Starch-based Active Packaging Film and its Application

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Montmorillonite (MMT) was used to improve the performance of starch and nano-ZnO was added to act as antibacterial agent for developing a starch-based active packaging material *via* solution mixing and casting methods. The effect of MMT and ZnO on the performance of starch films was investigated, and then the composite films were used for cherry preservation. The results indicated that the addition of MMT could improve the mechanical and water-vapor barrier properties of starch films, but it had only a slight impact on optical properties. However, the addition of nano-ZnO could reduce the optical, mechanical, and water vapor barrier properties of composite films. In preservation applications, compared to the other films, the composite films containing nano-ZnO could be beneficial to maintain quality and nutritional value of cherries by slowing down the growth of bacteria and thereby delaying weight loss, decreasing hardness, and decreasing pH and soluble solid content, resulting in a better preservation effect. Therefore, a starch-based active packaging film with good properties and preservation effect was developed with nano-ZnO and MMT, which could be applied in the field of preservation.

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Keywords: Starch; Bio-based materials; Active food packaging; Mechanical and physical properties

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INTRODUCTION

With the improvement of living standards, consumers' demand for fresh food is increasing. Traditional packaging materials can no longer meet consumers' usage needs. Active packaging has received great attention in the field of food preservation because of its ability to extend the shelf life of food by protecting food from physical and chemical damage, such as moisture loss, oxidation, and bacterial infection, *via* adding appropriate antibacterial agents (Karine *et al.* 2018; Khalida *et al.* 2018; Ezati *et al.* 2022; Zheng *et al.* 2023).

Nano zinc oxide (ZnO) is a common inorganic antibacterial agent with good antibacterial effect on most common microorganisms, such as *Escherichia coli*, *Staphylococcus aureus, etc.* (Liu *et al.* 2018; Hamid *et al.* 2021; Guerra *et al.* 2023). It is cheap, non-toxic, and environmentally friendly and has been widely used in the field of antibacterial materials and food preservation. Adding nano ZnO to composite materials could not only exhibit certain antibacterial properties, but also improve the mechanical properties of the composite material with a low content (Kim *et al.* 2019; Mojtaba *et al.* 2019; Li and Zhou *et al.* 2021). A polyvinyl alcohol based active packing film with incorporation of nano ZnO@Xylan had low water vapor permeability and good antibacterial properties, which could extend the shelf life of cherry tomatoes (Wang *et al.* 2022).

Given the urgent need for environmental protection, green, and biodegradable packaging materials are highly favored. Starch is a common biodegradable polymer material that is non-toxic and inexpensive. It has good development prospects in the field of green materials. However, its poor mechanical properties and water resistance limit its practical applications. To promote its applications, the properties of starch-based materials should be improved through physical mixing or chemical modification methods (Liu *et al.* 2019; Almeida *et al.* 2020).

Montmorillonite (MMT) is a type of silicate product with a unique layered nanostructure with a large specific surface area and aspect ratio. Due to these attributes, it could also extend the gas permeation path and increase the gas permeation time. In view of the above excellent performance, MMT is often used in the field of composite materials to improve the mechanical properties, barrier properties, and thermal stability, adsorption performance, *etc.* (Jiang *et al.* 2021; Li and Zhang *et al.* 2022). Research has shown that MMT could be uniformly dispersed in nanocellulose networks at a nanoscale to form intercalation structures, and the effective modulus of nanocomposites could reach up to 129.9 GPa (Xu *et al*. 2021). Similarly, MMT could also be dispersed in bamboo textile/kenaf fiber/epoxy resins to improve the properties of the composite films, where the tensile strength and Young's modulus increased 44.3% and 96.5%, respectively (Chee *et al*. 2021).

Additionally, when MMT was added to carboxymethyl cellulose at a mass content of 5%, the water vapor permeability of the composite films could be reduced 39% (Achachlouei and Zahedi 2018). Chitosan/hydroxyethyl cellulose (CS/HEC)-based active packaging films containing organophilic nanoclay showed a 52.6% improvement in tensile strength, a 31% enhancement in water vapor barrier property and a 58.0% improvement in oxygen barrier property compared to neat CS/HEC film and the composite films had good antibacterial properties, which could protect cherry tomatoes and extend the preservation time up to 21 days (Tamer 2023).

Cherry is a popular fruit with a round shape, bright red color, sweet and sour taste, and rich nutrition (Blando and Oomah 2019). In recent years, cherry has been favored by consumers because of its polyphenolic compounds that have good antioxidant capacity and are beneficial to human health (Correia *et al*. 2017). However, cherries are highly susceptible to external factors, such as water, bacteria, and physical forces, leading to spoilage and deterioration and resulting in huge waste. Zein/gelatin-based composite nanofiber films containing ZnO nanoparticles showed good inhibitory activity against *B. cinerea*, and they could be used for cherry preservation, reducing the losses of weight and firmness, delaying the respiration time by 5 days, and decreasing the peak of ethylene release by nearly half (Yuan *et al.* 2023).

Therefore, the aim of this study was to develop a starch-based active packaging by adding MMT and nano-ZnO *via* solution mixing and casting methods. The optical, mechanical, and water vapor permeability properties of the composite films were characterized. Then, the composite films were used for cherry preservation to evaluate its preservation effect by analyzing the related parameters of cherry during storage.

EXPERIMENTAL

Materials

Potato starch and sodium carboxymethyl cellulose were purchased from Rhawn reagent Co., Ltd., Tianjin, China. The MMT (< 50 μm, the density was 2.2 g/cm^2) was purchased from Zhejiang Fenghong New Materials Co., Ltd., Hangzhou, China. Nano zinc oxide (30 ±10 nm) was purchased from Aladdin Biotechnology Co., Ltd., Ontario, CA, USA. Glycerol was purchased from Tianjin Fuyu Fine Chemical Reagent Technology Co., Ltd., Tianjin, China. All the regents used were analytically pure.

Methods

Preparation of MMT/starch composite films

To begin, a certain amount of MMT was added to deionized water, and the mixture was mechanically stirred to evenly disperse the solids in the water at 80 ℃. After cooling to 70 ℃, 6 g of starch and 2 g of glycerol were added and mechanical stirring continued to evenly mix the solution. The MMT/starch composite films with MMT content of 1%, 3%, 5%, and 7% of starch were obtained by a casting method. Starch film without addition of MMT was prepared as a control by the same method.

Preparation of ZnO/MMT/starch composite films

Different masses of nano ZnO were added to the mixed solution, and mechanical stirring was continued at 70 ℃ to evenly mix the solution. The ZnO/MMT/starch composite films with ZnO content of 1%, 5%, and 10% of starch were ultimately obtained by the same method.

All films were stored in a constant humidity environment at room temperature before testing.

Characterization

The optical properties

The transmittance/haze of the composite films was measured by a transmittance haze tester (WAT-S, Shanghai Shenguang Instrument Co., Ltd., Shanghai, China). Each sample was tested at least 10 times and the average value was used.

The mechanical properties

The mechanical properties of the composite films were measured using an intelligent electronic tensile testing machine (XLW, Jinan Languang Electromechanical Technology Co., Ltd., Jinan, China). The samples were cut to a strip of size 80 mm × 15 mm. The gauge length used was 50 mm, the width was 15 mm, and the stretching speed was 200 mm/min. At least 10 sets of data were tested on each sample and the average value was taken.

The water vapor permeability

The water vapor permeability properties of the composite films were measured by a water vapor transmittance tester (W3/031, Jinan Languang Electromechanical Technology Co., Ltd., Jinan, China). The samples were cut into a circular piece with a diameter of 75 mm. Three data sets were tested for each sample and the average value was obtained.

X-Ray diffraction (XRD)

The XRD patterns of the composite films were measured by X-ray diffractometer (D8 Advance, Bruker, Germany) from 5° to 80° with a scan speed of 10°/min.

Antimicrobial activity

The antimicrobial activity levels of the films against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria *Escherichia coli* (*E. coli*) were evaluated by the agar disk diffusion method. The sample films were cut into small discs with a diameter of 1.2 cm and then sterilized under ultraviolet light. Each disc was placed in the middle of nutrient agar medium. It was previously inoculated with 50 μL of bacterial suspensions (approximately 107 CFU/mL). The plates were incubated at 37 °C for 24 h to measure the inhibition zone diameter with a caliper.

Preservation testing

Fresh cherries were purchased at a local market in Zhengzhou, China. The fresh cherries were placed in a beaker, and the beaker was sealed tightly with the composite films. Four groups were set: (1) the blank group without film covering, (2) the control group with 3% MMT/starch composite film, (3) the experiment group with 1% ZnO/3% MMT/starch composite film, and (4) the experiment group with 5% ZnO/3% MMT/starch composite film group for preservation testing.

The various indicators of cherries were measured regularly at room temperature. The mass of cherries was weighed and recorded. The weight loss of cherries was calculated by the ratio of weight reduction to original mass. The firmness of cherries was tested by a fruit firmness tester (GY-3, Quzhou Aipu Measuring Instruments Co., Ltd, Quzhou, China). The pH of cherries was tested using a pH meter (PHB-5, Hangzhou Aolilong Instrument Co., Ltd., Hangzhou, China). For the measurement of soluble solids content, cherries were ground in a mortar, and the juice was centrifuged at 10,000 rpm for 10 min. The supernatant was used for the soluble solids content test by a Pocket Refractometer (TD32, Shanghai Boji Instrument Co., Ltd, Shanghai, China).

RESULTS AND DISCUSSION

The Properties of MMT/Starch Composite Films

The effect of MMT on the optical properties

As shown in Fig. 1a, the transmittances of the MMT/starch composite films were gradually decreased by the addition of MMT. The starch film indicated the highest transmittance of 92.5%, and the composite films with MMT content of 7% exhibited the lowest transmittance of 90.4%, which was not remarkably different from starch film, indicating that the addition of MMT had little effect on the transmittance of the starch film, and MMT could be uniformly dispersed in the starch film. Moreover, the haze of the composite films increased with the addition of MMT. The haze of the starch film was 42.1%, and it gradually increased to 62.3% when the MMT content was increased to 7%. This haze increase may be caused by the addition of MMT, which could break the uniformity of starch film and promote light scattering.

The effect of MMT on the mechanical properties

The mechanical properties of MMT/starch composite films are shown in Fig. 1b. The starch film had the lowest tensile strength of 6.28 MPa and the highest elongation at break of 94.6%, which shows the beneficial effect of glycerol. With increased MMT content, the tensile strength of the composite film first increased and then gradually decreased. When the MMT content was 3%, the tensile strength of the composite film reached a maximum of 16.93 MPa, which was 170% higher than that of starch film. However, the tensile strength of the composite film began to decrease when the MMT content continued to increase. This was because MMT could be well dispersed in the starch film at a low content, thereby improving the tensile strength. However, it could cause self-aggregation and stress concentration when the content was high, leading to a decrease in tensile strength (Jiang *et al*. 2020). Meanwhile, the elongation at break of the composite films showed a decreasing trend with the addition of MMT. This was because MMT is a rigid substance. Thus, it had a negative effect on the flexibility of the molecular chain, further reducing the elongation at break of the composite films. Accordingly, MMT/starch composite film exhibited the best mechanical properties when the MMT content was 3%.

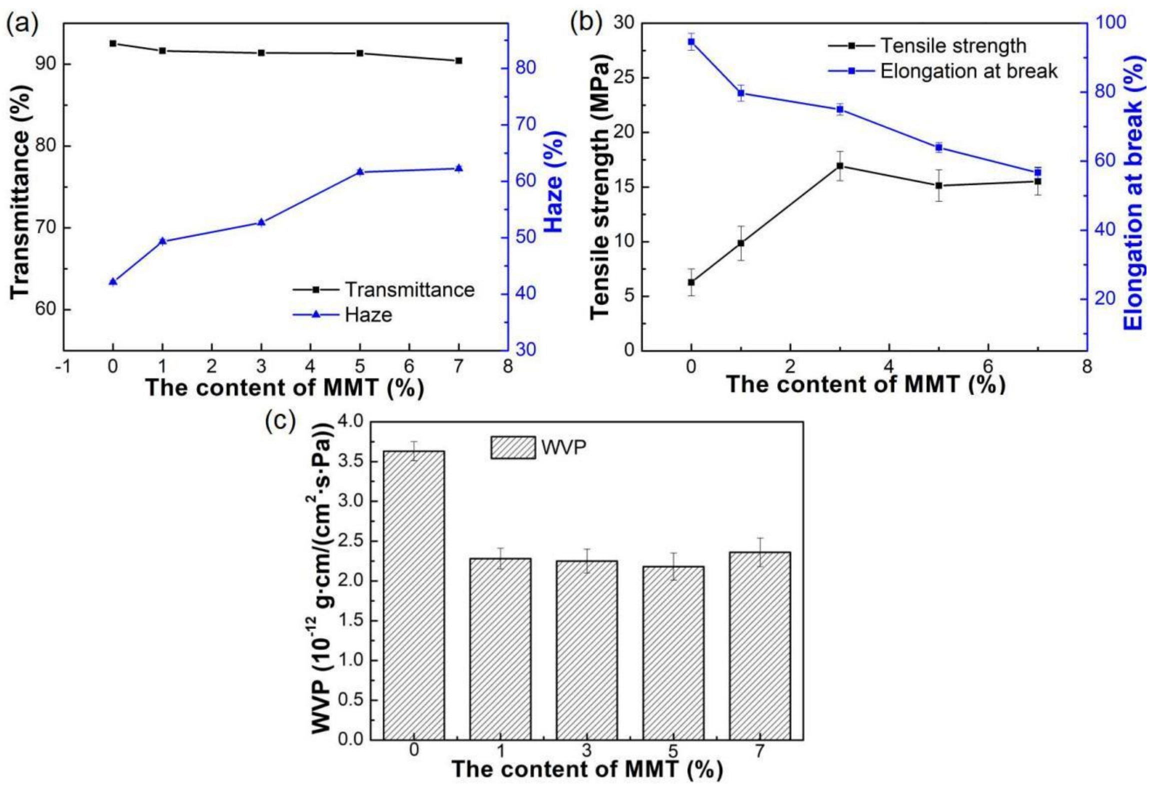

Fig. 1. The optical properties (a); mechanical properties (b); and WVP(c) of MMT/starch composite films

The effect of MMT on the water vapor permeability (WVP) of starch film

It could be seen that with the increase of MMT content, the WVP of the composite films first decreased and then increased slightly, as shown in Fig. 1c. This indicated that the addition of MMT could reduce the WVP of the composite film and improve its water vapor barrier performance. When the MMT content was 5%, the WVP coefficient was the lowest of 2.18×10^{-12} g·cm/(cm^2·s·Pa), and the water vapor barrier was the best, due to the layered structure of MMT, hindering the passage of water vapor. When the MMT content was 3%, the WVP coefficient was 2.25×10^{-12} g·cm/(cm^2·s·Pa), and there was no big difference compared to that of composite films with a 5% MMT content. When the MMT content continued to increase to 7%, the WVP coefficient slightly improved to 2.36×10^{-12} g·cm/(cm^2·s·Pa), but it was still much lower than the starch film. This may be due to the large agglomeration size of MMT, which affected the density of the composite films, and reduced the water vapor barrier performance (Jiang *et al*. 2021; Li and Zhang *et al*. 2021).

Based on the optical, mechanical, and water vapor barrier properties of MMT/starch composite films, it could be concluded that the optimal comprehensive performance was achieved when the MMT content was 3%. Therefore, the MMT content was fixed as 3% in subsequent experiments.

The Properties of ZnO/MMT/Starch Composite Films

The effect of ZnO on the optical properties

It could be seen from Fig. 2a that the transmittance of the ZnO/MMT/starch composite films gradually decreased, and the haze gradually increased with the addition of nano ZnO. The transmittance of 3%MMT/starch composite film was 91.4%. After adding nano ZnO, the transmittance of composite films decreased substantially, and the lowest transmittance was 57.7%. This big difference resulted from ZnO nanoparticles, which could hinder the passage of light. Larger nanoparticles resulted in greater obstruction, and lower transmittance. The haze of 3%MMT/starch composite film was 52.6%, and the haze of ZnO/MMT/starch composite films increased up to 81.9% with addition of nano ZnO, which could further disrupt the uniformity of starch film, resulting in an increase in light scattering and haze.

The effect of ZnO on the mechanical properties

The tensile strength of the composite films was gradually decreased by adding nano ZnO, as shown in Fig. 2b. It was 12.09, 12.21, and 10.14 MPa when the ZnO content was 1%, 5%, and 7%, respectively. The elongation at break first increased and then decreased by adding nano ZnO. When the content of nano ZnO was 1%, the composite films exhibited the maximum elongation at break of 84.6%, and then it decreased to 74.1% when the nano ZnO content was 1%. There was a sharp decrease to 26.1% when the content of nano ZnO increased to 10%.

It could be explained that nano ZnO dispersed between macromolecular chains, weakening the intermolecular forces, and increasing the molecular flexibility, thus reducing the tensile strength and increasing the elongation at break with a low nano ZnO content. However, nano-ZnO agglomeration occurred when the content was high, causing stress concentration and then resulting in a decrease of mechanical properties (Pan *et al*. 2022). Considering the practical application, when the content of nano ZnO was 5%, the mechanical properties of the composite film were good with tensile strength of 12.2 MPa and elongation at break of 74.1%. Compared with starch films, these values increased 94.4% and decreased 21.7%, respectively.

The effect of ZnO on the water vapor permeability (WVP)

The WVP of ZnO/MMT/starch composite films are shown in Fig. 2c. The WVP gradually increased with the addition of nano ZnO, indicating an increase in water vapor permeability but a decrease in water vapor barrier performance. The WVP coefficient of the composite film with nano ZnO content of 5% was 2.96×10^{-12} g·cm/(cm^2·s·Pa), which was higher than that of the 3% MMT/starch film but was lowered by 18.4% compared starch film with a WVP coefficient of 3.63 × 10^{-12} g·cm/(cm^2·s·Pa). Similar to the mechanical properties, this phenomenon could be explained by the aggregation of nano ZnO particles that weakened the compactness of the composite films, causing internal pores and making it easier for water vapor to pass through (Roy and Rhim 2019). In addition, both nano ZnO and MMT are inorganic materials, and their poor compatibility also affected the uniformity of the composite films, thereby affecting the water vapor permeability and mechanical properties.

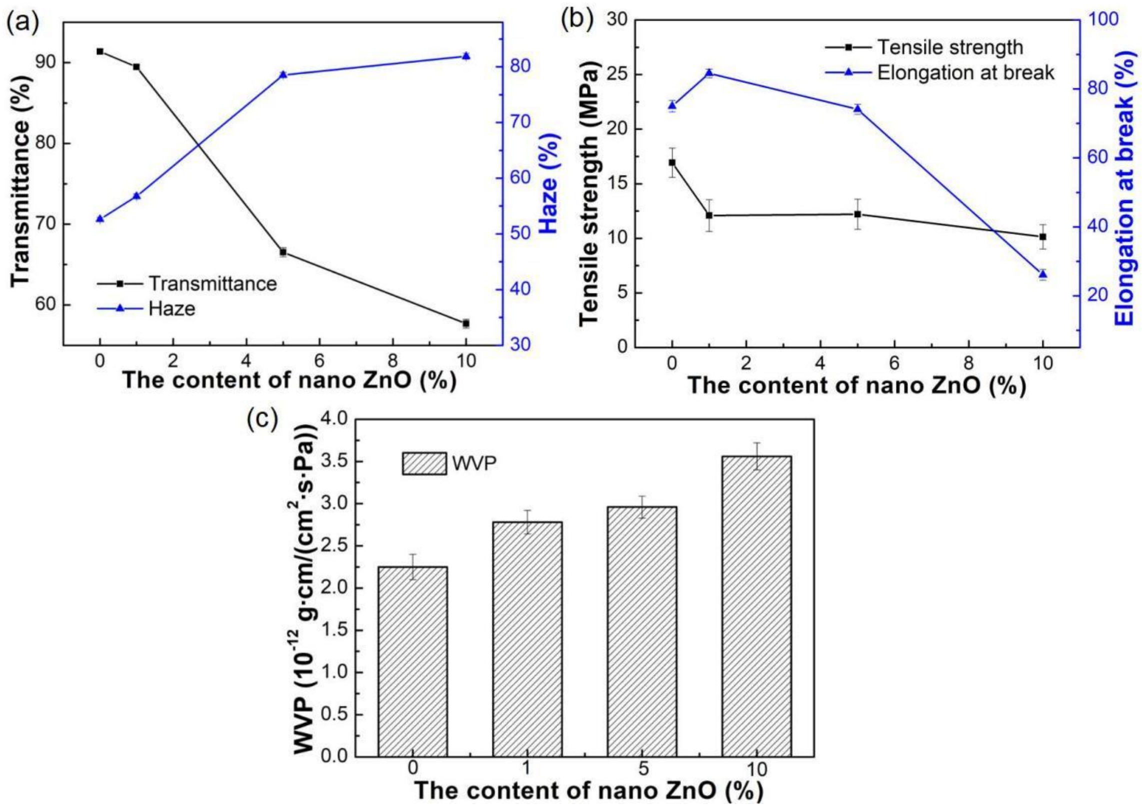

Fig. 2. The optical properties (a); mechanical properties (b); and WVP(c) of ZnO/MMT/starch composite films

The effect of MMT and ZnO on the XRD pattern of starch film

XRD patterns could be used to identify the crystalline structure. Figure 3 shows the effect of MMT and nano ZnO on the XRD of composite films. The XRD diffraction peaks at near 7° was the characteristic crystalline peaks of MMT. The diffraction peaks at 31°, 34°, 36°, 47°, 56°, 62° and 67° were obvious when the nano ZnO content was 5%, which were the characteristic crystalline peaks of ZnO and were attributed to facets (100), (002), (101), (102), (110), (103) and (112) of ZnO crystals respectively, according the standard XRD pattern of ZnO (JCPDS card No 76-0704). The diffraction peak of composite film with 1% nano ZnO was not noticeable due to its low content. There was no remarkable difference at other peak positions, indicating that the addition of MMT and nano ZnO had no effect on the XRD diffraction of starch.

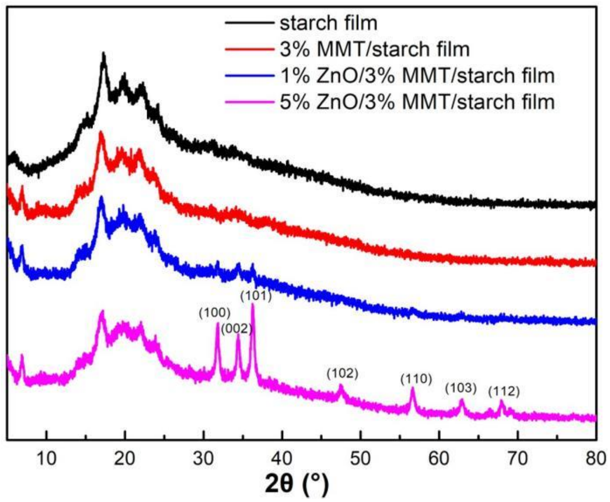

Fig. 3. The XRD patterns of composite films

Antimicrobial Activity

The antimicrobial activity of the starch film and 5% ZnO/3% MMT/starch composite film against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Gram-negative bacteria Escherichia coli* (*E. coli*) was evaluated by the agar disk diffusion method, which were commonly found in food, and the results are shown in Fig. 4.

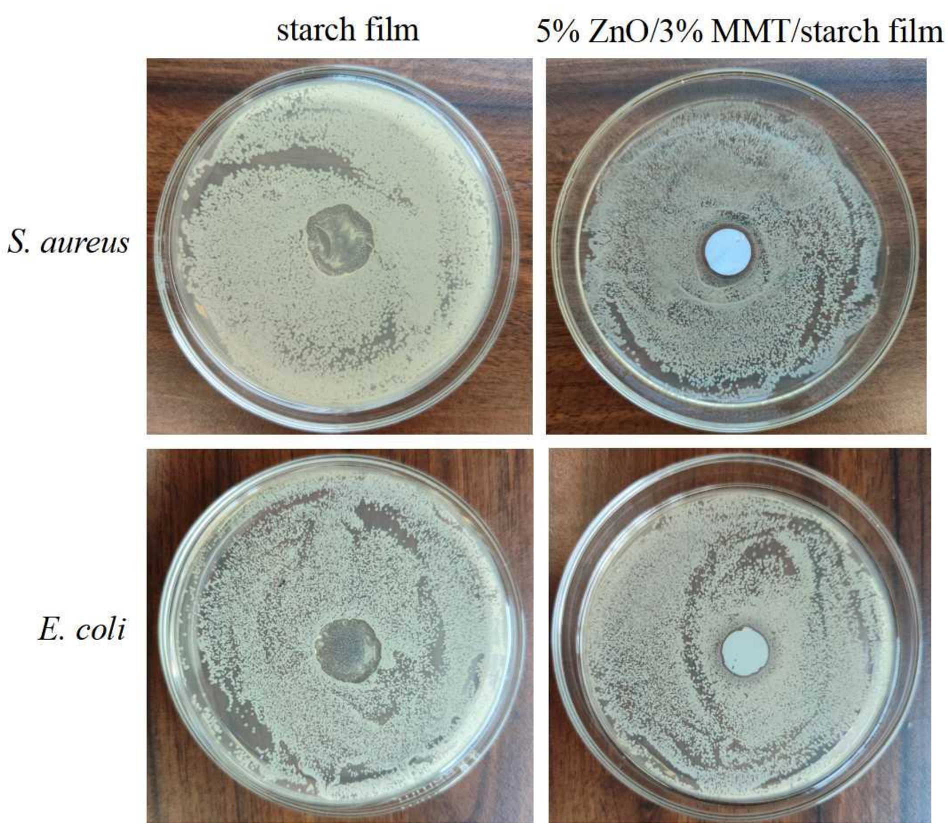

Fig. 4. The antimicrobial activities of composite films against *S. aureus* and *E. coli*

The starch film showed no inhibition zone against both *S. aureus* and *E. coli*. Bacteria grew on the surface of the film, due to the non-antibacterial property of starch film. And the starch film was partially destroyed, resulting from its good hydrophilicity. By contrast, inhibition zone diameters for 5% ZnO/3% MMT/starch composite film against *S. aureus* and *E. coli* were 15.8 mm and 12.1 mm, respectively. The antimicrobial activity of the composite film benefitted from the good antibacterial properties of nano-ZnO (Kim *et al.* 2019; Mojtaba *et al.* 2019; Li and Zhou *et al.* 2021).

Cherry Preservation Analysis

The preservation effect of the experimental films was higher than that of the blank group and the control group based on naked eye observation. The cherries in the blank group showed obvious suppuration and moldy phenomena on the 6th day, while the cherries in the experimental group only showed softening on the 6th day without any suppuration or moldy phenomena. With higher content of nano-ZnO, a better preservation effect by the composite film was observed. Antibacterial tests showed that the composite films containing nano ZnO exhibited a certain degree of antibacterial activity. Similar results had also been reported in many studies, such as Kim *et al*. 2019; Mojtaba *et al*. 2019; and Li and Zhou *et al*. 2021. Therefore, it could be considered that the good preservation effect was due to the addition of nano ZnO, making the composite film exhibit good antibacterial effects, which inhibited the growth and reproduction of bacteria and thus slowed down the spoilage of cherries.

Firmness and weight loss

Figure 5a shows that the weight loss of cherries gradually increased during storage time, due to the transpiration and cellular respiration of cherry (Wani *et al*. 2014).

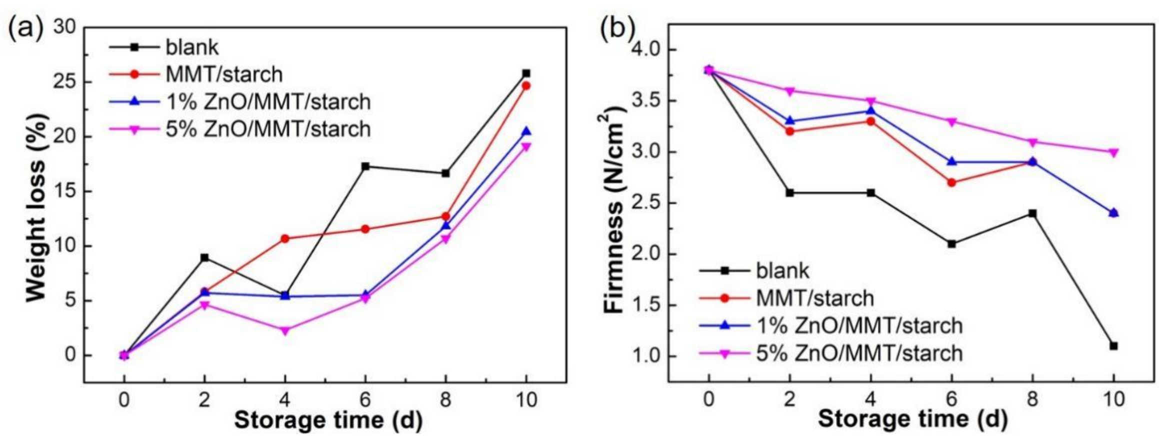

Fig. 5. The weight loss rate (a) and firmness (b) of cherries during storage time

The blank group and control group had higher weight loss, while the experimental group had lower weight loss. The WVP of the composite film with nano ZnO was higher than that without nano ZnO, which may have promoted weight loss. However, nano ZNO could inhibit the invasion and growth of microorganisms and then inhibit cellular respiration. The antibacterial effect of nano ZnO was more pronounced. So the cherry respiration was inhibited, and the weight loss was reduced. The firmness of cherries gradually decreased during storage time, as shown in Fig. 5b.

The experimental group had the highest firmness, followed by control group, and the blank group had the lowest firmness. A higher content of nano ZnO in the composite film resulted in a lower weight loss rate and higher firmness of cherries. This indicated that the composite films could prevent the water exchange of cherries and then suppress the weight loss and softening trend. Additionally, the more content of nano ZnO revealed a better preservation effect.

Effect of pH and soluble solids content

It could be seen from Fig. 6a that the pH of cherries first showed an increasing trend, followed by a decreasing trend. This could have been because the protein of cherries was decomposed to produce amine compounds in the early storage time, leading to the first increasing trend of pH (Guerraf *et al*. 2022), but with the deepening of decay, water loss, and mildew, the nutrients were decomposed by microorganisms, and then the flesh was soured, so the pH began to decline.

The pH of cherries in the blank group began to decrease on the 4th day, while the control group and experimental group both began to decrease on the 6th day. The experimental group with 5% ZnO always remained at a high pH level during storage time. This indicated that the composite film with 5% nano-ZnO could effectively prevent bacterial invasion, thus delaying decay and nutrient loss.

The changes of soluble solids content could reflect the degree of nutrient consumption, which is an important indicator for measuring the preservation effect (Zhang *et al*. 2021; Choi *et al*. 2023). It could be seen that the soluble solids content in each group first increased and then decreased, as seen from Fig. 6b. This may be because the cherries were not fully mature, so the soluble solids content increased in the early time (Blando *et al*. 2019). After reaching full maturity, the soluble solids content began to decrease, which was consumed by bacteria and cellular respiration. The soluble solids content in the experimental group was at a high level and it began to decrease on the 6th day, while it was low and began to decrease on the 4th day for the control and blank groups. This indicated the composite film with nano-ZnO had good antibacterial effect, which could inhibit microbial growth and slow down the consumption of nutrients in cherries. However, the antibacterial effect of control group and blank group was poor, so the cherries were prone to spoilage and deterioration, resulting in a decrease in soluble solids content.

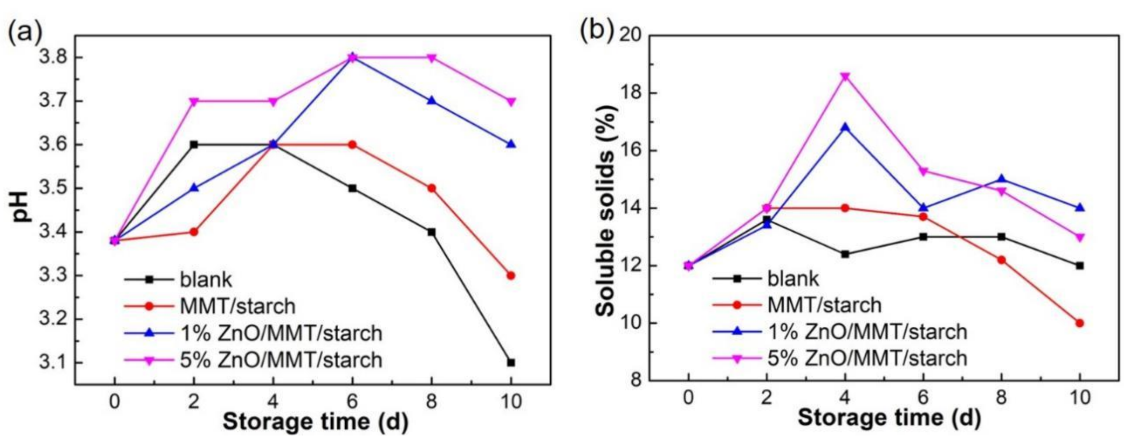

Fig. 6. The pH (a) and soluble solids content (b) of cherries during storage time

CONCLUSIONS

1. A starch-based active packaging film was prepared by combining montmorillonite (MMT) and nano ZnO *via* solution mixing and casting methods. The composite films exhibited good properties and preservation effects and could be applied in the field of food preservation.
2. From a comprehensive perspective, when the MMT content was 3% and the nano ZnO content was 5%, the composite film exhibited good properties. The tensile strength was 12.2 MPa, elongation at break was 74.1%, and the water vapor permeability coefficient was 2.96×10^{-12} g·cm/(cm^2·s·Pa), which increased 94.4%, decreased 21.7%, and 18.4%, compared to starch film, respectively.
3. The 5% nano ZnO/3%MMT/starch composite films could delay the decay and spoilage of cherry by suppressed bacterial growth, presented as restraining weight loss, softening the cherry, and decreasing pH and soluble solid content. In summary, the composite films revealed good properties and preservation effects and can be applied in the field of food preservation.

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