Development of Biodegradable Packaging from Tilapia Fish Scales Incorporated with Zinc Oxide Nanoparticles for Inhibiting Fungal Growth In Shallots

Boonyapon Sawangthaisong[1] and Chayanuch Yodpikul[1]
Watcharaporn Saenna[1] Rapeephon Takhianram[1]
[1]Princess Chulabhorn Science High School Buriram, Satuek, Satuek, Buriram, 31150, Thailand
[*]Email: 467boonyaponsawangthaisong@pcshsbr.ac.th

Abstract

This study developed an active biodegradable packaging film from tilapia (*Oreochromis niloticus*) scale gelatin incorporated with zinc oxide nanoparticles (ZnO-NPs) to inhibit post-harvest fungal growth and extend the shelf life of shallots (*Allium ascalonicum*). The physical, mechanical, and antifungal properties of the composite films were systematically evaluated. Gelatin extracted from tilapia scales waste using hydrochloric acid (HCl) was blended with carboxymethyl cellulose (CMC), glycerol, and varying concentrations of ZnO-NPs, followed by casting and drying at 60°C.

The optimized composite film exhibited an average thickness of 0.561 ± 0.031 mm and a low moisture content of 3.46 ± 0.38%. Mechanical characterization revealed a tensile strength of 3.07 MPa, an elastic modulus of 228 MPa, and a maximum stress of 0.93 MPa, indicating adequate structural integrity for packaging applications. Antifungal assays against *Aspergillus niger* and *Penicillium* sp. demonstrated that the incorporation of ZnO-NPs at 10,000 mg/L achieved the highest inhibitory efficacy, producing a distinct zone of inhibition. These findings suggest that tilapia scale-derived gelatin films reinforced with ZnO-NPs serve as a promising, eco-friendly alternative to synthetic plastic packaging. This approach successfully upcycles aquaculture waste, aligns with the Bio-Circular-Green (BCG) economy model, and mitigates post-harvest agricultural losses.

Keywords: Tilapia scales, Biodegradable packaging, Zinc oxide nanoparticles, Shallots, Antifungal activity

Introduction

The management of organic waste and industrial by-products has become an important challenge in achieving sustainable development, particularly in aquaculture communities. In Satuek District, Buriram Province, large quantities of tilapia scales are generated as waste from local fishery activities. These residues are commonly discarded without proper utilization, causing environmental problems such as unpleasant odors, pathogen accumulation, and contamination of natural water sources. To address these issues, this study applies the concept of the Circular

Economy by transforming fishery waste into value-added biodegradable packaging materials. This approach not only helps reduce environmental pollution and community waste, but also increases the economic value of local by-products, generates additional income for local fisheries, and supports sustainable community development.

This research focuses on the development of biomaterials derived from tilapia scales through the extraction of gelatin and collagen for use in biodegradable film packaging. The developed films are intended for agricultural applications, particularly for preserving shallots. Currently, shallot farmers experience significant post-harvest losses caused by fungal contamination, especially from *A.niger* and *Penicillium* sp. These fungi are major causes of black mold disease and product spoilage, leading to rapid quality deterioration, economic losses, and potential health risks due to mycotoxin contamination in the food chain.

To improve the performance of biodegradable films, zinc oxide nanoparticles (ZnO nanoparticles) were incorporated into the film matrix. ZnO nanoparticles exhibit strong antimicrobial activity by inhibiting and destroying fungal cells responsible for spoilage. In addition, the nanoparticles enhance the mechanical properties of the films, including tensile strength, durability, and moisture barrier performance.

The developed Nano-ZnO biodegradable film is expected to overcome the limitations of conventional synthetic plastics such as LDPE, which are non-biodegradable and lack antimicrobial properties.

Therefore, this innovation presents a sustainable alternative packaging material capable of extending the shelf life of agricultural products, reducing economic losses, minimizing environmental impacts, and promoting food safety for both producers and consumers.

Methodology

1) Raw Material Sourcing

In the case of this research, the researchers collected raw materials consisting of tilapia scales from local fishermen and fishing communities in Satuek District, Buriram Province.

This approach aimed to utilize aquaculture industrial by-products and organic waste, thereby mitigating environmental pollution, supporting the Bio-Circular-Green (BCG) economy model, and adding economic value to local fishery residues.

2) Extraction and Formulation of Biodegradable Films

1. Extract gelatin from the collected local tilapia scales using hydrochloric acid (HCl).

2. Blend the extracted tilapia scale gelatin with carboxymethyl cellulose (CMC), glycerol, and zinc oxide nanoparticles (Nano-ZnO).

3. Vary the composition ratios of carboxymethyl cellulose (CMC) and glycerol to enhance film-forming efficiency, optimize the formulation of biodegradable film sheets, and improve antifungal performance.

4. Cast the formulated biopolymer mixtures into film sheets and dry them at 60°C. A total of five film samples (Films 1–5) were prepared for the experiment alongside a conventional biodegradable film formulation, which served as the control group without additives.

3) Evaluation of Physical and Mechanical Properties

1. Measure the thickness of the five film samples (Films1-5) using micrometer measurements to evaluate biopolymer uniformity and surface distribution during film formation, which directly affects gas permeability resistance.

2. Evaluate the mechanical properties of the prepared tilapia scale gelatin films using a Universal Testing Machine (UTM).

3. Measure and record key mechanical parameters, including tensile strength, elastic modulus, and maximum stress.

4) Antifungal Efficiency Evaluation

This section analyzed the factors affecting the antifungal efficiency of the packaging by determining the Minimum Inhibitory Concentration (MIC) of Nano-ZnO solutions using the PDA culture medium technique. The experimental process commenced with the isolation of fungal spores, specifically *Aspergillus niger* and *Penicillium*, from shallots to serve as the target pathogens responsible for black mold disease and product spoilage. Following isolation, PDA (Potato Dextrose Agar) culture media were prepared in petri dishes containing Nano-ZnO solutions at three distinct concentrations 2,500 mg/L, 5,000 mg/L, and 10,000 mg/L alongside an untreated control group. The isolated fungal spores from the shallots were then inoculated onto each prepared culture medium. Subsequently, the culture plates were incubated at 27°C for 24, 48, and 72 hours in accordance with AOAC testing standards. Finally, the antifungal activity was evaluated by observing fungal growth and measuring the diameter of the inhibition zone (clear zone) to investigate the efficacy of the nanoparticles in destroying fungal cell walls and disrupting cellular respiration.

5) Moisture Analysis and Packaging Standard Evaluation

This phase focused on improving and verifying the efficiency of the biodegradable packaging films in reducing moisture accumulation to minimize microbial growth within the film.

1. Cut the developed biodegradable film sheets into 1x1 cm samples.

2. Analyze the moisture content of the film samples (Sample 1, 2, and 3) using a Moisture Analyzer.

3. Evaluate the results to ensure that the average moisture content is controlled to remain below the standard packaging threshold of 10%, thereby preserving the post-harvest storage quality of shallots.

6) Statistical Analysis

1. Compute the physical properties (film thickness) and the moisture content of the developed biodegradable packaging film using the arithmetic mean and the standard deviation ($\bar{x}$ ± SD). Compare the mechanical properties (tensile strength, elastic modulus, and maximum stress) of the improved tilapia scale gelatin film formulations against the conventional formulation (control group) using the arithmetic mean to evaluate polymer distribution and mechanical performance.

Result and Discussion

Part 1: focused on comparing the biodegradability and polymer distribution efficiency of biodegradable polymers extracted from tilapia scales with conventional

film formulations, including thickness analysis of all five film samples using micrometer measurements.

1.1 Comparison of Tensile Strength and Flexibility of Different Tilapia Scale Gelatin Film Formulations. The results demonstrate the tensile strength properties of gelatin films analyzed using a Universal Testing Machine.

Table 1 Mechanical Properties by Universal Testing Machine (UTM)

Mechanical Property	Average (MPa)
Tensile Strength	3.07
Elastic Modulus	2.28
Maximum Stress	0.93

The mechanical properties of the film showed an average tensile strength of 3.07 MPa, an elastic modulus of 228 MPa, and a maximum stress of 0.93 MPa. The results showed that the conventional biodegradable film formulation (control group without additives) exhibited the lowest tensile strength and flexibility.

1.2 Comparison of Film Thickness and Polymer Distribution Using Micrometer Analysis

Table 2 Film Thickness Measurement by Micrometer

Film Sample	Film Thickness (mm) ± SD
Film 1	0.593 ± 0.031
Film 2	0.587 ± 0.031
Film 3	0.523 ± 0.031
Film 4	0.566 ± 0.031
Film 5	0.535 ± 0.031
Average	**0.561 ± 0.031**

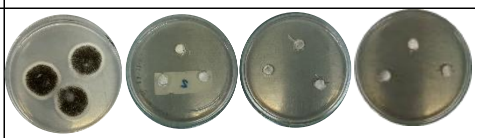
Table 2 shows that the film had an average thickness of 0.561 ± 0.031 mm.

The results indicated that the improved formulation containing carboxymethyl cellulose (CMC) exhibited greater thickness and more uniform surface distribution than the conventional formulation. These findings were subsequently used for further investigation.

Part 2: Analysis of Factors Affecting the Antifungal Efficiency of the Packaging

2.2 Effects of Nano-ZnO Concentration on Fungal Inhibition in Culture Media 2.2 Effects of Nano-ZnO Concentration on Fungal Inhibition in Culture Media

Table 3 Minimum Inhibitory Concentration (MIC) Test Results of Nano-ZnO Solution Against Fungal Growth

Time (Hours)	Control Group	Nano-ZnO Concentration (mg/L)		
		10,000	5,000	2,500
24				
	Positive*	Negative	Negative	Negative
48				
	Positive*	Negative	Positive*	Positive*
72				
	Positive*	Negative	Positive*	Positive*

Note: Positive and negative denote the presence and absence of fungal growth, respectively.

The study investigated the effects of different concentrations of zinc oxide nanoparticle solutions on fungal growth in shallots by determining the Minimum Inhibitory Concentration (MIC) of Nano-ZnO solutions against *Aspergillus niger* and *Penicillium* sp.

Part 3: Moisture Analysis and Packaging Standard Evaluation

The phase focused on improving the efficiency of biodegradable packaging films in reducing moisture accumulation by incorporating carboxymethyl cellulose (CMC) and glycerol into tilapia scale gelatin.

Table 4 Moisture Content Analysis Using a Moisture Analyzer

Film Sample	Moisture Content (%) ± SD
Sample 1	3.03 ± 0.38
Sample 2	3.39 ± 0.38
Sample 3	3.95 ± 0.38
Average	**3.46 ± 0.38**

The moisture content test showed that Sample 1, 2, and 3 had values of 3.03 ± 0.38%, 3.39 ±0.38%, and 3.95 ± 0.38% respectively, resulting in a mean of 3.46 ± 0.38%. This average value falls within the general packaging standard (less than 10%), thereby reducing the likelihood of microbial growth within the film and improving the storage quality of shallots.

Conclusions

The development of a biodegradable packaging film from tilapia scale gelatin incorporated with zinc oxide nanoparticles (Nano-ZnO) aimed to inhibit fungal growth and extend the shelf life of shallots. The study investigated the physical properties, mechanical properties, and antifungal activity of the film formulations.

The evaluation of mechanical properties using a Universal Testing Machine (UTM) revealed that the developed film demonstrated an average tensile strength of 3.07 MPa, an average elastic modulus of 228 MPa, and an average maximum stress of 0.93 MPa. In comparison, the conventional biodegradable film formulation without additives (control group) exhibited the lowest tensile strength and flexibility. Thickness analysis using micrometer measurements showed that the film had an average thickness of 0.561 ± 0.031 mm. The improved formulation containing carboxymethyl cellulose (CMC) exhibited a greater thickness and a more uniform surface distribution than the conventional formulation.

This enhancement is primarily attributed to the interfacial hydrogen bonding network established among gelatin, CMC, and glycerol. Glycerol acts as a plasticizer, inserting itself between the polymer chains of gelatin and CMC to form strong intermolecular hydrogen bonds. This interaction restricts polymer chain mobility while forming a dense, cohesive cross-linked matrix, resulting in a mechanically stronger film and a highly tortuous path for water molecules, which significantly improves moisture resistance.

For antifungal activity, the analysis of factors affecting packaging efficiency via the Minimum Inhibitory Concentration (MIC) test showed that zinc oxide nanoparticles at a concentration of 10,000 mg/L demonstrated the best performance against *Aspergillus niger* and *Penicillium* sp. isolated from shallots. The potent antifungal mechanism of ZnO-NPs is driven by two synergistic processes: the continuous release of zinc ions (Zn^{2+}) and the generation of Reactive Oxygen Species (ROS) such as hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2) under ambient conditions. The released Zn^{2+} ions interact with the negatively charged fungal cell walls, causing structural damage, while the accumulated ROS induce severe oxidative stress. This potent antifungal activity, characterized by a distinct clear zone, effectively destroys fungal cell walls, damages cellular membranes, and disrupts cellular respiration, thereby minimizing black spots and preventing shallot spoilage.

Moisture analysis using a Moisture Analyzer indicated that the film samples had an average moisture content of 3.46 ± 0.38%. This average value successfully falls within the general packaging standard threshold of less than 10%, thereby reducing the likelihood of microbial growth within the film matrix and enhancing the storage quality of shallots.

In conclusion, the biodegradable film developed from tilapia scale gelatin incorporated with zinc oxide nanoparticles shows strong potential for use as an antimicrobial packaging material to extend the shelf life of shallots, reduce post-harvest fungal contamination, and minimize synthetic plastic waste. This sustainable innovation successfully upcycles community fishery waste into high-value biomaterials, supporting the Bio-Circular-Green (BCG) economy model.

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