Development of advanced biopolymer thin films of Carboxymethyl cellulose, Silver, and Zinc Oxide nanoparticles for avocado fruit preservation applications

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ABSTRACT

Nanomaterials in post-harvest preservation offer advantages such as non-toxicity, chemical residue-free protection, and effective inhibition of mold and bacteria. Due to their strong antimicrobial properties and their ability to form a thin nano-coating on fruit surfaces, even small amounts of nanoparticles provide extensive coverage, preventing microbial penetration. This technology significantly improves fruit quality and longevity during storage and transportation. In this work, we introduced an advanced biopolymer film of carboxymethyl cellulose (CMC) incorporating ZnO and Ag nanoparticles (NPs). Ag NPs and ZnO NPs demonstrated an outstanding antimicrobial inhibition property that can be used for fruit preservation. As a proof of concept, the biopolymer thin films enable to extend avocado ripening from up to 35 days. The sugar concentration variation, the hardness, and the weight loss of the avocado with and without the advanced biopolymer thin film integrated ZnO and Ag nanoparticles in 35 days were compared with each other to clarify the ripening reduction ability of the biopolymer thin film.

Keywords: Carboxymethyl cellulose CMC; Ag nanoparticles; ZnO nanoparticles; Antibacterial; Fruit preservation.

1. INTRODUCTION

Effective post-harvest fruit preservation techniques in Vietnam need to be simple, affordable, safe for consumption, and capable of extending the shelf life of fruits. Using biofilm coatings in conjunction with nanoparticles to prevent ripening and increase shelf life is one appropriate strategy [1].

In practice, the integration of nanotechnology in fruit preservation and the monitoring of fruit ripeness have been tested by many research groups, yielding remarkable results. This is evidenced by the diversity of nanomaterials used. Depending on the intended use and type of produce, various films have been developed.

When integrated into biopolymers such as alginate, chitosan, cellulose, or pectin to create advanced films, nanomaterials like ZnO, TiO_2, Ag, and chitosan nanoparticles [2] exhibit impressive antibacterial properties against both Gram-positive and Gram-negative bacteria such as Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and Bacillus subtilis. ZnO nanoparticles may be able to produce coatings that are resistant to UV light and minimize water loss on the surfaces of strawberries, guavas, mangoes, pomegranates, or papaya slices, according to Anugrah et al.'s findings [1]. The findings demonstrated that guavas could be kept at about 20°C and 80% humidity for up to 20 days. During storage, the morphology and weight of the fruit remained largely unchanged, indicating that ZnO nanomaterial films could reduce water loss and thus slow down the ripening process. The hardness and color of the fruits were also preserved. Arroyo et al. demonstrated that surface damage on guavas was not observed when coated with the film [3]. Saba's research group also indicated that the presence of ZnO nanomaterials could slow the ripening process of pomegranates and protect the fruit from weight loss, as well as minimize the loss of vitamins and minerals such as Vitamin C, anthocyanins, and phenols [4]. Additionally, ZnO nanomaterials may boost the tensile strength and durability of the film by up to 190% [5, 6].

Apart from ZnO nanomaterials, nano-Ag is well-known for its antibacterial properties. It can inhibit the growth of numerous molds, bacteria, and even viruses such as S. aureus, E. coli, Klebsiella pneumonia, Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella, and some parasites, yeasts, and algae. Ag nanomaterials can suppress and kill bacteria by destroying the nutrition transport enzymes in bacterial cells, weakening cell walls and membranes, and interfering with metabolic processes [5]. The antibacterial capability of Ag nanomaterial is 20-50 thousand times higher than Ag ions and they can inhibit 650 species of microorganisms, including bacteria, fungi, and even viruses. Hence, nano-Ag is also interesting for fruit and food preservation [7].

Given the advantages of nanomaterials and biopolymers, the primary objective of this study is to develop biopolymer coatings containing nanomaterials based on two main materials, ZnO and Ag nanoparticles, combined with CMC biopolymer to extend the shelf life of avocados. Based on this primary goal, this research focuses on the study, fabrication, and evaluation of the antibacterial and antifungal capabilities of ZnO and Ag nanomaterials, proposing suitable materials for developing avocado preservation films.

2. EXPERIMENTAL

2.1. Materials and reagents

Zinc nitrate ($Zn(NO_3)_2$) and citric acid (CA) were obtained from Xilong. Other high-purity materials were sodium carboxymethyl cellulose ($C_8H_{16}NaO_8$); glycerol ($C_3H_8O_3$) and calcium chloride ($CaCl_2$) from Xilong; sodium borohydride ($NaBH_4$) and trisodium citrate ($Na_3C_6H_5O_7$) from Shandong; silver nitrate ($AgNO_3$) and ethanol (CH_3CH_2OH, >99% purity) from Merck.

2.2. Synthesis and characterization of Ag NPs, ZnO NPs, and biopolymer thin films

A simple chemical reduction process is used to create Ag NPs [8]. First, silver nitrate ($AgNO_3$) was dissolved in water with trisodium citrate (TSC) as a surfactant. Concurrently, a reducing agent solution containing BH_4^- ions was prepared using sodium borohydride ($NaBH_4$). Ag NPs were then created by progressively adding the reducing agent solution to the Ag+ ion solution. Lastly, the solution was diluted or additional surface stabilizers were added to bring the pH down to a level suitable for storage.

The sol-gel technique with three main steps was used to produce ZnO NPs [9]. First of all, zinc nitrate ($Zn(NO_3)_2$) was mixed with citric acid (CA) in a 1:6 molar ratio in water. Next, this mixture was heated for 20 hours at 80 °C. After two hours of drying at 200 °C, the mixture became a porous grey xerogel. Ultimately, white ZnO nanopowder with an average particle size of 50 nm was formed by granulating the xerogel powder and calcining it for four hours at 650 °C.

Biopolymer thin films containing ZnO and Ag nanoparticles were synthesized using a detailed procedure. A 100 mL solution of Ag nanoparticles was combined with 1.5 g of sodium carboxymethyl cellulose (CMC), 2 g of glycerol, and 10 mg of ZnO powder. The mixtures were continuously stirred at 70 °C for 40 minutes. Following this, the resulting mixture, which included 1.5% CMC, 2% glycerol, and ZnO nanoparticles, was evenly applied to a glass slide and allowed to dry at 70 °C for 2 hours, producing the final biopolymer thin film.

2.3. Optical and microbial inhibition investigations

The morphology of ZnO NPs, Ag NPs, and the composite CMC-ZnO-Ag biopolymer film was analyzed using a scanning electron microscope (SEM) (FE-SEM S-4800, Hitachi), and a transmission electron microscope (TEM) (JEOL JEM-1200EX). The nanoparticle sizes within the biopolymer film were measured using the ImageJ software. The samples' absorbance was studied using a UV−VIS spectrometer (UV−2450, Shimadzu), and a 325 nm He-Cd laser (Kimmon) was used to excite the samples for photoluminescence (PL) analysis. Additionally, the possibility of any chemical interactions among functional groups of CMC-ZnO-Ag was examined using Fourier transform infrared spectroscopy (FTIR).

The microbial inhibition of ZnO NPs and Ag NPs was assessed by the accredited Quality Assurance and Testing Center 1 (QUATEST 1) under the Directorate for Standards Metrology and Quality.

2.4. Fruit preservation investigation

The process for fabricating CMC biopolymer films with ZnO and Ag NPs to preserve avocados involves these steps: Avocados were washed with water and soaked in a 5% $CaCl_2$ solution at 4 °C for five minutes. They were then divided into two groups: one group was the untreated control, and the other was coated with the CMC-ZnO-Ag film by immersion for one minute. Both groups were kept at 8 °C and 80% humidity, and daily checks were conducted to track any changes to the avocados' surface and overall quality.

A French refractometer (Alla) determined sugar concentration changes at each stage. The formula for defining sugar concentration changes: $B = (B_2 – B_1)/B_1$, where B_1 is the initial sugar concentration in the avocado, and B_2 is the concentration after each monitoring session. Natural weight loss was determined by using the highly accurate Sartorius analytical balance from Germany. Natural weight loss was calculated using the formula: $X = (M_1 – M_2)/M_1$, where, X = Natural weight loss at each monitoring session, M_1 = Weight of fruit before preservation (grams), M_2= Weight of fruit at each monitoring session (grams). The hardness of the fruit was determined using a handheld FHT-1122 Stiffness tester from China. Hardness was calculated using the formula: $X = F/S$, where X = hardness of the fruit (kg/cm^2); F = reading on the hardness tester; S = Area of the needle tip.

3. RESULTS AND DISCUSSION

3.1. Surface morphology investigation of the CMC-ZnO-Ag biopolymer film

ZnO NPs were effectively synthesized by the sol-gel method and thermal annealing, which produced particles that were about 50 nm in size (figure 1). These nanoparticles tend to form larger clusters approximately 500 nm in size (figure 1C, F). Additionally, Ag NPs, which ranged from 5 to 15 nm, were uniformly dispersed within the solution (figure 1D). The microstructure morphology of the biopolymer film was observed using optical microscopy. The film's thickness is less than 20 μm (figure 1B). This thin layer permits gas exchange through the film in addition to aiding in lowering the respiration rate, which in turn reduces ethylene formation and extends the fruit's ripening period. Scanning electron microscopy (SEM) images of the CMC biopolymer film integrating ZnO NPs and Ag NPs (CMC-ZnO-Ag) are shown in figure 1E and figure 1F. As observed, the entire surface is covered by spherical nanostructures, with the nanoparticles tending to aggregate to form larger particles.

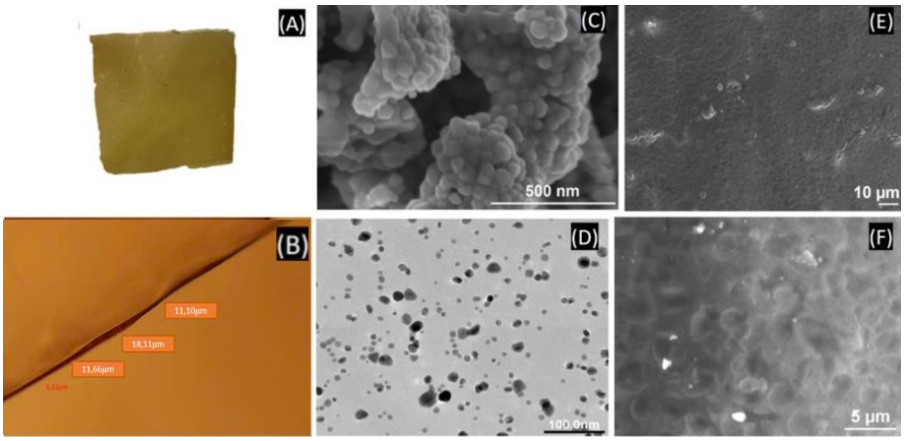

Figure 1. *(A) Image taken by a phone camera of the CMC-ZnO-Ag biopolymer film; (B) Optical microscopy of the CMC-ZnO-Ag biopolymer film; (C) SEM image of ZnO powder; (D) TEM image of the solution containing Ag NPs in TSC surfactant solution; (E, F) SEM images of the CMC-ZnO-Ag biopolymer film.*

3.2. Optical property investigation of the CMC-ZnO-Ag biopolymer film

The CMC-ZnO-Ag biopolymer thin film was characterized by some optical measurements as illustrated in figure 2. The UV-Vis spectra (figure 2A) of this film show the typical UV absorption peak of ZnO NPs at around 280 nm and a peak surface plasmon resonance (SPR) of Ag NPs at 421 nm. In figure 2B, the PL spectra have two peaks. The first peak is at 378 nm resulting in exciton-exciton recombination [10]. The low broad luminescence band centered around 486 nm can be explained by defects in the ZnO nanocrystals [11].

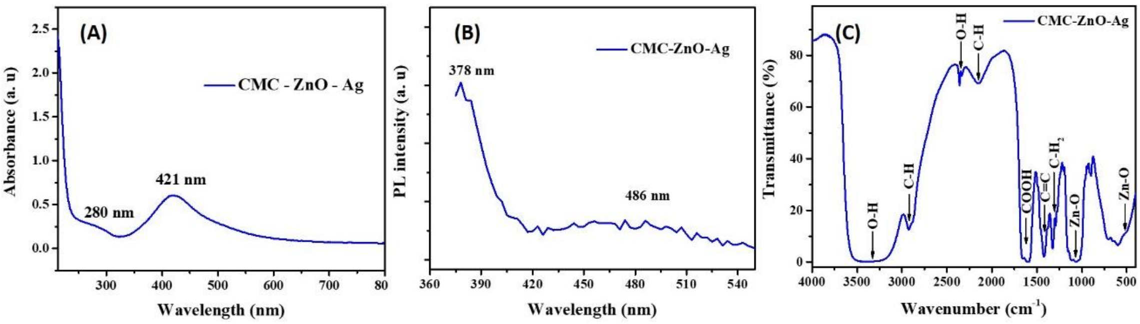

Figure 2. *(A) UV-Vis spectra; (B) PL spectra; (C) FTIR spectra of the CMC-ZnO-Ag biopolymer film.*

In addition, the existence of nanoparticles in the CMC biopolymer film was verified by FTIR measurement (figure 2C). By revealing the functional groups and chemical interactions, this technique confirms the successful integration of nanoparticles within the biopolymer matrix. A significant absorbance band between 3545 cm^{-1} and 3174 cm^{-1}, as well as at 2355 cm^{-1}, is associated with the intramolecular hydrogen bond (O-H) vibrations. The peak at 2920 cm^{-1} and 2147 cm^{-1} is linked to C-H bond vibrations in various modes. The vibrational activity of the carboxyl group (COOH) is shown by a strong peak at 1602 cm^{-1} [12]. Moreover, the peak at 1418 cm^{-1} corresponds to C = C bond vibrations, while the peak near 1319 cm^{-1} is related to the cleavage of $C\text{-}H_2$. Peaks observed around 1063 cm^{-1} and 521 cm^{-1} are indicative of Zn-O interactions [13].

3.3. Antibacterial activities test of ZnO and Ag nanoparticles

Table 1 shows the antimicrobial effects, specifically against E. coli, S. aureus, and the mold Aspergillus niger (A. niger), of the solution samples containing Ag NPs and ZnO NPs. It can be easily seen that after 24 hours of exposure to each type of nanomaterial, the density of microorganisms in the test samples was significantly lower than that in the control samples. This suggests that Ag and ZnO nanoparticles have outstanding antimicrobial properties. In particular, Ag nanoparticles achieved antimicrobial efficiencies of 99.99%, 99.99%, and 80.13%, and ZnO nanoparticles demonstrated efficiencies of 99.96%, 99.95%, and 13.24% against E. coli, S. aureus, and A. niger, respectively.

Table 1. *The evaluation results of the antimicrobial inhibition of Ag NPs.*

Item	Test sample	Parameters	Test method	Result			
				The microbial density of the control at 0h (N_0) (CFU/mL)	The microbial density of the control sample after 24 hours of exposure (N_1) (CFU/mL)	The microbial density of the test sample after 24 hours of exposure (N_2) (CFU/mL)	Antimicrobial efficiency (%) R= $(N_1-N_2)/N_1*100$
1.	Ag NPs	E. coli ATCC 8739	TN8/HD/P/93	3.2×10^4	1.4×10^7	<1.0	99.99
2.	ZnO NPs					7.3×10^3	99.96
3.	Ag NPs	S. aureus ATCC 6538	TN8/HD/P/93	2.0×10^4	4.9×10^6	1.0×10^1	99.99
4.	ZnO NPs					2.3×10^3	99.95
5.	Ag NPs	A. niger ATCC 16888	TN8/HD/P/93	3.4×10^4	2.8×10^5	5.6×10^4	80.13
6.	ZnO NPs					2.4×10^5	13.24

Based on the Quatest 1 results, Ag and ZnO nanoparticles have proven to have superior antimicrobial properties. Therefore, these materials have enormous potential for application in combination with CMC biopolymer films in the preservation of avocados.

3.4. Application of CMC-ZnO-Ag nanocomposite film to preserve avocado

3.4.1. The results of avocado preservation using bio-polymer coating film for 35 days

Two groups of avocados were created: a control group (untreated) and a group coated with a CMC-ZnO-Ag nanocomposite film. For 35 days, both groups were subjected to ocular evaluations at 1, 7, 14, 28, and 35 days (table 2).

Table 2. *The color variation of control and preservation sample in 35 days.*

	1 day	7 days	14 days	28 days	35 days	
Control sample						
Preservation sample						

After 7 days, both groups remained fresh. By day 14, the coated avocados were still fresh, but the control avocados started to show signs of spoilage, such as dark green tint and black spots. On day 35, the control avocados had turned black and shrunk, indicating substantial ripening and deterioration. In contrast, the coated avocados showed very few dark spots and remained their original size and freshness.

This implies that the CMC-ZnO-Ag nanocomposite film efficiently preserves avocados by improving the barrier qualities of the coating, reducing moisture loss [1], and inhibiting the oxidation [14]. Similar preservation outcomes have been observed in studies on strawberries [15], kiwifruits [16], and pomegranate arils [4]. The CMC matrix's ability to hold onto fruit longer after coating them is greatly enhanced by the addition of ZnO and Ag NPs.

3.4.2. The influence of the film on the hardness, the weight, and the sugar concentration of avocados during 35 days

The CMC-ZnO-Ag film coating resulted in the greatest reduction in fruit hardness, dropping from 14.7 kg/cm^2 to 13.1 kg/cm^2 after five weeks (figure 3A). In contrast, fruits with polymer solutions containing nanomaterials saw a slower decrease, from 14.7 kg/cm^2 to 14.6 kg/cm^2. This pattern of keeping hardness matches closely with the natural weight loss and color changes.

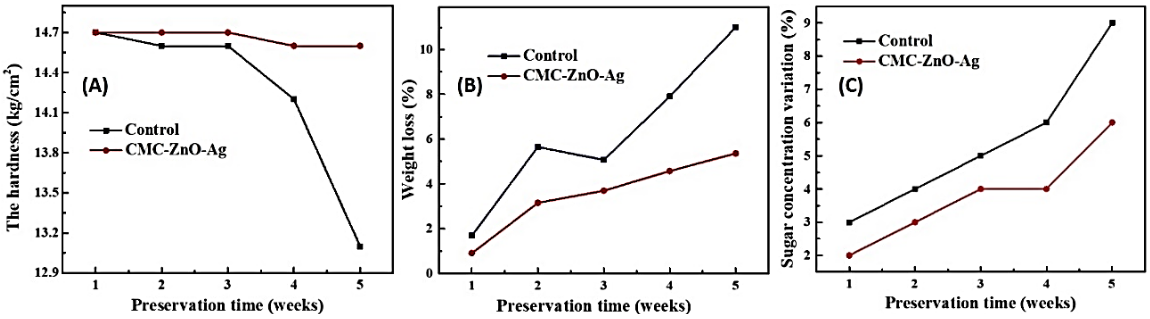

Figure 3. *(A) The hardness; (B) The weight loss;*
(C) The sugar concentration variation of avocado after 35 days.

Natural weight loss (NWL) was greatly reduced over five weeks by the coated formula (figure 3B), with only 8.61% weight loss as opposed to 4.88% in the immersion sample. Weight loss was decreased as a result of the coating's reduction of water evaporation and respiration. The coated formula showed a slower rate of rise (9%) than the control formula (6%), indicating that the coating slowed the ripening process. However, the sugar levels in all formulations increased (figure 3C). This suggests that the coated formulas effectively slow down the avocados' ripening. The data reveal a significant difference in sugar concentration tendency between the two types of formulas. The control formula exhibited a sharp increase in sugar, suggesting a more accelerated ripening. In contrast, the coated formula's sugar concentration rose more gradually, representing a more controlled ripening environment.

These results show that the coated methods are useful in moderating avocado ripening by creating an ideal preservation environment. This approach lessens variations in the sugar concentration and stabilizes the ripening process. The findings highlight the potential for further research and optimization to improve preservation methods to meet quality standards and satisfy evolving consumers and market demands.

4. CONCLUSIONS

We have effectively developed a Carboxymethyl cellulose (CMC) biopolymer thin film incorporating ZnO NPs and Ag NPs (CMC-ZnO-Ag). The ZnO NPs show irregular agglomeration, tending to cluster into larger formations, while the Ag NPs, range in size from 5 to 15 nm. This integration of CMC, ZnO NPs, and Ag NPs has resulted in the successful production of the CMC-

ZnO-Ag composite film. The UV-Vis, PL, and FTIR spectra of this film indicate high purity and crystallinity. According to Quatest 1 results, Ag NPs and ZnO NPs have proven to have superior antimicrobial properties. Moreover, the CMC-ZnO-Ag biopolymer film can extend the ripening period of avocados to up to 35 days. The nanocomposites' improved gas and water barrier characteristics also help prevent degradation from water loss and oxidation. Due to its eco-friendliness and biocompatibility, the CMC-ZnO-Ag biopolymer film holds significant potential for use in fruit and food product packaging and preservation.

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TÓM TẮT

Phát triển màng mỏng polymer sinh học tiên tiến từ Carboxymethyl cellulose, các hạt nano bạc và các hạt nano ZnO định hướng ứng dụng bảo quản trái bơ

Vật liệu nano trong bảo quản sau thu hoạch mang lại nhiều ưu điểm như không độc hại, không gây tồn dư hóa chất, và ngăn chặn hiệu quả sự phát triển của nấm mốc và vi khuẩn. Nhờ tính kháng khuẩn mạnh mẽ và khả năng tạo lớp phủ nano mỏng trên bề mặt trái cây, ngay cả lượng nhỏ hạt nano cũng cung cấp khả năng bao phủ rộng rãi, ngăn chặn sự xâm nhập của vi sinh vật. Công nghệ này cải thiện đáng kể chất lượng và thời gian bảo quản của trái cây trong quá trình bảo quản và vận chuyển. Trong nghiên cứu này, chúng tôi đã giới thiệu một màng sinh học tiên tiến từ carboxymethyl cellulose (CMC) kết hợp với hạt nano ZnO và hạt nano Ag (NPs). Hạt nano Ag và hạt nano ZnO đã cho thấy tính chất kháng khuẩn xuất sắc, có thể được sử dụng để bảo quản trái cây. Màng mỏng sinh học cho phép kéo dài thời gian chín của quả bơ lên đến 35 ngày. Sự biến đổi nồng độ đường, độ cứng và độ giảm khối lượng của quả bơ khi có và không có màng sinh học tiên tiến tích hợp hạt nano ZnO và Ag trong 35 ngày đã được so sánh với nhau để làm rõ khả năng giảm quá trình chín của màng sinh học.

Từ khóa: Carboxymethyl cellulose CMC; Hạt nano Ag; Hạt nano ZnO; Kháng khuẩn; Bảo quản trái cây.