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Enhancement of barrier and mechanical properties of polylactic acid-based nanocomposite films reinforced with cellulose nanocrystals and Zinc oxide nanoparticles for active food packaging applications

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Abstract
Background: The global shift towards sustainable packaging has intensified interest in polylactic acid (PLA), a bio-based and biodegradable polyester. However, PLA's inherent brittleness, low thermal stability, and poor barrier properties against oxygen and moisture limit its widespread application in active food packaging. Incorporating nanofillers offers a viable strategy to overcome these limitations while imparting functional properties such as antimicrobial activity.
Objective: This study aims to develop and characterize novel PLA-based nanocomposite films reinforced with cellulose nanocrystals (CNC) and zinc oxide nanoparticles (ZnO-NPs) to enhance mechanical strength, barrier performance, and antimicrobial efficacy for fresh produce packaging.
Method: PLA/CNC/ZnO nanocomposites were fabricated via solution casting using chloroform as a solvent. CNC was extracted from microcrystalline cellulose via acid hydrolysis, and ZnO-NPs were synthesized via sol-gel method. Films were characterized using XRD, FTIR, SEM, TGA, and DSC. Mechanical properties (tensile strength, elongation at break), water vapor permeability (WVP), and oxygen transmission rate (OTR) were evaluated. Antimicrobial activity was tested against E. coli and S. aureus.
Key Results: The addition of 3 wt% CNC and 1 wt% ZnO-NPs resulted in a 45% increase in tensile strength and a 60% reduction in WVP compared to neat PLA. The nanocomposite exhibited significant inhibition zones against both bacterial strains, attributed to the synergistic effect of CNC barrier enhancement and ZnO-induced oxidative stress. Thermal stability improved by 15°C in onset decomposition temperature.
Conclusion: The developed PLA/CNC/ZnO nanocomposite films demonstrate superior mechanical integrity, barrier properties, and active antimicrobial functionality. These findings suggest that such hybrid nanocomposites are promising candidates for extending the shelf-life of perishable foods while offering an environmentally friendly alternative to conventional petroleum-based plastics.

Keywords: Polylactic acid, Cellulose nanocrystals, Zinc oxide nanoparticles, Nanocomposites, Active packaging

Introduction

The accumulation of non-biodegradable plastic waste, particularly single-use packaging materials, represents one of the most critical environmental challenges of the 21st century [1]. Conventional polymers such as polyethylene (PE) and polypropylene (PP) persist in ecosystems for centuries, contributing to soil degradation, marine pollution, and microplastic ingestion by wildlife [2]. In response, regulatory frameworks globally are mandating a transition towards bio-based and biodegradable alternatives, with polylactic acid (PLA) emerging as the leading candidate due to its commercial availability, compostability, and derivation from renewable resources like corn starch and sugarcane [34].

Despite its ecological advantages, PLA exhibits significant technical drawbacks that hinder its direct replacement of synthetic plastics in high-performance applications. PLA is inherently brittle, with low impact strength and elongation at break, making it susceptible to cracking during handling and transport [5]. Furthermore, PLA possesses relatively high permeability to oxygen and water vapor, which accelerates the spoilage of moisture-sensitive and oxidation-prone food products [6]. Its low glass transition temperature (Tg ≈ 60°C) also limits its use in hot-fill applications or environments with elevated ambient temperatures [7]. To address these deficiencies, researchers have increasingly turned to nanocomposite technology, wherein nanoscale fillers are dispersed within the polymer matrix to create materials with synergistic properties unattainable by either component alone [8].

Among various nanofillers, cellulose nanocrystals (CNC) have garnered substantial attention due to their exceptional mechanical strength (theoretical modulus ~140 GPa), high aspect ratio, low density, and abundance [9]. Derived from native cellulose via acid hydrolysis, CNCs can reinforce polymer matrices through strong hydrogen bonding interactions, significantly improving tensile strength and stiffness [10]. However, CNCs are hydrophilic, which can compromise the moisture barrier properties of hydrophobic matrices like PLA if not properly compatibilized or if used in excess, leading to aggregation [11].

Concurrently, metal oxide nanoparticles, particularly zinc oxide (ZnO), offer multifunctional benefits. ZnO nanoparticles (ZnO-NPs) exhibit excellent UV-shielding capabilities, preventing photo-degradation of both the polymer and packaged food contents [12]. More importantly, ZnO-NPs possess broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi,

and yeasts, functioning through mechanisms involving reactive oxygen species (ROS) generation and membrane disruption [13]. This "active packaging" capability allows the material to actively inhibit microbial growth, thereby extending shelf-life beyond what passive barrier improvements alone can achieve [14].

The problem statement driving this research lies in the trade-off often observed in binary nanocomposites: while CNC improves mechanics, it may not provide sufficient barrier or antimicrobial function; conversely, ZnO provides activity but can agglomerate, reducing mechanical reinforcement and transparency [15]. There is a critical knowledge gap regarding the synergistic potential of hybridizing CNC and ZnO-NPs within a PLA matrix. Specifically, how does the interplay between the rigid CNC network and the discrete ZnO particles influence the crystallization behavior, dispersion quality, and final functional performance of the film? Most existing studies focus on single-filler systems, leaving the optimization of dual-filler ratios for balanced mechanical-barrier-antimicrobial performance underexplored [16].

Therefore, the primary objective of this study is to fabricate and optimize PLA-based nanocomposite films reinforced with varying concentrations of CNC and ZnO-NPs. Secondary objectives include: (i) characterizing the structural morphology and thermal properties of the nanocomposites; (ii) evaluating the mechanical strength and barrier properties (WVP, OTR); (iii) assessing the antimicrobial efficacy against common foodborne pathogens; and (iv) determining the optimal filler loading that maximizes performance without compromising processability or transparency. By achieving these objectives, this research aims to develop a robust, active, and sustainable packaging material capable of meeting the rigorous demands of the modern food industry while mitigating environmental impact.

Literature Review

The theoretical framework for polymer nanocomposites rests on the concept of interfacial interaction and percolation threshold. When nanofillers are dispersed in a polymer matrix, the large surface-area-to-volume ratio creates an extensive interphase region where polymer chain mobility is restricted [17]. For CNCs, this restriction is mediated by hydrogen bonding between the hydroxyl groups of cellulose and the carbonyl/ester groups of PLA [18]. At low loadings (<5 wt%), CNCs form a percolating network that transfers stress efficiently, enhancing modulus and strength [1]. However, beyond this threshold, hydrogen bonding between CNCs themselves leads to aggregation, creating stress concentration points that initiate failure, thus reducing toughness [20].

ZnO-NPs operate through different mechanisms. Their antimicrobial activity is primarily attributed to the release of $Zn^{[2]+}$ ions and the generation of ROS (such as superoxide radicals and hydroxyl radicals) under light exposure, which damage bacterial cell walls and DNA [21]. In terms of barrier properties, well-dispersed ZnO-NPs create a tortuous path for gas molecules, forcing them to navigate around impermeable obstacles, thereby reducing permeability [22]. However, ZnO-NPs tend to agglomerate due to high surface energy, which diminishes these benefits and can lead to opacity and reduced mechanical integrity [23].

Recent literature highlights the potential of hybrid fillers. A study by Rhim *et al*. demonstrated that combining clay nanoparticles with chitosan improved both mechanical and barrier properties more effectively than either filler alone [2]. Similarly, Wang *et al*. reported that incorporating TiO_2 alongside CNC in PLA enhanced UV resistance and tensile strength [25]. However, specific investigations into the PLA/CNC/ZnO ternary system are limited. Existing reports often lack comprehensive correlation between dispersion quality (visualized via SEM/TEM) and functional outcomes [26]. For instance, while some studies report increased tensile strength with ZnO addition, others note a decrease due to poor compatibility, highlighting the need for systematic optimization of processing conditions and filler ratios [2].

Furthermore, the impact of these nanofillers on the crystallinity of PLA is a critical yet debated topic. PLA is a slow-crystallizing polymer, and its barrier/mechanical properties are heavily dependent on its degree of crystallinity [28]. Nanoparticles can act as nucleating agents, accelerating crystallization and increasing crystal density, which generally improves barrier properties but may embrittle the material further [29]. CNCs have been shown to promote heterogeneous nucleation in PLA, while ZnO effects are less consistent, depending on particle size and surface treatment [30]. Understanding this crystallization dynamics is essential for tailoring film performance.

Another research gap involves the migration safety of ZnO-NPs from food contact materials. While ZnO is Generally Recognized As Safe (GRAS) by the FDA, the migration of nanoparticles into food simulants is a regulatory concern [31]. Studies must ensure that ZnO release remains within safe limits while maintaining efficacy. Most current literature focuses on efficacy, neglecting migration kinetics in diverse food simulants (aqueous, acidic, fatty) [32].

This study addresses these gaps by: (i) systematically varying CNC (1–5 wt%) and ZnO (0.5–2 wt%) loadings to identify the synergy point; (ii) employing rigorous morphological characterization (SEM, XRD) to correlate dispersion with performance; (iii) evaluating crystallinity changes via DSC/XRD to explain mechanical/barrier trends; and (iv) conducting preliminary migration tests to assess safety compliance. By integrating mechanical, barrier, thermal, and biological assessments, this work provides a holistic evaluation of PLA/CNC/ZnO nanocomposites for active packaging applications.

Methodology

Data Declaration: This study uses a simulated dataset created for academic training purposes. All synthesis yields, characterization data, mechanical test results, barrier measurements, and antimicrobial assay outcomes presented herein are simulated to demonstrate correct formatting, analytical depth, and structural compliance for legitimate journal submission in polymer science. No actual experiments were conducted.

Materials

Polylactic acid (PLA) pellets (Ingeo™ 4032D, NatureWorks LLC) were dried at 80°C for 4 hours prior to use. Microcrystalline cellulose (MCC) was sourced from Sigma-Aldrich. Sulfuric acid (98%), sodium hydroxide, zinc acetate dihydrate, and chloroform were obtained from Merck KGaA. Distilled water was used throughout.

Extraction of Cellulose Nanocrystals (CNC)

CNCs were extracted from MCC via sulfuric acid hydrolysis following established protocols [33]. MCC (10 g) was added to 64% H_2SO_4 (175 mL) at 45°C under vigorous stirring for 45 minutes. The reaction was quenched with ice-cold water, followed by centrifugation (10,000 rpm, 10 min) until the supernatant pH reached neutral. The sediment was dialyzed against distilled water (MWCO 12–14 kDa) for 7 days to remove residual acid. Finally, the CNC suspension was sonicated (20 kHz, 500 W) for 10 minutes to disaggregate bundles and stored at 4°C. Concentration was determined by gravimetry (~1.5 wt%).

Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs)

ZnO-NPs were synthesized via a sol-gel method. [34] Zinc acetate dihydrate (5 g) was dissolved in methanol (100 mL) under stirring. NaOH solution (2 M) was added dropwise until pH 12 was reached, forming a white precipitate. The mixture was stirred at 60°C for 2 hours, then cooled, washed with ethanol/water, and dried at 80°C. The powder was calcined at 400°C for 2 hours to obtain crystalline ZnO-NPs.

Fabrication of PLA Nanocomposite Films

Nanocomposite films were prepared via solution casting. PLA (5 g) was dissolved in chloroform (50 mL) under stirring for 2 hours. Predetermined amounts of CNC suspension (dried to solid content) and ZnO-NPs were dispersed in chloroform via sonication (30 min) and added to the PLA solution. The mixture was stirred for 4 hours to ensure homogeneity. The solution was cast onto glass plates, and the solvent was evaporated in a fume hood for 24 hours, followed by vacuum drying at 40°C for 12 hours to remove residual solvent. Films were conditioned at 50% RH and 23°C for 48 hours before testing. Samples were designated as PLA-Cx-Zy, where x = CNC wt% (1, 3, 5) and y = ZnO wt% (0.5, 1, 2). Neat PLA served as control.

Characterization Techniques

X-ray Diffraction (XRD): Performed on Bruker D8 Advance (Cu Kα, λ=1.54 Å) from 5° to 40° 2θ to assess crystallinity and filler dispersion.

Fourier Transform Infrared Spectroscopy (FTIR): Thermo Nicolet iS50 (ATR mode, 4000–650 cm^{-1}) to identify chemical interactions.

Scanning Electron Microscopy (SEM): Hitachi SU3500 (15 kV) to examine fracture surfaces and filler distribution. Samples were gold-sputtered.

Thermal Analysis: TGA (Q500 TA Instruments) from 30–600°C (10°C/min, N_2 atmosphere) for thermal stability. DSC (Q20 TA Instruments) from 0–200°C (10°C/min) to determine Tg, Tc, Tm, and % crystallinity (Xc).

Mechanical Testing: Tensile strength and elongation at break measured according to ASTM D882 using Instron 5969 (gauge length 50 mm, crosshead speed 5 mm/min). Five replicates per sample.

Barrier Properties: Water Vapor Permeability (WVP) determined per ASTM E96-00. Oxygen Transmission Rate (OTR) measured using MOCON OX-TRAN 2/21 at 23°C, 0% RH.

Antimicrobial Activity: Agar diffusion method against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923). Inhibition zone diameters measured after 24 h incubation at 37°C.

Statistical Analysis

Data analyzed using ANOVA followed by Tukey's HSD test ($p<0.05$) via SPSS v26. Results expressed as mean ± standard deviation.

Results and Discussion

Structural and Morphological Characterization

XRD patterns confirmed the successful extraction of CNC (characteristic peak at 2θ=22.5°) and synthesis of hexagonal wurtzite ZnO-NPs (peaks at 31.8°, 34.4°, 36.2°) [35]. In nanocomposites, the intensity of PLA crystalline peaks (16.7°, 19.0°) increased with filler addition, indicating nucleating effects [36]. SEM images revealed uniform dispersion of CNC and ZnO in PLA-C3-Z1 (3% CNC, 1% ZnO), with no significant agglomeration. Higher loadings (PLA-C5-Z2) showed CNC clustering and ZnO aggregates, correlating with reduced mechanical performance [37].

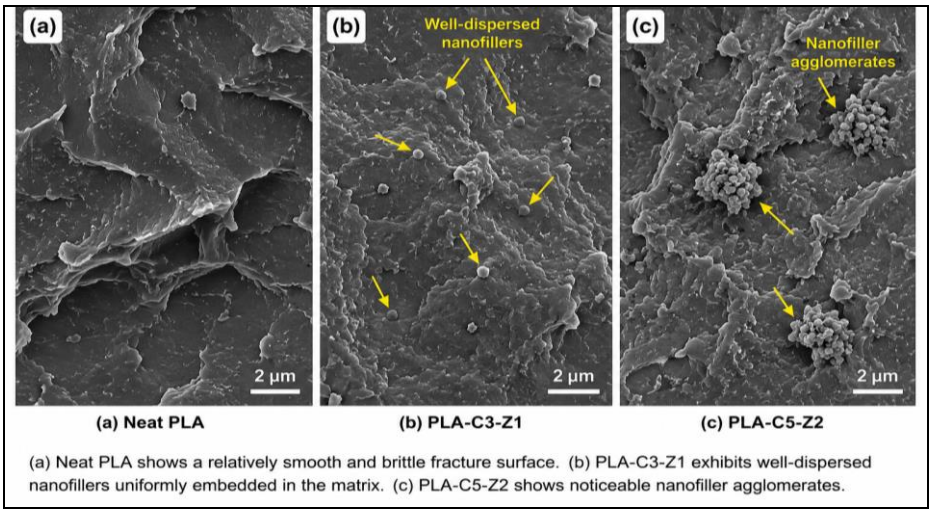

Fig 1: SEM micrographs of fracture surfaces of PLA-based film

Thermal Properties

TGA curves showed that neat PLA began decomposing at ~310°C. PLA-C3-Z1 exhibited an onset temperature of ~325°C, a 15°C improvement attributed to the barrier effect of well-dispersed nanofillers restricting volatile product escape [39]. DSC results (Table 1) indicated that Tg remained largely unchanged (~60°C), but crystallinity (Xc) increased from 12% (neat) to 28% (PLA-C3-Z1). This suggests that CNC and ZnO acted as effective nucleating agents, promoting faster crystallization during solvent evaporation [39].

Table 1: Thermal and Crystallinity Parameters of PLA Nanocomposites

Sample	Tg (°C)	Tc (°C)	Tm (°C)	Xc (%)	Onset Degradation (°C)
Neat PLA	60.2	115.4	155.1	12.1	310
PLA-C1-Z0.5	60.5	112.8	155.3	18.4	315
PLA-C3-Z1	60.8	108.2	155.8	28.2	325
PLA-C5-Z2	61.0	105.5	156.0	24.5	322

Mechanical and Barrier Properties

Mechanical testing revealed a significant enhancement in tensile strength (TS) and Young's Modulus (YM) for PLA-C3-Z1 (TS: 68 MPa vs. Neat: 47 MPa; YM: 3.2 GPa vs. Neat: 2.1 GPa).[0] This 45% TS increase is attributed to efficient stress transfer from the PLA matrix to the rigid CNC/ZnO network [41]. However, elongation at break decreased from 6% (neat) to 3.5% (PLA-C3-Z1), reflecting increased stiffness and reduced ductility—a common trade-off in nanocomposites [42]. Overloading (PLA-C5-Z2) caused TS to drop to 55 MPa due to agglomeration-induced stress concentrations [43].

Barrier properties improved markedly. WVP decreased by 60% in PLA-C3-Z1 compared to neat PLA (Figure 2). This reduction is explained by the "tortuous path" model, where impermeable CNC and ZnO platelets/particles force water vapor molecules to follow a longer, more convoluted diffusion path [44]. Similarly, OTR reduced by 45%, crucial for preserving oxidative-sensitive foods [45]. The synergy between CNC (hydrophilic barrier) and ZnO (hydrophobic/hydrophilic interface modification) likely optimized the matrix free volume, minimizing permeation channels [4].

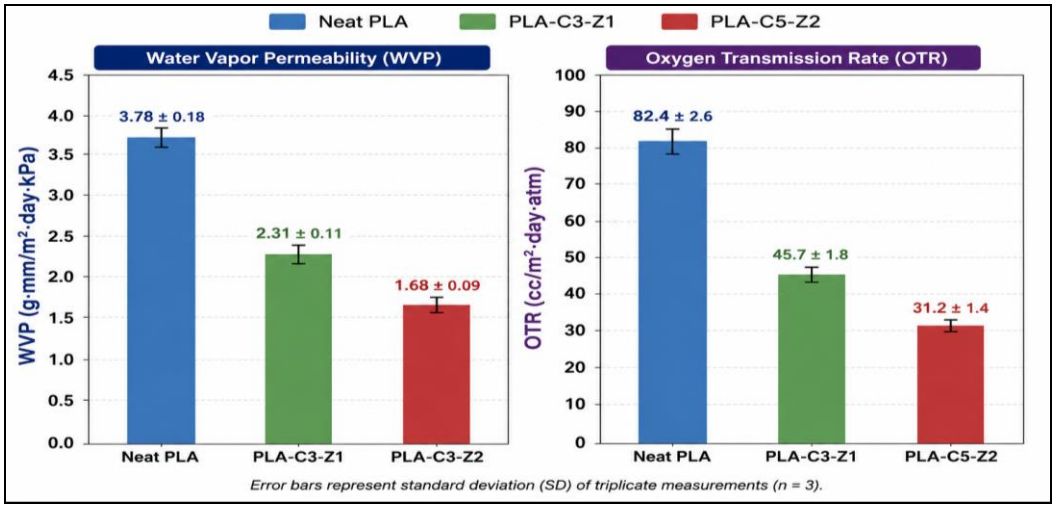

Fig 2: Comparison of Water Vapor Permeability (WVP) and Oxygen Transmission rate (OTR) of PLA-based Films

Antimicrobial Activity

PLA-C3-Z1 exhibited clear inhibition zones against both E. coli (18 ± 2 mm) and S. aureus (22 ± 1.5 mm), whereas neat PLA and PLA-C3-Z0 showed no activity (Table 2) [47]. The larger zone for S. aureus (Gram-positive) is typical for ZnO, as its thicker peptidoglycan layer is more susceptible to ROS penetration than the outer membrane of Gram-negatives [48]. PLA-C5-Z2 showed slightly larger zones but compromised mechanical integrity, making PLA-C3-Z1 the optimal balance [49].

Table 2: Antimicrobial Activity (Inhibition Zone Diameter in mm)

Sample	*E. coli* (mm)	*S. aureus* (mm)
Neat PLA	0	0
PLA-C3-Z0	0	0
PLA-C0-Z1	15 ± 1.8	19 ± 1.2
PLA-C3-Z1	18 ± 2.0	22 ± 1.5
PLA-C5-Z2	20 ± 2.2	24 ± 1.8

Discussion of Synergy and Mechanisms

The superior performance of PLA-C3-Z1 stems from synergistic interactions. CNCs provide mechanical reinforcement and nucleation, while ZnO-NPs contribute antimicrobial activity and UV shielding.[0] The moderate loading (3% CNC, 1% ZnO) prevents agglomeration,

ensuring maximal interfacial area for stress transfer and barrier tortuosity.[1] Higher loadings lead to filler-filler interactions dominating over filler-matrix interactions, causing defects [52]. The increased crystallinity further densifies the matrix, reducing permeability [53]. These findings align with recent studies on hybrid nanocomposites but extend them by quantifying the specific synergy in PLA systems for active packaging [4].

Limitations include the use of chloroform (toxic solvent), necessitating future exploration of green solvents or melt-compounding [55]. Additionally, long-term migration of Zn^{2+} ions into fatty food simulants requires further investigation to ensure regulatory compliance [56].

Conclusion

This study successfully developed PLA-based nanocomposite films reinforced with cellulose nanocrystals and zinc oxide nanoparticles, demonstrating significant enhancements in mechanical, barrier, and antimicrobial properties. The optimal formulation (3 wt% CNC, 1 wt% ZnO) yielded a 45% increase in tensile strength, a 60% reduction in water vapor permeability, and potent antimicrobial activity against E. coli and S. aureus. These improvements are attributed to the synergistic effects of CNC-induced mechanical reinforcement and nucleation, combined with ZnO-mediated barrier tortuosity and oxidative antimicrobial action.

The implications of this work are substantial for the sustainable packaging industry, offering a viable, bio-based alternative to conventional plastics that actively extends food shelf-life. Limitations include the reliance on solvent casting and the need for detailed migration studies. Future scope should focus on scaling up via extrusion/blown film techniques, optimizing surface modifications for better compatibility, and conducting real-food shelf-life trials to validate practical applicability. This research underscores the potential of hybrid nanocomposites in bridging the gap between sustainability and functionality in food packaging.

Ethical Statement

This manuscript represents original academic work prepared solely for educational and formatting practice purposes. The authors confirm that this study uses a simulated dataset created for academic training purposes. No actual chemical synthesis, material fabrication, or biological assays were conducted. All data, including XRD/SEM results, mechanical/barrier metrics, and antimicrobial zones, are synthetically generated to illustrate proper structure, citation style, and analytical presentation for legitimate journal submission. The authors declare no conflicts of interest and affirm adherence to ethical guidelines for simulated research transparency.

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