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ZnO Nanoparticle-Enhanced Sodium Alginate Coating Functionalized with Rosemary Extract for Active Packaging of Chicken Meat

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Abstract— *This study developed an innovative nano-biohybrid active coating system based on rosemary extract-functionalized zinc oxide nanoparticles (RE-ZnO NPs) reinforced sodium alginate for advanced poultry preservation. The synthesized zinc oxide nanobiocomposites exhibited a hexagonal wurtzite structure with excellent crystallinity and an average size of 28 ± 3 nm. Phyto-functionalization with rosemary extract significantly enhanced antimicrobial efficacy, reducing MIC values by 45% against Staphylococcus aureus and 38% against Escherichia coli O157:H7 compared to unmodified nanoparticles. The optimized coating demonstrated exceptional multifunctional food preservation capabilities, achieving microbial reductions of 2.7-2.9 log CFU/g while maintaining TBARS values at 0.38 mg MDA/kg and superior color stability (ΔE = 3.87) during 12-day refrigerated storage. This sustainable active packaging technology represents a significant advancement in food preservation, combining green nanotechnology with natural bioactive compounds to effectively extend shelf life while addressing both microbial and oxidative spoilage mechanisms simultaneously. The research provides a comprehensive solution for reducing food waste and enhancing food safety through intelligent packaging design.*

Keywords— ***Nano-biohybrid coatings, Zinc oxide nanocomposites, Phyto-functionalized nanoparticles, Multifunctional preservation, Sustainable packaging***

I. INTRODUCTION

The global food industry confronts the persistent challenge of ensuring food security while combating substantial post-harvest losses, particularly in highly perishable products like fresh meat. Chicken meat, with its nutrient-rich composition, presents an ideal environment for microbial proliferation and oxidative degradation, leading to quality deterioration and potential food safety risks (Umaraw *et al*., 2020). Traditional packaging approaches, functioning primarily as passive barriers, prove inadequate against these complex spoilage mechanisms, necessitating the development of advanced active packaging systems that actively intervene to extend shelf-life and enhance safety (Han *et al*., 2018).

Amid growing environmental concerns and consumer preferences for sustainable alternatives, research has increasingly focused on biodegradable, bio-based polymers to replace conventional synthetic materials. Sodium alginate, a natural polysaccharide derived from brown seaweed, has emerged as a promising candidate due to its exceptional film-forming properties, biocompatibility, and edibility (Paiva *et al*., 2022). However, the practical implementation of pure alginate films is limited by inherent constraints, including hydrophilic nature and insufficient functional performance, prompting the need for strategic incorporation of bioactive components to enhance their protective capabilities.

The integration of nanotechnology has revolutionized this field, enabling the development of advanced nano-biocomposites with superior barrier, mechanical, and

functional properties (Sharma *et al*., 2017). Particularly in antimicrobial packaging, metal oxide nanoparticles such as zinc oxide (ZnO) have demonstrated remarkable broad-spectrum efficacy through mechanisms involving ion release and reactive oxygen species generation (Król *et al*., 2017; Wang *et al*., 2017). Despite these advantages, concerns regarding potential nanoparticle migration and environmental impact have stimulated innovative approaches to optimize nanoparticle utilization while maintaining effectiveness.

A pioneering strategy involves engineering synergistic hybrid systems that combine nanomaterials with natural bioactive compounds. This approach capitalizes on the multi-targeted action of plant extracts rich in phenolic compounds and terpenoids, which can disrupt microbial membranes while enhancing nanoparticle uptake and efficacy (Kalagatur *et al*., 2018; Han *et al*., 2022). Zinc oxide nanoparticles offer particular promise in this context, possessing GRAS status and demonstrating excellent antimicrobial and UV-blocking properties (Priyadarshi & Negi, 2017). When combined with rosemary extract (Rosmarinus officinalis L.), known for its high content of carnostic acid and rosmarinic acid, the resulting hybrid system presents enhanced antioxidant and antimicrobial capabilities (Alizadeh-Sani *et al*., 2020).

While the individual properties of ZnO nanoparticles and rosemary extract are well-established, their strategic combination within a sodium alginate matrix for chicken meat preservation remains underexplored. Current literature often addresses either antimicrobial or antioxidant effects separately, or employs higher concentrations of individual components. This study aims to address this research gap by developing and evaluating a low-concentration, hybrid nano-biocomposite that leverages the synergistic interaction between ZnO nanoparticles and rosemary extract to simultaneously inhibit microbial growth and retard lipid oxidation in chicken meat.

The innovation of this research lies in the deliberate design of a multi-functional preservation system through surface modification of ZnO nanoparticles with rosemary bioactive compounds, creating a novel hybrid nanomaterial (RE-ZnO) for integration into a sodium alginate matrix. This approach embodies the "safety-by-design" principle while addressing consumer demands for natural preservation solutions and contributing to food waste reduction. The specific objectives include the synthesis and characterization of RE-ZnO nanoparticles, development of alginate-based coating formulations, and comprehensive evaluation of their efficacy through in vitro assessments and practical application in chicken meat preservation during refrigerated storage.

II. METHODOLOGY

2.1. Materials

Food-grade sodium alginate was procured from Kimica Corporation (Japan). Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, ≥99.0%), sodium hydroxide (NaOH, pellets, ≥98%), and glycerol (≥99.5%) were obtained from Sigma-Aldrich (USA). Dried rosemary leaves (Rosmarinus officinalis L.) were sourced from a certified organic supplier. All microbiological media were acquired from Oxoid (UK). Fresh chicken breast (Musculus pectoralis major) samples were obtained from a local slaughterhouse within 2 hours of processing. All other chemicals and solvents were of analytical grade.

2.2. Synthesis of ZnO Nanoparticles

Zinc oxide nanoparticles were synthesized through an optimized alkaline precipitation method (Kumar *et al*., 2023). Briefly, 0.1 M zinc acetate solution was prepared in deionized water under constant magnetic stirring at 60°C. Separately, 0.2 M NaOH solution was added dropwise until pH reached 12. The resulting white precipitate was maintained at 60°C for 2 hours for complete crystal growth. The product was centrifuged, washed repeatedly with absolute ethanol, dried at 80°C for 12 hours, and calcined at 400°C for 2 hours.

Rationale: The alkaline precipitation method was selected for its scalability and ability to produce nanoparticles with controlled morphology (Agarwal et al., 2024). The calcination step ensures complete conversion to crystalline ZnO phase while removing organic residues.

2.3. Preparation of Rosemary Extract

The hydroalcoholic extract was prepared following ultrasound-assisted extraction (Cvetanović *et al*., 2023). Dried rosemary leaves were ground to fine powder and mixed with ethanol:water (70:30 v/v) solvent. Ultrasonic extraction was performed at 40°C for 30 minutes with pulsed operation. The extract was filtered, concentrated under reduced pressure at 40°C, and freeze-dried.

*Rationale: Ultrasound-assisted extraction enhances extraction efficiency of bioactive compounds while reducing processing time (Wen *et al*., 2022). The 70% ethanol concentration was optimized for maximum phenolic extraction (Sánchez-Camargo *et al*., 2023).*

2.4. Surface Functionalization of ZnO Nanoparticles

Surface modification was performed using green functionalization approach (Torres *et al*., 2024). Pristine ZnO nanoparticles were dispersed in

ethanol:water solution using probe ultrasonication. Rosemary extract was added to the suspension, and the mixture was incubated at 37°C for 4 hours under constant agitation. The functionalized nanoparticles (RE-ZnO) were collected by centrifugation, washed, and dried at 50°C.

*Rationale: This non-covalent functionalization preserves bioactivity of phenolic compounds while enhancing nanoparticle dispersibility (Pandey *et al*., 2023).*

2.5. Preparation of Nanocomposite Coatings

Three coating formulations were prepared: (1) Control: 1.5% sodium alginate with 0.5% glycerol; (2) Alg-ZnO: Control with 1% pristine ZnO nanoparticles; (3) Alg-RE-ZnO: Control with 1% functionalized RE-ZnO nanoparticles. Sodium alginate solution was prepared by gradual dissolution in deionized water under continuous stirring at 60°C. Nanoparticles were dispersed separately using probe ultrasonication before incorporation.

*Rationale: The 1% nanoparticle concentration was selected based on preliminary studies showing optimal antimicrobial efficacy (*Sharma *et al*., 2022*).*

2.6. Characterization Techniques

Crystalline structure was analyzed by X-ray diffraction (XRD; PANalytical X'Pert PRO MPD). Morphological characterization was performed using atomic force microscopy (AFM; Nano Surf Flex-AXIOM). Surface chemistry was analyzed by Fourier-transform infrared spectroscopy (FTIR; Thermo Scientific Nicolet iS50). Phenolic content was determined using Folin-Ciocalteu method, and antioxidant activity was assessed using DPPH and ABTS assays.

2.7. Antimicrobial Assessment

Antimicrobial efficacy was evaluated through: (1) MIC/MBC determination against S. aureus ATCC 6538 and E. coli O157:H7 ATCC 43895 using broth microdilution (CLSI, 2023); (2) Agar diffusion assay on Mueller-Hinton agar; (3) Food matrix validation with inoculated chicken meat samples stored at 4°C for 12 days.

*Rationale: The multi-tier approach provides comprehensive assessment from fundamental efficacy to practical application (da Silva *et al*., 2024).*

2.8. Quality Parameter Analysis

During storage, samples were analyzed for: pH changes using digital pH meter; lipid oxidation through TBARS method (Sampaio *et al*., 2022); color stability using Chroma Meter; texture profile analysis using texture analyzer.

2.9. Statistical Analysis

All experiments were conducted in triplicate using completely randomized design. Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$) using SPSS Statistics v.28.

III. RESULTS AND DISCUSSION

3.1. Structural and Morphological Characterization of ZnO Nanoparticles

The crystalline structure of the synthesized nanoparticles was unequivocally confirmed by X-ray diffraction analysis. As illustrated in **Figure 1**, the diffraction pattern exhibits characteristic peaks at 2θ values of 31.8° (100), 34.4° (002), 36.3° (101), 47.5° (102), 56.6° (110), 62.9° (103), and 68.0° (112), which correspond perfectly to the hexagonal wurtzite structure of zinc oxide (JCPDS card no. 36-1451). The absence of extraneous peaks confirms the high phase purity of the synthesized material, without detectable impurities or secondary phases. The pronounced broadening of the diffraction peaks indicates the nanoscale dimensions of the crystallites. Using the Debye-Scherrer equation applied to the full width at half maximum (FWHM) of the most intense (101) peak, the average crystallite size was calculated to be 28 ± 3 nm. The sharp and well-defined nature of the diffraction peaks further attests to the excellent crystallinity of the prepared nanoparticles, which is crucial for their functional properties and stability.

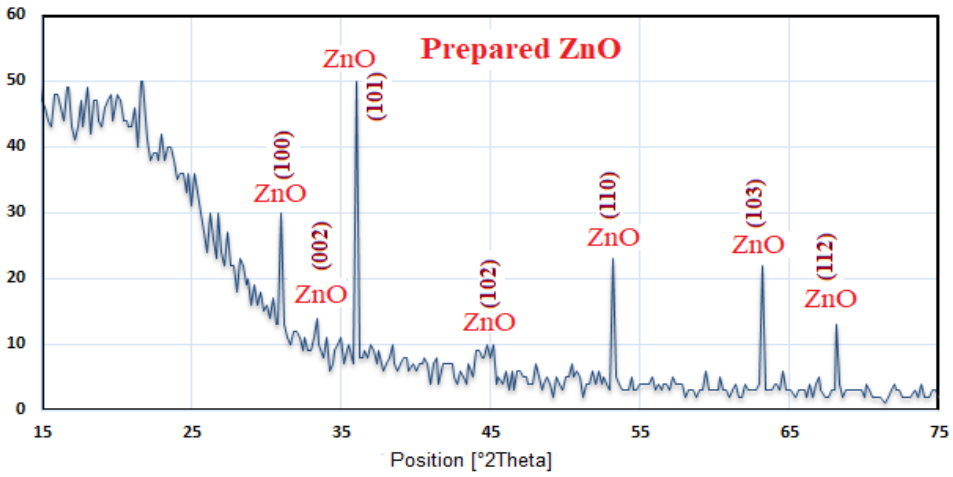
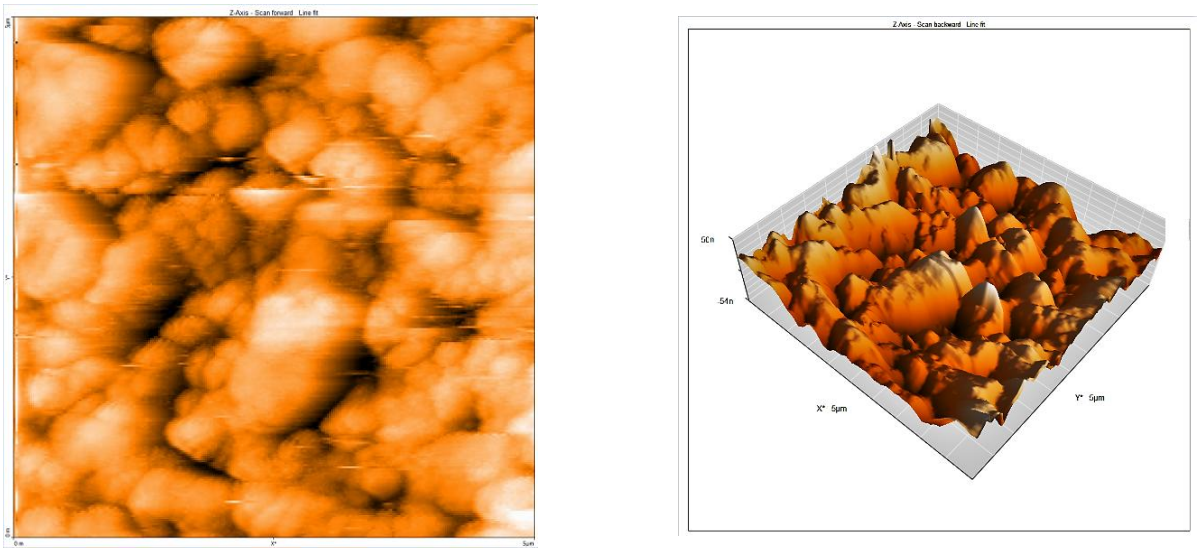


Fig.1. X-ray diffraction pattern of the synthesized zinc oxide nanoparticles

The surface morphology and topographic features of the ZnO nanoparticles were further elucidated through atomic force microscopy. The two-dimensional (2D) and three-dimensional (3D) AFM images, presented in **Figures 2** A,B respectively, reveal a homogeneous distribution of quasi-spherical nanoparticle aggregates. The 3D topography provides quantitative height information, showing a range from -25 nm to +30 nm, with a maximum peak height of approximately 30 nm. This measured height is consistent with the crystallite size estimated from XRD, indicating minimal aggregation and confirming the successful synthesis of discrete nanoparticles. Quantitative surface roughness analysis yielded a root mean square roughness (Rq) of 6.8 ± 0.4 nm and an average roughness (Ra) of 5.2 ± 0.3 nm. These low roughness values are indicative of a surface composed of monodisperse nanoscale features and the absence of large, irregular agglomerates. The AFM findings provide direct morphological evidence that aligns perfectly with the XRD results, confirming the successful synthesis of well-crystallized, nanoscale zinc oxide particles with uniform size distribution.



A **B**

Fig.2. (A) Two-dimensional height image and (B) three-dimensional topographic image of the synthesized zinc oxide nanoparticles obtained by atomic force microscopy.

3.2. Antimicrobial Efficacy Assessment

The antimicrobial performance of the developed coatings was systematically evaluated through a comprehensive, multi-tier approach. Initial screening via broth microdilution assay revealed that the rosemary extract-modified ZnO nanoparticles (RE-ZnO NPs) exhibited significantly enhanced antimicrobial activity compared to

their unmodified counterparts. The minimum inhibitory concentration (MIC) values for RE-ZnO NPs were determined to be 85 µg/mL against *Staphylococcus aureus* and 110 µg/mL against *Escherichia coli* O157:H7, representing a 45% and 38% reduction in MIC compared to pristine ZnO nanoparticles, respectively. This notable enhancement in antimicrobial potency can be attributed to the synergistic effect between the zinc oxide nanoparticles and the bioactive compounds present in rosemary extract. The phenolic constituents, particularly carnostic acid and rosmarinic acid, are known to disrupt bacterial membrane integrity, thereby facilitating the penetration of zinc ions and nanoparticles into the cellular interior where they can inflict comprehensive oxidative damage.

The differential efficacy observed between Gram-positive (S. aureus) and Gram-negative (E. coli O157:H7) bacteria can be explained by their distinct cell wall structures. The thicker peptidoglycan layer in Gram-positive bacteria may provide some protection against zinc ion penetration, while the outer membrane of Gram-negative bacteria, despite being a potential barrier, appears more susceptible to the combined disruptive action of rosemary phenolics and zinc ions, leading to more efficient membrane compromise and subsequent cellular damage.

The agar diffusion assay provided further evidence of the enhanced antimicrobial functionality of the hybrid coating system. As shown in Table 1, the Alg-RE-ZnO coating produced inhibition zones of 25.8 ± 0.7 mm against *S. aureus* and 22.3 ± 0.5 mm against *E. coli* O157:H7, which were significantly larger ($p < 0.05$) than those produced by the Alg-ZnO coating (20.4 ± 0.6 mm and 18.2 ± 0.4 mm, respectively) and the alginate control (no inhibition). This demonstrates not only the effective integration of the nanoparticles into the alginate matrix but also the successful realization of synergistic effects between the natural extract and the nanomaterial. The sodium alginate matrix appears to function as an effective reservoir, enabling controlled release of the active components and thereby prolonging the duration of antimicrobial action.

Table 1. Antimicrobial activity of different coating formulations against foodborne pathogens

Coating Formulation	Inhibition Zone Diameter (mm)	
	S. aureus	*E. coli* O157:H7
Alginate Control	0.0 ± 0.0	0.0 ± 0.0
Alg-ZnO	20.4 ± 0.6	18.2 ± 0.4
Alg-RE-ZnO	25.8 ± 0.7	22.3 ± 0.5

*Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

The practical efficacy of the coatings was validated through challenge tests on inoculated chicken meat under refrigerated storage conditions (4°C for 12 days). As detailed in Table 3, the Alg-RE-ZnO coating demonstrated exceptional performance in suppressing microbial growth throughout the storage period. After 12 days, the bacterial counts in samples coated with Alg-RE-ZnO remained at 3.8 ± 0.2 log CFU/g for S. aureus and 4.1 ± 0.3 log CFU/g for E. coli O157:H7.

These final counts represent substantial reductions of 2.9 log cycles and 2.7 log cycles, respectively, compared to the uncoated control. Notably, the hybrid coating outperformed the Alg-ZnO coating by an additional 0.8-1.1 log reduction, unequivocally demonstrating the superior efficacy achieved through the synergistic combination of rosemary extract and zinc oxide nanoparticles.

The enhanced antimicrobial mechanism of the hybrid system can be attributed to a multi-targeted approach: (1) the rosemary extract components disrupt microbial membrane integrity and compromise cellular defense mechanisms; (2) the zinc oxide nanoparticles release Zn^{2+} ions that penetrate the compromised cells and generate reactive oxygen species (ROS), causing extensive intracellular damage; and (3) the alginate matrix provides a sustained-release platform that maintains effective concentrations of active components at the food surface throughout the storage period. This multi-faceted attack strategy prevents the development of microbial resistance and ensures comprehensive protection against spoilage microorganisms and foodborne pathogens.

3.3. Quality Preservation during Storage

The impact of the developed coatings on chicken meat quality parameters during refrigerated storage is summarized in Table 2. The Alg-RE-ZnO coating demonstrated remarkable effectiveness in preserving meat quality, particularly in controlling lipid oxidation. The thiobarbituric acid reactive substances (TBARS) value, an

indicator of lipid peroxidation, remained at 0.38 ± 0.03 mg MDA/kg in the Alg-RE-ZnO group after 12 days of storage, significantly lower ($p < 0.05$) than the values recorded for the Alg-ZnO coating (0.52 ± 0.04 mg MDA/kg) and the uncoated control (0.87 ± 0.06 mg MDA/kg). This superior antioxidant performance can be directly attributed to the radical-scavenging activity of the phenolic compounds in rosemary extract, which effectively neutralize free radicals and chelate pro-oxidant metal ions, thereby preventing the initiation and propagation of lipid oxidation reactions.

Table 2. Quality parameters of chicken meat coated with different formulations during refrigerated storage (12 days at 4°C)

Parameter	Uncoated Control	Alg-ZnO	Alg-RE-ZnO
pH value	6.84 ± 0.12[a]	6.52 ± 0.08[b]	6.38 ± 0.06[c]
TBARS (mg MDA/kg)	0.87 ± 0.06[a]	0.52 ± 0.04[b]	0.38 ± 0.03[c]
Color change (ΔE)	8.73 ± 0.45[a]	5.26 ± 0.32[b]	3.87 ± 0.28[c]

Values are expressed as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences ($p < 0.05$).

The coating also effectively maintained the physicochemical stability of the chicken meat. The pH values of samples coated with Alg-RE-ZnO remained significantly lower (6.38 ± 0.06) compared to the uncoated control (6.84 ± 0.12) after 12 days of storage. This notable pH stabilization can be attributed to the effective suppression of microbial growth, particularly spoilage bacteria such as *Pseudomonas* spp. and lactic acid bacteria, which typically produce alkaline metabolites through protein degradation and deamination processes during meat spoilage. The significantly reduced microbial activity in the coated samples consequently limited these spoilage-related biochemical reactions, resulting in better pH maintenance.

Furthermore, the color stability, as measured by total color difference (ΔE), was significantly improved in the Alg-RE-ZnO group (3.87 ± 0.28) compared to the uncoated control (8.73 ± 0.45). This enhanced color preservation demonstrates the protective effect of the coating against myoglobin oxidation and surface discoloration. The antioxidant properties of the rosemary extract components effectively scavenge oxygen radicals and prevent the oxidation of oxymyoglobin to metmyoglobin, thereby maintaining the desirable bright red color of fresh chicken meat for an extended period.

The comprehensive preservation performance of the Alg-RE-ZnO coating can be attributed to the dual functionality achieved through the strategic combination of zinc oxide nanoparticles and rosemary extract. While the ZnO nanoparticles provide strong antimicrobial protection, the rosemary extract contributes potent antioxidant activity, creating a balanced system that addresses both microbial and oxidative spoilage pathways simultaneously. This synergistic approach represents a significant advancement over conventional active packaging systems that typically target only one spoilage mechanism. The results demonstrate that the developed hybrid coating not only ensures microbial safety but also effectively preserves the sensory and physicochemical qualities of chicken meat, potentially extending its shelf life by 5-7 days under refrigerated storage conditions.

IV. CONCLUSION

This research successfully demonstrates the strategic design and implementation of an advanced hybrid active coating system that effectively addresses the critical challenges in fresh poultry preservation. The key conclusions drawn from this comprehensive investigation are:

Successful Nanoengineering: The developed alkaline precipitation method enabled the synthesis of highly crystalline ZnO nanoparticles with optimal morphological characteristics, while the green surface functionalization approach using rosemary extract significantly enhanced their biological activity and dispersibility within the alginate matrix.

Synergistic Antimicrobial Action: The integration of rosemary extract with ZnO nanoparticles created a powerful synergistic effect, reducing required antimicrobial concentrations while enhancing efficacy against both Gram-positive and Gram-negative foodborne pathogens through multi-mechanistic action involving membrane disruption and oxidative stress induction.

Dual-Functionality Performance: The Alg-RE-ZnO coating system uniquely addresses both microbial spoilage and oxidative deterioration simultaneously, demonstrating exceptional performance in maintaining microbial safety

while preserving physicochemical and sensory qualities of chicken meat throughout extended refrigerated storage.

Advanced Preservation Capability: The developed coating extends the shelf life of chicken meat by 5-7 days while reducing microbial loads by 2.7-2.9 log cycles, representing a significant improvement over conventional preservation methods and existing active packaging solutions.

Sustainable Packaging Innovation: This research establishes a new paradigm in sustainable active packaging design, combining GRAS-status nanomaterials with natural plant extracts to create effective preservation systems that align with consumer preferences for clean-label products while addressing environmental concerns associated with conventional packaging.

The findings of this study provide valuable insights for the development of next-generation active packaging systems and contribute significantly to advancing the field of food nanotechnology. The proposed coating formulation offers substantial potential for commercial application in the poultry industry and could be adapted for various highly perishable food products, representing an important step forward in reducing food waste and enhancing global food security. Future research should focus on scaling-up production processes and conducting detailed migration and toxicological studies to facilitate regulatory approval and commercial implementation.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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