts with our previous findings in suspension (Mendes et al., 2025), where freely dispersed ZnO NPs produced greater activity against *E. coli* than against *S. aureus*. In suspension, rapid Zn^{2+} release, enhanced particle–cell interactions, and ROS generation likely account for the stronger effect on *E. coli*, which is often more vulnerable to oxidative stress (Behera et al., 2024). However, when ZnO NPs are embedded in NC films, nanoparticle mobility and ion release are restricted, and the diffusion-based nature of the agar assay further limits the availability of antimicrobial agents. Under these conditions, the outer membrane of *E. coli* may provide additional protection, while *S. aureus*, lacking this outer layer, is comparatively more affected. Similar findings have been reported for bacterial cellulose films incorporated with ZnO NPs (Shahmohammadi Jebel & Almasi, 2016), although that study used commercial particles and did not examine NP morphology. In contrast, in the present study the NC control exhibited some baseline antimicrobial activity, which may have influenced the comparative outcomes.

Table 2 - Antimicrobial activity of NC/ZnO films determined by agar diffusion assay. Results are expressed as inhibitory halos (mm).

Microorganism	Neat NC	NC/ZnO-SP	NC/ZnO-SH	NC/ZnO-FL
S. aureus ATCC 6538P	17.1 ± 4.2	20.5 ± 1.0	21.8 ± 1.2	16.0 ± 1.0

E. coli *ATCC 8739*	10.5 ± 5.4	16.9 ± 1.1	17.5 ± 0.3	11.4 ± 5.6

Values represent the mean ± SD of three independent experiments, each performed in technical triplicate (three discs per plate; two diameter readings per disc).

372

373 **3.3. Shelf Life Evaluation of Fresh Melon Packaged with NC/ZnO Films**

374 **3.3.1. Microbial Growth Monitoring**

375 To evaluate the applicability of NC/ZnO films as a packaging material for extending food shelf
376 life, microbiological analyses were performed on fresh-cut melon samples stored at 4 °C for 7
377 days. As expected, both the melon control and the neat NC control showed increasing microbial
378 counts during storage, with counts consistently higher than those observed for melons packaged
379 with NC/ZnO films across all microbial groups (Figure 5). Notably, the melon with NC control
380 exhibited approximately 1 log CFU g^{-1} higher microbial counts than the melon control alone.
381 Although neat NC films displayed antimicrobial activity in the agar diffusion assay, likely due to
382 the presence of 1,2-Benzisothiazolin-3-one, this effect was not evident in the melon storage
383 tests, where microbial growth was even more pronounced. This apparent divergence can be
384 attributed to differences between the two experimental systems. In the agar diffusion assay,
385 antimicrobial compounds can easily diffuse through the agar medium because of its simple
386 matrix nature, facilitating the formation of inhibition zones. In contrast, in real food systems such
387 as fresh-cut melon, the diffusion of such compounds from the film to food surface may be
388 limited. In fact, the composition of the melon is a complex interaction of carbohydrates, fibers,
389 minerals, etc. Additionally, the NC film may reduce surface water loss and maintain a more humid
390 environment around the melon surface, which can favour microbial proliferation (Ahmad &
391 Siddiqui, 2015). Furthermore, interactions with the natural microbiota of melon may reduce the
392 effectiveness of weak antimicrobial compounds. Together, these factors may mask the intrinsic
393 antimicrobial effect observed for neat NC.

394 Melon samples packaged with NC/ZnO films exhibited a clear reduction in microbial growth
395 throughout storage, confirming the antimicrobial efficacy of the ZnO-containing films. However,
396 the magnitude of this effect strongly depended on nanoparticle morphology. NC/ZnO-SH films
397 were the most effective, maintaining the lowest microbial counts, followed by spherical particles,
398 while flower-shaped particles exhibited only limited activity. These results are consistent with
399 the agar diffusion assay and with previous reports on ZnO NPs tested in suspension (Mendes et
400 al., 2024, 2025), in which SP and SH morphologies demonstrated superior antimicrobial activity,
401 whereas FL displayed poor antimicrobial performance.

Initial populations of total mesophilic aerobic bacteria at 30 °C and 7 °C were approximately 2 and 1 log CFU g^{-1}, respectively, while *Enterobacteriaceae* as well as yeasts and moulds were below the detection limit, indicating the good initial microbiological quality of the fresh-cut melon. For total aerobic counts at 30 °C and 7 °C (Figure 5a,b), both storage time and packaging condition had a statistically significant effect on microbial counts ($p < 0.05$). However, the interaction between the two factors was only significant at 30 °C. Counts steadily increase in the control samples, reaching values close to or above 10^5 CFU g^{-1} by day 7, which corresponds to the upper acceptability limit for fresh-cut fruits (INSA, 2019; Oms-Oliu et al., 2010). In contrast, melon samples packaged with NC/ZnO films exhibited lower counts throughout storage. At 30 °C, all ZnO films resulted in statistically significant reductions in counts on day 7 compared with the cellulose control ($p < 0.05$). In particular, NC/ZnO-SH films were the most effective, with final counts of approximately 10^2 CFU g^{-1}, which were significantly lower than those of all other study groups (controls and the other ZnO films, $p < 0.05$). On the other hand, NC/ZnO-FL initially delayed microbial proliferation (at day 3, compared with NC control, $p < 0.05$), but this effect was not sustained, and by day 7, they displayed the highest microbial loads among the three morphologies, confirming their poor antimicrobial performance. At 7 °C (Figure 5b), the control groups (melon and NC film) were significantly higher than the ZnO-containing film groups ($p < 0.05$).

Enterobacteriaceae became detectable in the control samples after day 3 and increased sharply until the end of storage (Figure 5c). In the NC/ZnO films, detectable viable microorganisms appeared later during storage, and the microbial growth increased more slowly, remaining lower than in the controls. The interaction between packaging condition and storage time for *Enterobacteriaceae* counts was significant ($p < 0.05$). Higher counts were observed in melon packaged with NC films, reaching values of 10^5 CFU g^{-1} by day 7, which corresponds to the upper acceptability limit for fresh-cut fruits. According to microbiological guidelines, satisfactory levels of *Enterobacteriaceae* should remain below 10^4 CFU g^{-1} (INSA, 2019; Oms-Oliu et al., 2010). In contrast, populations remained very low or undetectable in melons packaged with NC/ZnO films, particularly with the SH morphology. On day 7, differences were observed for all three ZnO morphologies compared with both melon and NC controls ($p < 0.05$). Although *Enterobacteriaceae* are primarily used as hygiene indicators (relevant to initial contamination and processing), their marked inhibition during storage is also important because members of this group include potential foodborne pathogens (Schwan et al., 2022).

Yeasts and moulds also showed progressive growth in the control samples, reaching approximately 4 log CFU g^{-1} after 7 days, whereas their proliferation was significantly reduced in the presence of ZnO-containing films (Figure 5d). There were no statistically significant

differences in counts across packaging groups, nor any interaction between time and packaging groups. However, the effect of time showed that after one week, yeast and mould counts were significantly higher than at earlier time points ($p < 0.05$). According to microbiological guidelines, counts below 10^4 CFU g^{-1} are considered satisfactory, and all samples complied with this limit throughout the 7-day storage period (INSA, 2019; Oms-Oliu et al., 2010). Yeasts and moulds are generally present at lower levels than total mesophilic aerobic bacteria in fresh-cut melon, as previously reported by Ukuku et al., 2018, which is consistent with the trends observed in the present study.

In a previous study, cellulose-based absorbent pads containing silver nanoparticles, instead of ZnO-NPs, were applied to fresh-cut melon (Fernández et al., 2010). In that work, microbial growth in melon pieces was only moderately reduced: approximately 0.8 log CFU g^{-1} for total aerobic counts at 30 and 7 °C. This can be explained by the indirect contact of the silver-loaded pads with the fruit, as they became active mainly after being impregnated with melon exudates, thereby limiting their direct antimicrobial effect on the fruit surface. In contrast, the NC/ZnO films in the present study remained in continuous contact with the melon, leading to greater reductions in microbial counts. Similar antimicrobial trends in fresh-cut melon have been reported when incorporating silver NPs into other biopolymeric matrices, such as chitosan (Ortiz-Duarte et al., 2019) and alginate (Danza et al., 2015). However, the magnitude of microbial inhibition varied depending on the polymer and nanoparticle type.

The stronger antimicrobial effect observed in this study, particularly for the ZnO-SH films, can be explained by the multiple antimicrobial mechanisms of ZnO NPs. These include the release of Zn^{2+} ions, the generation of ROS, which induce oxidative damage to cellular components, and the direct contact of nanoparticles with microbial membranes, leading to structural disruption. The superior performance of the SH morphology can be attributed to its higher specific surface area, and the continuous contact of the films with the melon surface likely promoted enhanced Zn^{2+} release and ROS generation, thereby reinforcing their antimicrobial activity (Mendes et al., 2024). In contrast, FL showed lower surface area and poorer complex interactions with the matrix, reducing ion/ROS flux and direct cell contact.

In summary, these results clearly demonstrate that the incorporation of ZnO NPs delays microbial proliferation in fresh-cut melon, thereby extending its microbiological shelf life. NC/ZnO-SH films effectively maintained safe microbial levels for at least 7 days under refrigerated storage, whereas control samples reached the upper acceptability limit within 3–4 days. These findings highlight the potential of ZnO-containing cellulose films as active packaging materials to improve food safety, prolong shelf life, and reduce food waste in minimally processed fruits.

3.3.2. Evaluation of Zinc Migration in Packaged Melon

Zinc migration from NC/ZnO nanocomposite films into melon was assessed under realistic conditions. The results showed that melons in contact with all NC/ZnO films exhibited a similar migration profile, characterized by a rapid increase during the initial days of storage, followed by a stabilization after day 4 (Figure 6). This trend suggests an initial fast diffusion of loosely bound or surface-available Zn species, followed by the establishment of equilibrium between the film and food matrix. Although the three morphologies exhibited distinct particle sizes and specific surface areas, as represented in Figure S1, these differences did not influence Zn migration in the melon matrix. In fact, ZnO NPs morphology can influence dissolution behaviour, as smaller particles with higher surface areas tend to release more Zn^{2+} ions. However, under the present conditions, the migration process appears to be governed primarily by the properties of the food matrix rather than by NPs morphology. The high water content of melon may promote ZnO dissolution and Zn^{2+} release in a similar pattern for all the samples, regardless of NPs and morphology.

The SML for Zn (5 mg kg^{-1} of food) was reached after approximately 2 days, with concentrations further increasing to 7 - 8 mg kg^{-1} melon. These levels exceeded the migration limit, underscoring the importance of carefully controlling the interaction between ZnO NPs and the food matrix, as balancing antimicrobial efficacy with regulatory compliance is essential for the safe application of such materials in food packaging. It is important to note that the experimental setup represents a high contact surface area scenario, involving direct exposure of a small melon portion to a relatively large film area, which increases migration compared to typical packaging conditions. Therefore, these results should not be interpreted as an automatic disqualification of ZnO nanocomposites for food packaging applications. These findings represent a worst-case scenario and underscore the need for further optimization of overall packaging, considering that Zn migration behaviour strongly depends on several factors, including NPs loading, NPs/matrix interaction, food composition, storage time, temperature, and the surface area-to-mass ratio between packaging and food.

The control melon samples exhibited negligible Zn levels throughout the storage period, confirming that the detected Zn was originated from nanocomposite films. Two-way ANOVA revealed statistically significant interaction between storage time and packaging condition groups ($p < 0.05$) on Zn migration. Interaction post-hoc Tukey's test showed that, from day 1 onwards, all melons containing NC/ZnO NPs films exhibited significantly higher Zn levels

505 compared to the control ($p < 0.05$), and no significant differences were observed among the
506 different ZnO film morphologies at any time point.

507 The experimental Zn concentration data (Table S2) were converted to realistic packaging
508 conditions based on the actual surface area-to-mass ratio of a half melon. Average melon
509 dimensions of 25x15 cm and a total weight of 4 kg per fruit (2 kg per half fruit) were considered.
510 The edible portion was assumed to extend 4 cm from the surface. After subtracting the seed
511 cavity, the effective contact area was estimated as 2 dm^2. Normalized to the mass of 2 kg, this
512 corresponded to an A/M ratio of 1 $dm^2\ kg^{-1}$ (Figure S3). The detailed calculation is provided in
513 supplementary material. No previous studies on packaged melon have assessed the migration
514 of compounds directly to the fruit itself. One study developing cellulose pad with silver NPs
515 demonstrated silver migration into an external medium (Mueller-Hinton Broth) using graphite-
516 furnace atomic absorption spectrometry, rather than directly into the melon matrix (Fernández
517 et al., 2010).

518 The Zn migration profiles observed in melon differed from those previously reported for the
519 same films tested with food simulants (water and 10% ethanol) (Mendes et al., 2026). Higher Zn
520 migration rates were observed in the real food matrix than in food simulants. In water food
521 simulant, the SML was reached only after 29 days, and Zn levels approached 8 mg kg^{-1} after 35
522 days. In contrast, melon samples reached similar Zn concentrations after only 8 days of storage.
523 Moreover, when using food simulants, a clear difference in Zn migration was observed among
524 the three ZnO morphologies, whereas in melon, all samples exhibited similar migration
525 behaviour. In fact, melon has a complex composition, including organic acids, sugars, high water
526 content, and enzymatic activity, that may facilitate ZnO dissolution and Zn^{2+} release (Youn &
527 Choi, 2022). Studies with ZnO NPs incorporated in bacterial nanocellulose have also
528 demonstrated that Zn migration was much higher in chicken food than in ethanolic food
529 simulants (Soares Silva et al., 2023). This discrepancy highlights the limitations of simulant-based
530 testing as models of real food interactions, although such approaches remain important for
531 regulatory compliance when overestimation is assured (Lerch et al., 2023). It is important to note
532 that migration levels in real foods are expected to vary depending on food type, storage
533 conditions, packaging characteristics, and intrinsic food properties, such as composition, water
534 content, and pH. Under realistic exposure conditions, Zn migration from NC/ZnO films can
535 approach or surpass regulatory migration limits, especially in high-moisture and acidic food
536 matrices. Future studies should therefore extend to different food matrices and packaging
537 systems to better understand the NPs migration variation under real case scenarios. Considering
538 the relatively high Zn concentrations observed in melon, future strategies could also include the
539 application of surface coatings or barrier layers designed to delay Zn migration.

Another aspect to consider is that the present study addressed only total Zn migration, without differentiating between ionic Zn^{2+} and nanoparticulate forms. Based on previous results, also for NC films, obtained with food simulants, ca 18% of the total migrated Zn corresponded to ZnO NPs (Mendes et al., 2026). As a rough estimation, a similar ratio can be considered for melon samples. Considering the total Zn migration values of approximately 8 mg kg^{-1} melon, this corresponds to an estimated 1.3 – 1.5 mg kg^{-1} potentially attributed to ZnO NPs, while the remaining proportion would represent the ionic Zn^{2+}. However, this estimation should be interpreted with caution, as the complex composition of melon (organic acids, sugars, enzymes) may influence ZnO dissolution and migration dynamics, leading to a different ionic/nanoparticle ratio compared with that of food simulants. Differentiating between these two migrating forms is essential for accurate assessment of potential exposure and toxicity, since physicochemical properties, mechanisms and bioavailability of Zn^{2+} ions differ from those of ZnO NPs (EFSA, 2021; Wu et al., 2019). This distinction remains one of the major analytical challenges in the field, as well as regulation gap regarding nanoparticle migration (Ansari et al., 2026). Understanding the type of migration and the release mechanisms is indeed crucial for future toxicological evaluations of these materials.

3.3.3. Monitoring of pH in Packaged Melon

One crucial parameter related with microbiological and Zn migration results was the pH evolution of fresh-cut melon in contact with NC/ZnO films, stored at 4 °C for 7 days. Results for melon control and melon with nanocomposite films are shown in Figure 7. The initial pH of 6.5 exhibited a sharp decrease on the first day, followed by stabilization until day 7 for all the samples. This initial pH drop may be associated with physiological responses triggered by tissue disruption during cutting, which can induce stress and accelerate metabolic processes in fresh-cut fruits, thereby masking the immediate effect of the packaging (Guo et al., 2023). The subsequent observed fluctuations may be attributed to intrinsic variability of the melon metabolic activity, as well as the experimental measurements. Both melon control and the melon-NC control demonstrated a stronger decrease (to approximately 5.8), whereas melons packaged with NC/ZnO films maintained slightly higher pH values after 7 days. Two-way ANOVA showed that storage time and packaging condition significantly affected melon pH values ($p < 0.05$), while their interaction was not significant. Post-hoc Tukey's test revealed that melon packaged with NC control film exhibited significantly lower pH compared to melons packaged with NC/ZnO films ($p < 0.05$). The more acidic pH observed in the control samples can be explained by the higher microbial activity (Figure 5), since proliferation of microorganisms producing organic acids and other metabolites contribute to pH reduction and accelerate fruit

spoilage. In contrast, melons packaged with ZnO films maintained slightly higher pH values, likely due to microbial growth inhibition. Furthermore, pH is also a key factor influencing ZnO solubilization, as lower pH may promote dissolution and the subsequent release of Zn^{2+} ions (Hakeem et al., 2020). The gradual pH decrease observed over one week may therefore contribute for the increased Zn migration in melons packaged with NC/ZnO films (Figure 6).

A similar behaviour of an initial pH decline has been reported for fresh-cut melon fruit packaged with chitosan/graphene oxide films (Paiva et al., 2020). However, that study extended storage to 21 days, revealing a subsequent pH increase after the initial decline, which was not seen in the current work. This implies that multiphase dynamics may influence pH change based on storage duration and microbial activity. In food science, pH is a crucial quality parameter as it influences various aspects such texture, flavour, and microbial stability, while also serving as an indicator of food safety (Venkatesan & Muniyan, 2024). Nevertheless, the intrinsic characteristics of the food matrix, such as water activity and sugar content, can strongly influence the pH evolution. Additionally, extrinsic factors such as initial microbial load and storage conditions can also significantly affect these dynamics. As a result, the present outcomes cannot be directly generalized to other food matrices and should instead be evaluated on a case-by-case basis, considering their specific physicochemical and microbiological properties.

4. Conclusion

This work allowed for the development and evaluation of NC films containing three different ZnO NPs morphologies, assessing their capacity to extend melon shelf life, by reducing microbial proliferation, in parallel with zinc migration assessment.

SEM micrographs confirmed the nanofibrillar and porous NC network of the films, suggesting an appropriate structure for ZnO NPs linking, and cross-sectional SEM showed the distribution of the ZnO NPs within the matrix. *In vitro* antimicrobial activity of NC/ZnO films was assessed against *S. aureus* and *E. coli,* where results revealed a clear morphology effect: NC/ZnO–SH produced higher inhibitory halos, followed by NC/ZnO-SP, whereas FL films showed lower inhibition. The greater susceptibility of *S. aureus* compared with *E. coli* is consistent with the additional outer membrane of gram-negative *E. coli*, that limits the Zn^{2+}/ROS release from NPs.

When applied to a fresh-cut melon and stored at 4 °C for one week, NC/ZnO films slowed microbial proliferation compared with neat NC and control melon samples. The effect of NPs morphology was evident: ZnO-SH produced the slowest increase in microbial loads, followed by ZnO-SP, whereas ZnO-FL showed the weakest inhibition among the three shapes. Under a realistic area-to-mass scenario, total Zn reached the SML at day 2 and stabilized by 7–8 mg kg^{-1},

609 with no differences among morphologies. Melons packaged with NC/ZnO-NPs films presented
610 slightly higher pH compared with NC and melon control, consistent with antimicrobial and Zn
611 migration results.

612 Current EU Regulations and EFSA guidelines were developed for plastics FCM and do not yet
613 fully cover bio-based composites. Moreover, nanoparticle migration in FCM remains
614 insufficiently addressed and is handled on a case-by-case basis for risk assessment. In this study,
615 total Zn was quantified after acid digestion of the whole melon cube, so ionic and particulate
616 forms could not be differentiated. Future work should include particle/ion discrimination using
617 other analytical techniques for quantification, such as single particle inductively coupled plasma
618 mass spectrometry (spICP-MS) or Asymmetric-Flow Field-Flow Fractionation (AF4). Furthermore,
619 it would be valuable to analyse the concentration profile within the melon pulp by determining
620 the Zn levels in direct contact with the film and in regions progressively farther from the contact
621 surface, in order to evaluate the extent of Zn diffusion through the pulp. Additionally, the
622 quantification of the amount of Zn remaining in the film itself after the migration step would
623 have been of interest as a mass balance verification. Moreover, experiments were limited to one
624 food matrix and storage condition. Evaluation of different fruits and storage conditions,
625 combined with toxicological and sensory studies, would be relevant for better assessing
626 consumer safety and acceptance.

627 Overall, NC/ZnO films, particularly those with sheet- and spherical-shaped ZnO NPs,
628 effectively reduced microbial proliferation and maintained microbiological acceptability for at
629 least 7 days, extending food shelf life. Achieving an appropriate balance between antimicrobial
630 performance and zinc migration is required, give the potential toxicological implications. Overall,
631 the effectiveness of bio-based NC/ZnO film is determined by nanomaterial choice, particle
632 morphology, and the properties of the packaging system.

633

634 **Supplementary Materials**

635 The following supporting information can be downloaded at “link”. **Figure S1** – SEM (top row)
636 and TEM (bottom row) micrographs of ZnO NPs with different morphologies (Mendes et al.,
637 2024, 2025). **Table S1** - Summary of the main physicochemical and functional properties of ZnO
638 NPs with different morphologies (Mendes et al., 2024, 2025). **Figure S2** - NC/ZnO film placed on
639 a PCA agar plate after UV treatment to confirm the effectiveness of UV sterilization prior to
640 antimicrobial assays. No microbial growth was observed.; **Figure S3** – Schematic and calculation
641 of the area-to-mass (A/M) ratio used for realistic normalization. Assumptions: external
642 dimensions 25x15 cm; edible portion 4 cm; whole fruit mass 4 kg (2 kg per half). After subtracting

the seed cavity, effective contact area = 2 dm^2. Normalized to 2 kg, A/M = 1 dm^2 kg^{-1}.; **Table S2** - Zn migration (mg kg^{-1} melon) from NC/ZnO films into fresh-cut melon during storage at 4 °C. Results are expressed as mean ± standard deviation (n = 3). The melon control corresponds to samples stored without contact with the nanocomposite films.

CRediT Authorship Contribution Statement

Ana Rita Mendes: Investigation, Methodology, Formal analysis, Writing—original draft. **Francisco A.G. Soares Silva:** Investigation, Methodology, Writing—review and editing. **Cristina Mena:** Methodology, Writing—review and editing. **Fátima Silva:** Methodology, Writing—review and editing. **Cristina L. M. Silva:** Writing—review and editing. **Paula Teixeira:** Conceptualization, Writing—review and editing. **Fátima Poças:** Supervision, Conceptualization, Resources, Writing—review and editing.

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Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Figures

Figure 1 – Experimental procedure with melon samples: a) whole melon fruits purchased from a local Portuguese supermarket; b) melon slice (~3x3 cm) wrapped with the NC/ZnO film (5x5 cm); c) wrapped samples corresponding to one of the five experimental time points, stored in sterile bags for subsequent analysis.

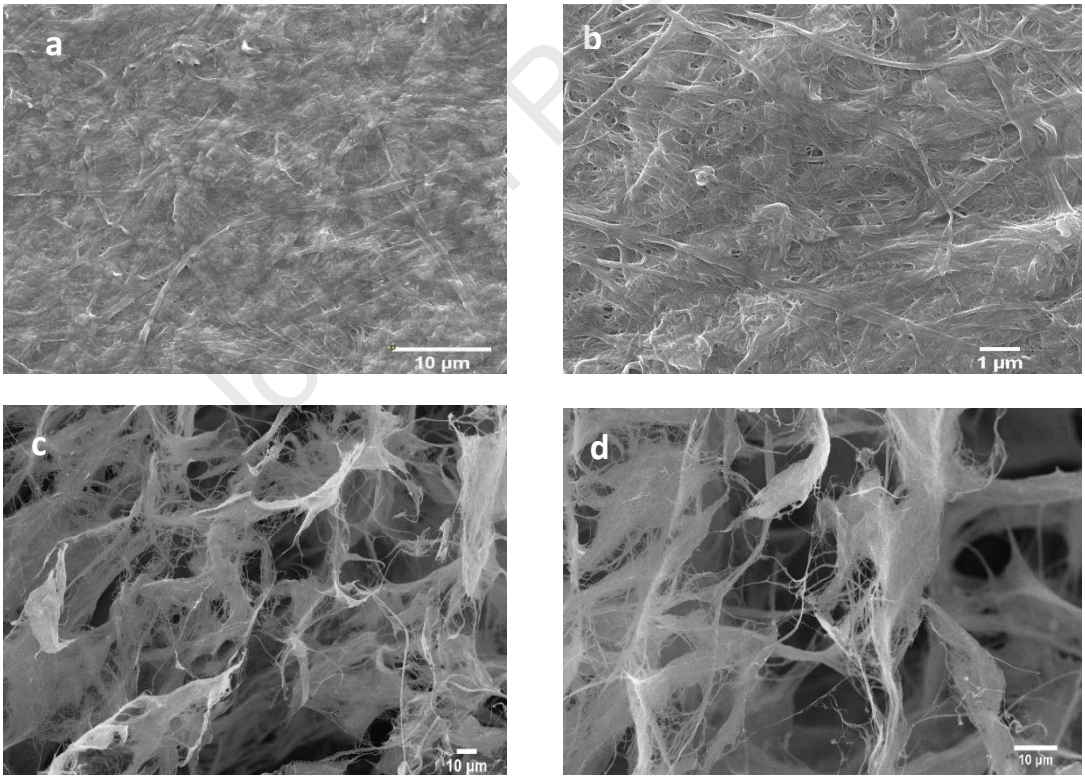

Figure 2 - SEM micrographs of the NC matrix surface at magnifications of (a) 5 000x and (b) 20 000x; and after freeze-drying process at magnifications of (c) 2 050x and (d) 4 300x.

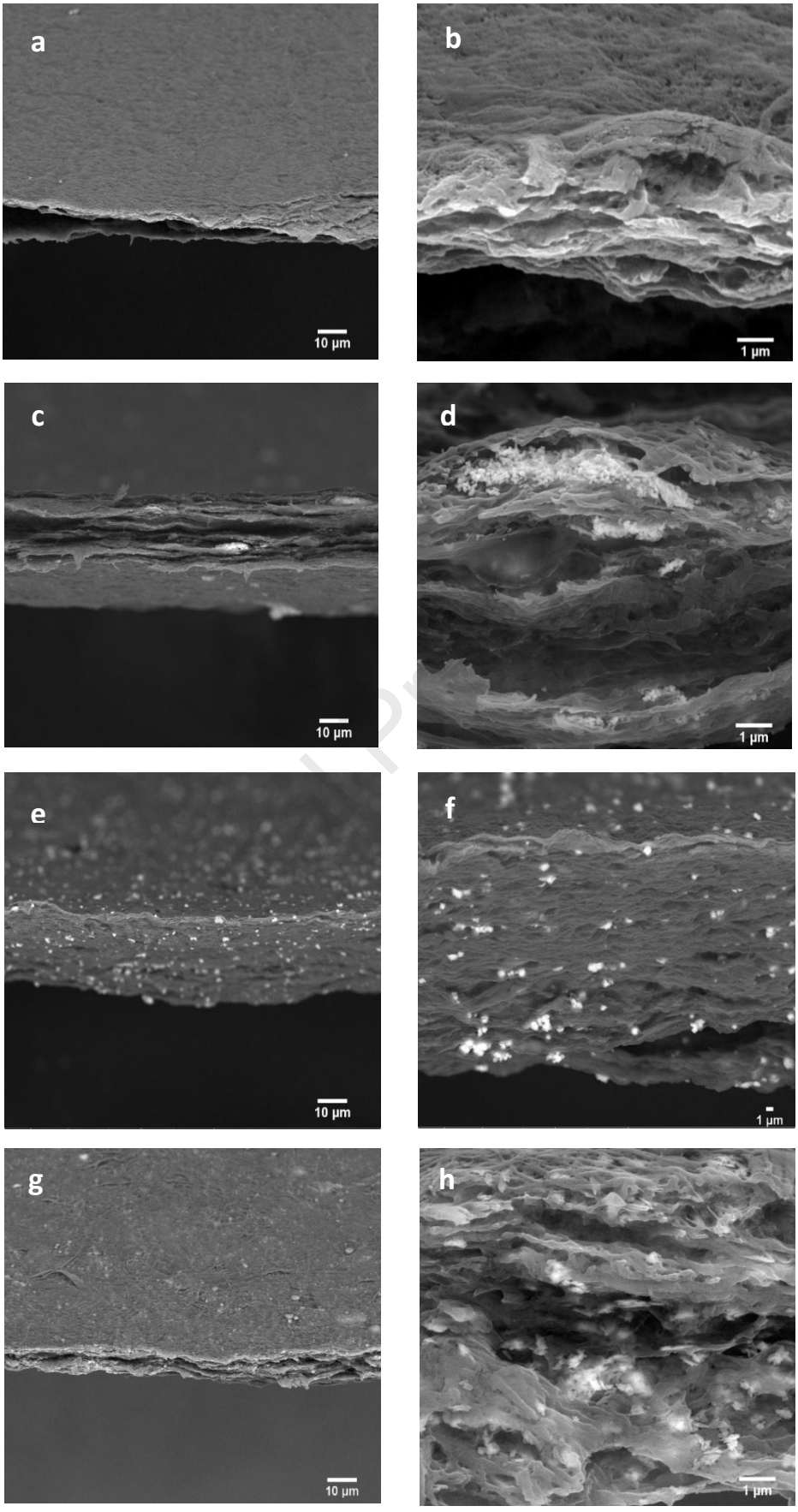

Figure 3 - Cross-section SEM micrographs of the films: NC (a, b), NC/ZnO-SP (c, d), NC/ZnO-FL (e, f), and NC/ZnO-SH (g, h). Magnifications: 2 000x (a, c, e, g); 5 000x (f); 25 000x (b, d, h).

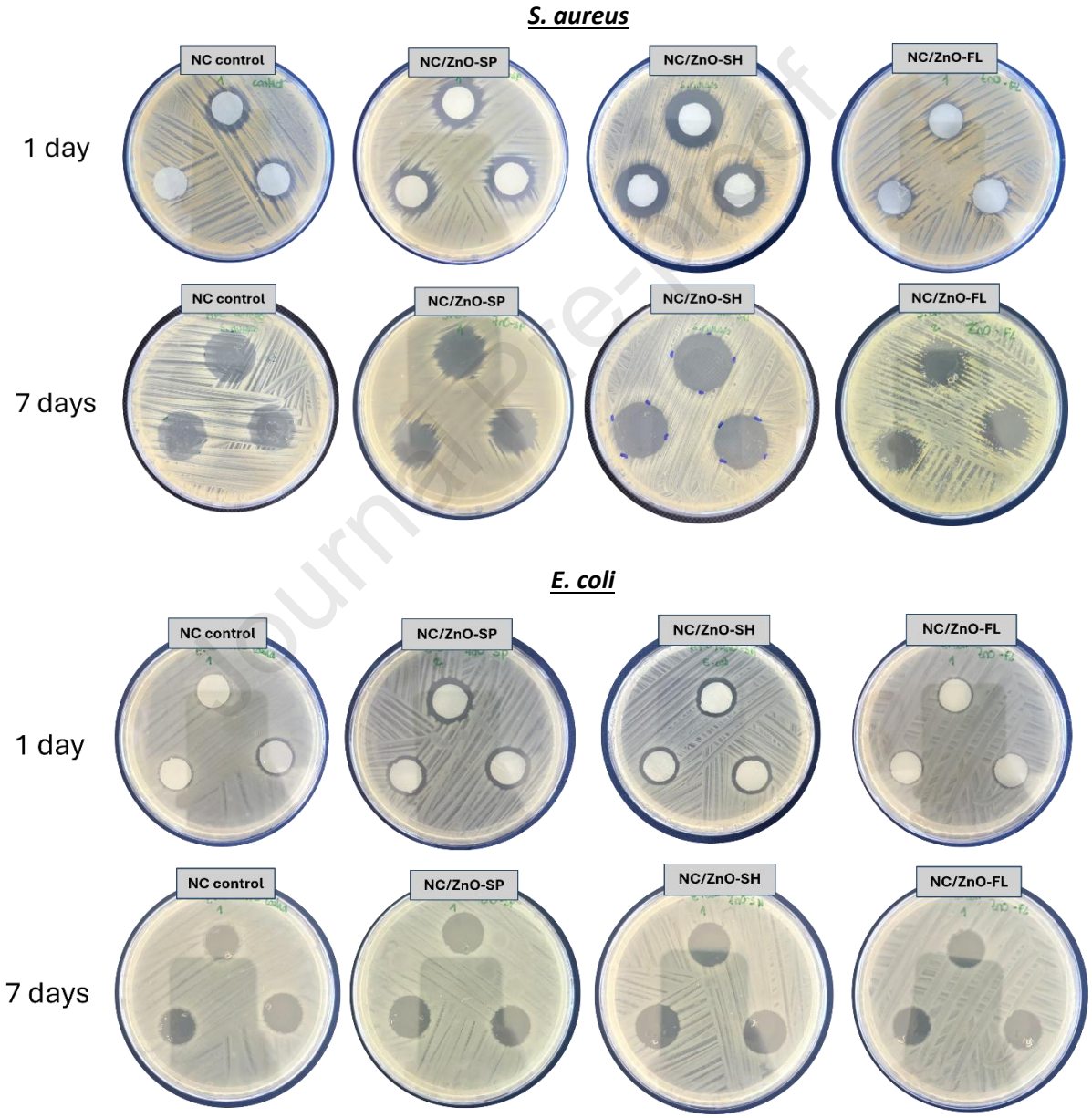

Figure 4 – Agar diffusion assay of NC/ZnO films against *S. aureus* and *E. coli* after 24 h of incubation and 7 days after disc removal.

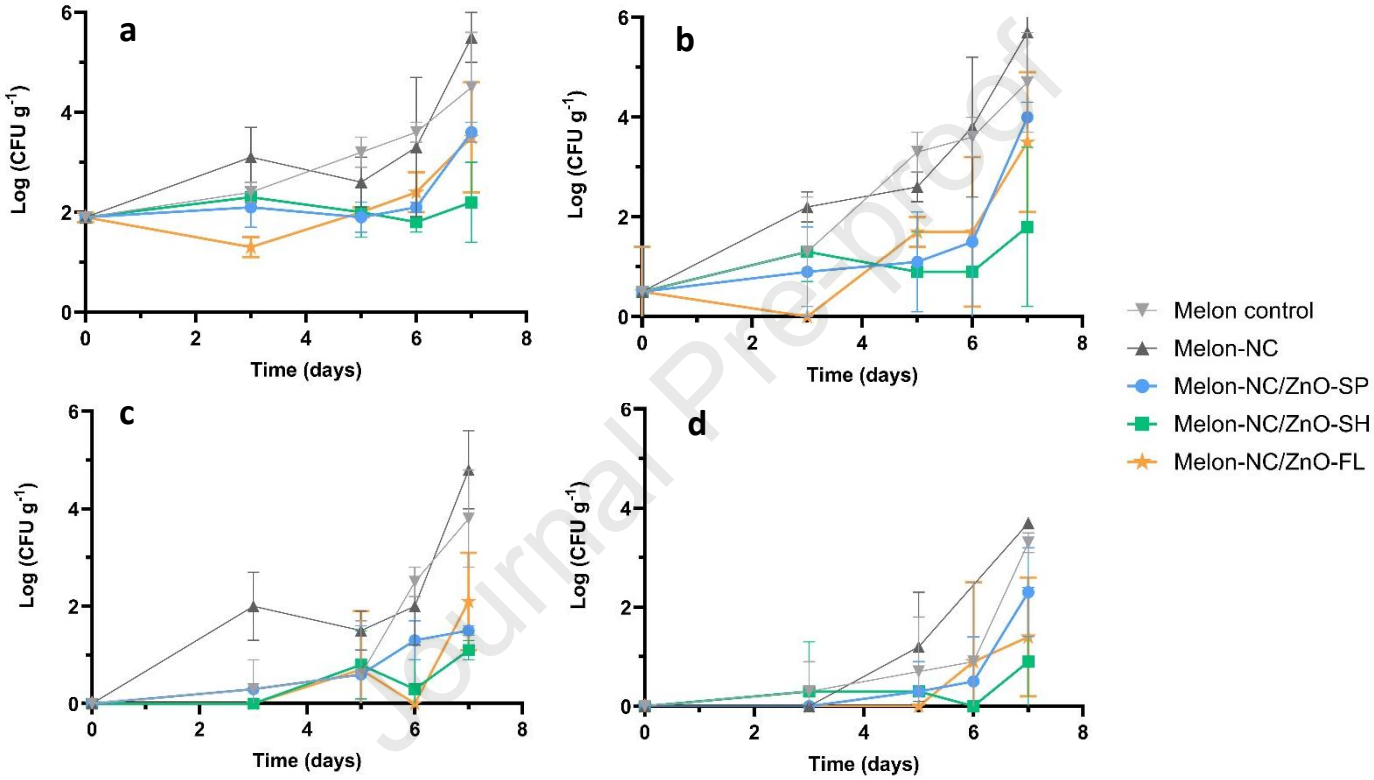

Figure 5 - Microbial counts of fresh-cut melon samples stored at 4 °C for 7 days: a) total aerobic counts at 30 °C, b) total aerobic counts at 7 °C, c) *Enterobacteriaceae*, and d) yeasts and moulds. Samples include melon control, NC film control, and NC films containing ZnO NPs (SP, SH, FL). Results are expressed as mean ± standard deviation (n = 3) and reported as log CFU g^{-1}.

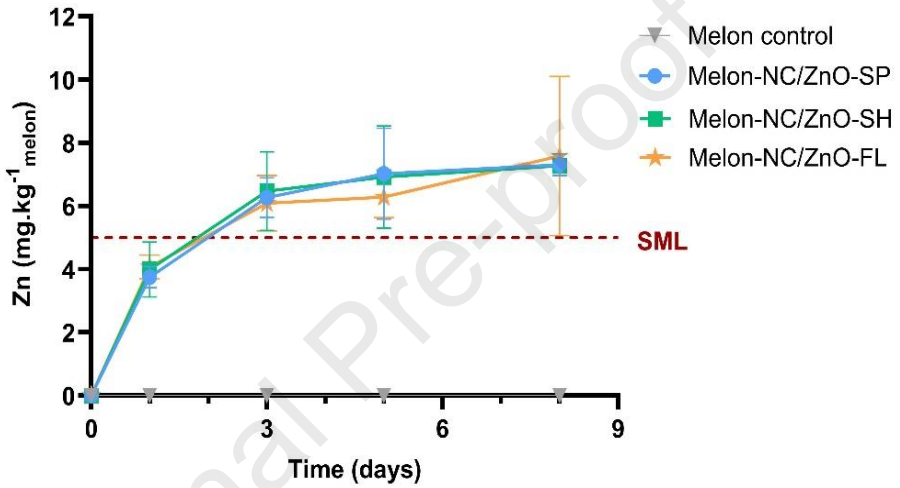

Figure 6 – Zn migration from NC/ZnO nanocomposite films into fresh-cut melon during storage at 4 °C for 8 days, under a realistic scenario (A/M = 1 dm^2 kg^{-1} food). Results are expressed as mean ± standard deviation (n = 3) and reported as mg kg^{-1} melon. The SML (5 mg kg^{-1}) food is indicated for reference.

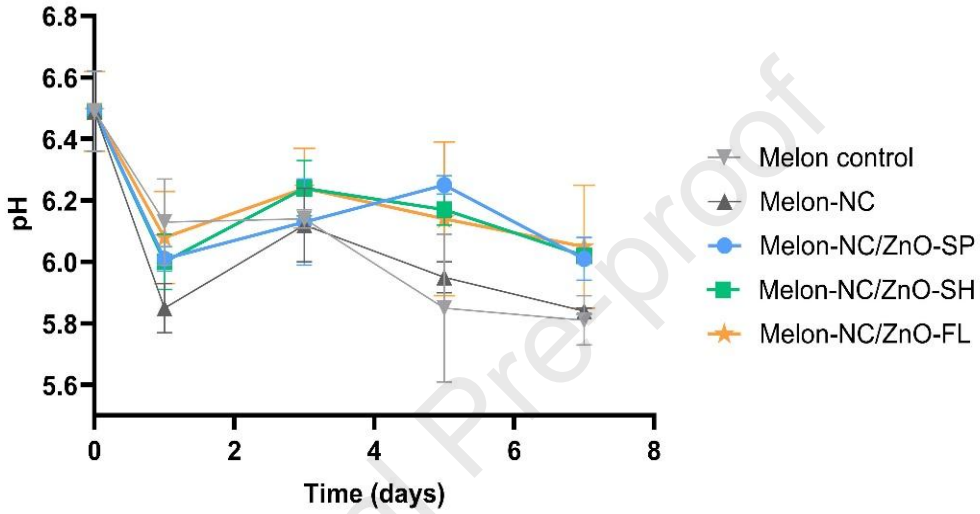

Figure 7 – Evolution of pH in fresh-cut melon samples stored at 4 °C for 7 days. Samples include melon control, NC film control, and NC films containing ZnO NPs (SP, SH, FL). Results are expressed as mean ± standard deviation (n = 3).

Highlights

- Nanocellulose films incorporating ZnO NPs were developed for active food packaging
- NC/ZnO films reduced microbial growth on refrigerated fresh-cut melon
- Sheet-shaped ZnO showed the highest antimicrobial efficacy in melon fruit
- Zn migration reached specific migration limit by day 2 remaining stable afterwards
- NC/ZnO films maintained higher melon pH consistent with microbial growth inhibition

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Porto 28th January 2026