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Development of Chitosan-Based Antimicrobial Nanocomposite Films for Extending the Shelf Life of Perishable Food Products

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Abstract--- Fruits, meats, and fish go bad within days, whereas the plastics covering them can live hundreds of years past their expiration date. Could we use chitosan films with zinc oxide (ZnO) nanoparticles that would retard the growth rate of bacteria and also be biodegradable? Chitosan films were cast with 0, 0.5, 1.0, 2.0 and 3.0 wt% ZnO, who's mechanical, barrier and optical properties were measured, along with their action on three types of food pathogens, before being used to wrap fresh strawberries kept at 4 °C for 12 days. Loading of 1.0 wt% ZnO was optimal. Tensile strength increased by 24.5%, water vapour permeability decreased by 27% and UV transmittance at 280 nm reduced by 38% down to 11%, whereas still retaining transparency. The chitosan films reduced Escherichia coli, Staphylococcus aureus and Salmonella contamination by 4.1, 4.7 and 3.8 log units in 24 hours. The time to reach 20% degradation of strawberries covered by the film was about 10.5 days compared with 4.9 days of unpacked and 6.8 days of pure chitosan film-covered fruits. Nanoparticles formed aggregates at higher concentrations, limiting their content to 1.0 wt%. The process of making chitosan into an active packaging film requires only one step.

Keywords--- Chitosan, Zinc Oxide Nanoparticles, Active Packaging, Antimicrobial Film, Shelf Life, Perishable Food.

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I. Introduction

Each year, significant quantities of vegetables, meat and seafood are wasted on their journey from field to table, and microbial spoilage is the most common cause. Despite being the least expensive solution, plastic films are the most commonly used form of packaging and do not provide any dynamic response to the microorganisms they cover, in addition to being environmentally harmful once they have served their purpose. The idea behind active packaging is to provide a more effective response by adding an antimicrobial agent on the food surface, using biopolymers as carriers (Jiang et al., 2023; Silva et al., 2023).

Chitosan is one that stands out. This is a cationic polysaccharide extracted from chitin, easy to make into films, and it prevents Gram-positive and Gram-negative bacteria, yeasts, and filamentous fungi, as a result of its positive charges reacting with the negatively charged microbes (Jiang et al., 2023). This is not enough, however. Pure films are fragile when dry and swell in humid air, providing poor protection, thus many recent developments have concentrated on improving their properties through additives (Barik et al., 2024; Karam & Mallah, 2023). Edible coatings with essential oils or extracts based on nanochitosan can be promising for fresh produce (Karthik et al., 2022).

Another solution comes from the use of nanofillers. Large surface areas increase the barrier effects and increase contact with microorganisms, yet concerns exist with respect to agglomeration, migration to food, and toxicity (Zhou et al., 2024). A good example would be zinc oxide. With chitosan, it increases tensile strength and elongation and preserves cherry tomatoes for 15 days (Li et al., 2021). Another example is the use of titanium

dioxide, which has produced similar results with chitosan coatings (Xing et al., 2020). However, there is one issue with the dosage. In a chitosan-cyclodextrin film, tensile strength decreased drastically with more than 1 wt% TiO_2 due to agglomeration.

Thus, the real question here is not whether the nanofiller benefits the film in any way but what quantity will be required. Most of the previous research works answer this question in respect to only one feature, one organism or one type of food. We needed one set of results which would describe how one loading regime influences the structure of the film, its biological and physical properties and finally its efficiency in preserving the food. This paper presents this study and aims at (i) quantification of the influence of ZnO loading on mechanical, barrier and optical properties of chitosan films; (ii) measurement of antibacterial activity against three foodborne pathogens; and (iii) evaluation of the possibility of using the most efficient film in preservation of strawberries.

II. Conceptual Framework

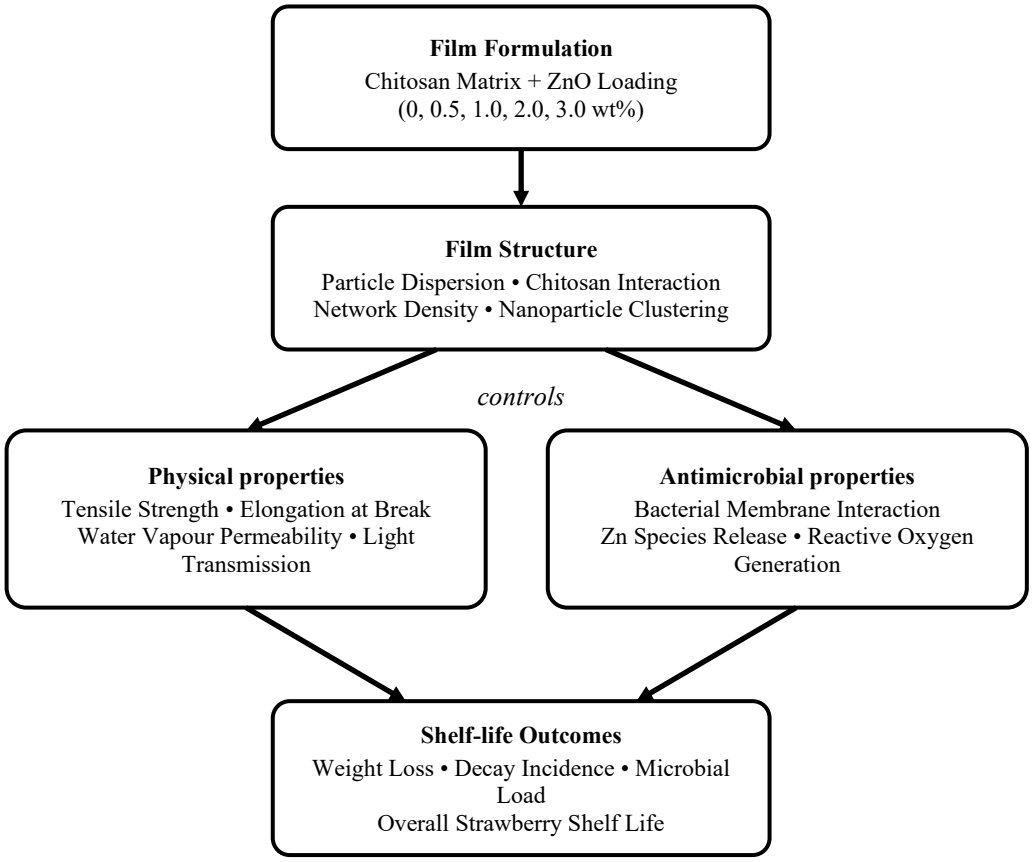


Figure 1: Conceptual Framework Linking Film Formulation, Structure, Physical and Antimicrobial Properties, and Shelf-Life Outcomes

Work organization was based on the cause-and-effect chain represented in figure 1. The film formulation – the combination of the chitosan matrix and certain ZnO loading – defines the film structure, i.e. particle distribution, interaction with chitosan chains, density of the network. Two categories of properties depend on structure. Physical properties – such as mechanical strength, flexibility, water vapour transmission and light transmission – determine the film capability of protecting food from moisture loss, mechanical impact and light exposure. Biological properties – such as bacterial membrane interaction, Zn species release, reactive oxygen generation – determine the capability of the film to inhibit microorganism proliferation (Jiang et al., 2023; Li et

al., 2021; Pandey et al., 2020). All that determines food-related parameters of weight loss, decay, microbial contamination – i.e. shelf life. Temperature and humidity in storage influence each link of the chain, while the main challenge for it is nanoparticle aggregation at higher ZnO loadings.

Two hypotheses emerge from this model. The first one is that the performance increases as the amount of ZnO increases before the dispersion is lost. The second one is that beyond this critical point, the strength decreases while the antimicrobial effect does not vanish. Section 4 will test both.

III. Materials and Methods

Film Preparation

Medium molecular weight chitosan (degree of deacetylation ~85%) was dissolved in 1% acetic acid at a concentration of 2% (w/v) with addition of glycerol as plasticizer in an amount of 30 wt.% of chitosan. The ZnO nanoparticles were sonicated in water with a probe for 20 minutes and then introduced in the chitosan solution in amounts of 0, 0.5, 1.0, 2.0 and 3.0 wt.% with respect to chitosan. Degassed solutions (40 g) were cast on level Petri dishes and dried for 24 h at 35 °C. The films were removed from Petri dishes and equilibrated in 25 °C and 50 % relative humidity for 48 hours (Barik et al., 2024; Li et al., 2021).

Physical Characterization

The thickness was calculated from the average of five readings from a micrometer. The tensile strength and elongation were determined using 10 × 50 mm films using a universal testing machine according to the ASTM D882 standard. The water vapour transmission rate was determined using the cup method at 25 °C. The light transmission values are reported for the range of 200 – 800 nm, specifically 280 and 600 nm.

Antibacterial Assay

The bacterial strains Escherichia coli, Staphylococcus aureus, and Salmonella were inoculated to 10^4 CFU/mL and incubated at 37°C with the film discs (1 cm^2) for 24 hours. The surviving bacteria were quantified using the spread plate technique, and log reduction was determined relative to the control without film disc.

Strawberry Storage Trial

The strawberries, which were firm and uniformly red in color, were washed, air dried and grouped into three lots consisting of 30 fruits each, which included the control lot, those wrapped using the clean chitosan film, and those wrapped using the 1.0 wt% ZnO film. The fruits were stored at 4 °C and 85% relative humidity for 12 days with measurements done after every 3 days. The weight loss was obtained from the initial weight of the fruits while the decay incidence was recorded based on the percentage of fruits showing moulds or softening. TVC was determined after 12 days. The shelf life was determined from 20% decay incidence using interpolation.

IV. Results and Discussion

Does ZnO make Chitosan Films Stronger and Tighter?

Certainly, to some extent. As shown in table 1, the addition of ZnO at 0.5 and 1.0 wt% enhanced tensile strength from 38.4 to 43.1 and 47.8 MPa, respectively, and elongation at break from 9.8% to 15.9% (see figure 2). Permeability to water vapour decreased from 3.42 to 2.51 × 10^{-10} g m^{-1} s^{-1} Pa^{-1}, i.e., by 27%. The presence of well-dispersed particles functions as physical cross-linking and increases the distance the molecules have to travel, as demonstrated in chitosan films containing nanocellulose (Stefanowska et al., 2024; Escamilla-García et al., 2022) and in chitosan films with ZnO content (Bahar et al., 2023). The increase in strength corresponds to previous data on nano-ZnO in chitosan (Li et al., 2021) and green-synthesised ZnO-chitosan films (Ardra Lekshmi & Smitha Chandran, 2025).

Table 1: Physical Properties Of Chitosan Films With Different ZnO Loadings

ZnO Loading (wt%)	Thickness (µm)	Tensile Strength (MPa)	Elongation at Break (%)	WVP ($\times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1})	Transmittance at 600 nm (%)
0 (neat chitosan)	52	38.4	9.8	3.42	88.2
0.5	54	43.1	12.6	2.96	86.5
1.0	55	47.8	15.9	2.51	84.0
2.0	58	44.2	13.1	2.78	79.5
3.0	61	39.6	10.7	3.05	73.8

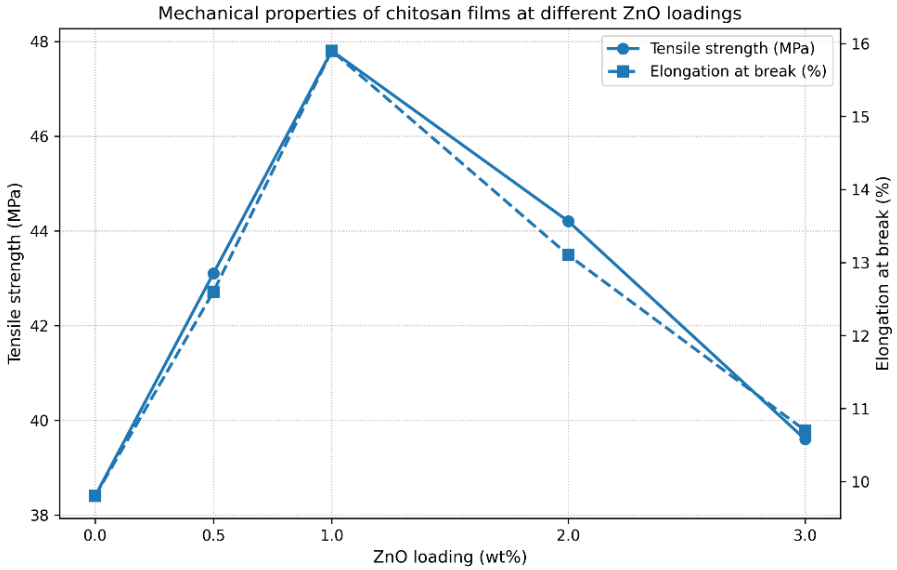


Figure 2: Tensile Strength and Elongation at Break of Chitosan Films as a Function of ZnO Loading

Above 1.0 wt% there was a reversal in the trend. The tensile strength fell to 44.2 MPa at 2.0 wt% and 39.6 MPa at 3.0 wt% slightly above that of the pure film, whereas the permeability started rising. This is known as the clustering effect reported for TiO2 in chitosan films (Pham et al., 2024) where the particles when in contact tend to create vulnerable spots rather than making cross-links. Moreover, the transparency decreased consistently from 88.2% at 600 nm to 73.8%, and at 280 nm, the UV transmittance dropped from 38% to 4%. This is advantageous for food that is light sensitive, although the consumer expects clarity in the pack, hence, 1.0 wt% film is the preferable choice with 84.0% transmittance.

How Strongly do the Films Act on Bacteria?

Extremely well, but with diminishing effects. Neat chitosan showed reductions of 1.0-1.8 log units of the three bacteria within 24 h; this corresponds to its activity against both Gram-positive and Gram-negative bacteria (Jiang et al., 2023). At a 1.0 wt% ZnO content, the reductions were 4.1 log units for E. coli, 4.7 for S. aureus, and 3.8 for Salmonella (Table 2, Figure 3). Increasing the loading to 3.0 wt% resulted in just one more log unit of reduction. Gram-positive S. aureus remained the most sensitive bacterium, a trend also observed in other systems (chitosan-TiO_2 and chitosan-ZnO films).

Table 2: Log Reduction (log CFU mL^{-1}) After 24 h Contact with the Films

ZnO loading (wt%)	E. coli	S. aureus	Salmonella
0 (neat chitosan)	1.2	1.8	1.0
0.5	2.8	3.4	2.5
1.0	4.1	4.7	3.8
2.0	4.9	5.5	4.6
3.0	5.2	5.8	4.9

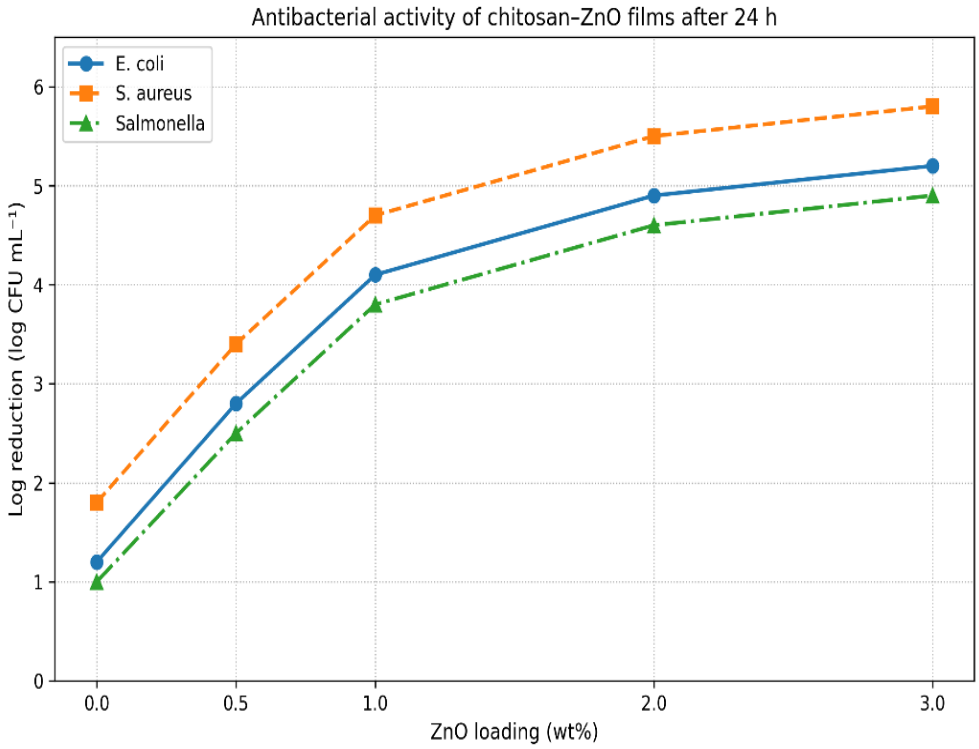


Figure 3: Log Reduction of E. coli, S. aureus and Salmonella by Chitosan Films with Different ZnO Loadings

Curve flattening is significant for the design process. Beyond 1.0 wt% the additional lethality comes at the expense of weakening and reduced clarity; therefore, the conclusions about antimicrobial action and strength are in agreement regarding an optimum value. This confirms our second hypothesis within the framework, as the reduction in strength occurred first, followed by leveling off of antimicrobial activity. It must be noted that the experiment was conducted in a liquid medium; the food test follows.

Does it Keep Strawberries Fresh Longer?

After 12 days at 4 °C, there is no doubt that the packaging treatment affected the quality of strawberries (Table 3, Figure 4). Unpacked fruits decreased by 15.2% their initial mass and demonstrated 81% decay, whereas packed fruits in neat chitosan by 10.5% and 55% decay. Packed fruits in 1.0 wt% ZnO film showed the least weight loss, i.e. 6.7%, and the lowest incidence of decay - 26%. The total count of viable microorganisms after 12 days was 7.9 log CFU g^{-1} in the control, 6.8 in neat chitosan, and 5.4 in ZnO film. Extrapolated to 20% decay, the shelf life of unpacked fruits was 4.9 days, for neat chitosan fruits – 6.8 days, and for packed in ZnO film fruits – 10.5 days, twice as much as in unpacked case. Probably, two factors contribute simultaneously: reduced moisture loss due to low water vapour permeability and decreased spoilage bacteria growth due to antibacterial properties of the surface, which corresponds to the results obtained with chitosan-based packaging for other perishable products.

Table 3: Weight Loss, Decay Incidence, Total Viable Count and Shelf Life of Strawberries After 12 Days At 4 °C

	Weight loss	Decay	Total viable count (log CFU g^{-1})	Shelf life
Unwrapped	15.2%	81%	7.9	4.9 days
Neat chitosan	10.5%	55%	6.8	6.8 days
1.0 wt% ZnO film	6.7%	26%	5.4	10.5 days

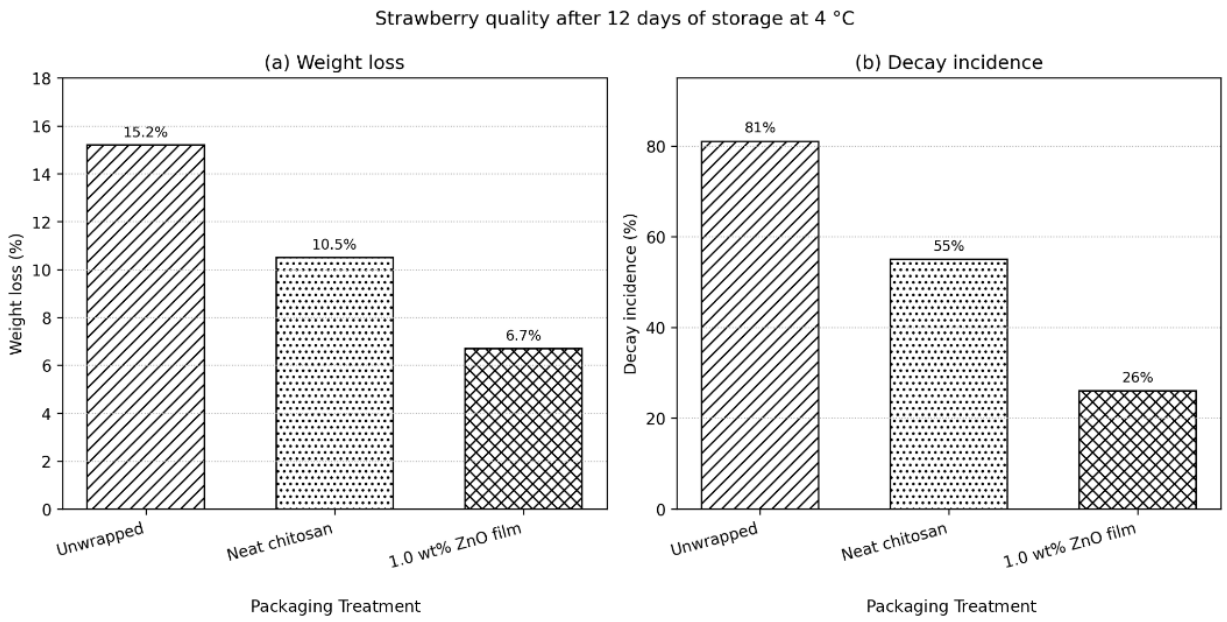


Figure 4: Weight Loss (a) and Decay Incidence (b) of Strawberries Stored at 4 °C for 12 Days

How does this Compare with other Work?

Table 4 establishes the optimal film with respect to relevant recent research on perishable food products. Both tensile strength and shelf-life enhancement capabilities are within the same ballpark as those of ZnO-chitosan and TiO_2-chitosan films on cherry tomatoes and avocado, and as electro spun chitosan films containing silver for meat preservation. Since the conditions (types of test foods, temperatures, definitions of shelf life) vary between these studies, the comparison is illustrative rather than exact. The superiority of our material lies in its relative simplicity.

Table 4: Comparison of the Optimum Film with Recent Chitosan-Based Nanocomposite Films

Film	Food tested	Reported Outcome	Ref.
Chitosan–nano-ZnO (0.6%)	Cherry Tomatoes	Tensile Strength 46.79 MPa; 15-Day Preservation	(Li et al., 2021)
Chitosan–Cyclodextrin/TiO_2	Avocados	Over 99.5% Bacterial Kill in 15 min; 7 Days at 30 °C	(Pham et al., 2024)
Chitosan/AgNP–PVA electrospun layer	Meat	Shelf Life Extended by About One Week	(Pandey et al., 2020)
Green ZnO–chitosan	Fruits	Tensile strength 47.5 MPa; life extended by up to 7 days	(Daeialiakbar et al., 2025)
Chitosan–1.0 wt% ZnO (this work)	Strawberries	Tensile Strength 47.8 MPa; shelf life 10.5 vs 4.9 days	This Work

Limits of the Study

Three constraints need to be discussed. We have studied a single food sample at a single temperature; therefore, we have not studied other conditions of misuse, for instance, 20 °C storage. Further, we have not measured the amount of migration of Zn to the food samples or simulants, which is necessary as per regulations, and migration and toxicity of nanofillers are already recognized issues.

V. Conclusion

Chitosan films incorporating 1.0 wt% ZnO showed superior mechanical properties (47.8 MPa), low water vapour permeability, UV protection and 3.8 to 4.7 log reductions in three foodborne microorganisms, and increased the shelf life of refrigerated strawberries by about twice. With higher concentrations, there was evidence of clustering, poor mechanical properties, decreased transparency and no further antimicrobial effect,

meaning that approximately 1 wt% is the optimum loading. This demonstrates the effectiveness of the casting process for producing active and biodegradable packaging. Future work involves migration studies, testing on meat, fish and dairy products, as well as composting studies.

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