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Characterization of polysaccharide-based antibacterial films properties of loaded with Nisin and preservation of fresh-cut watermelon

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Abstract

The study aimed to improve polysaccharide-based packaging to extend the shelf life of fresh produce; a composite film with antimicrobial function was developed and tested. The packaging film is a blend of agar, a film-forming substrate; carrageenan, as a reinforcing agent; glycerol, as a plasticizer; and Nisin, an antimicrobial agent. The film was evaluated for its antibacterial, mechanical, and barrier properties at different humidity levels, using fresh-cut watermelon as the test food material. The film effectively inhibited the growth of *Staphylococcus aureus* and *Listeria monocytogenes*. Under relative humidity of 20%, 50%, and 90%, the tensile strength of the antibacterial film containing 0.28% Nisin was 23.08 ± 0.65, 16.09 ± 1.73, and 6.52 ± 0.56 MPa, respectively, and the film also had excellent barrier and heat-sealing properties. The packaging test using fresh-cut watermelon sealed in the antibacterial film containing 0.28% Nisin, under controlled atmosphere storage at either 4 °C or 20 °C, effectively inhibited microbial colonization in the melon and slowed the deterioration of the fruit, as indicated by measures of hardness, weight loss, and soluble solids. This method can extend the shelf life of fresh-cut fruit and provide a reference for further research on polysaccharide-based protective film for fresh produce.

Keywords: agar; Nisin; fresh cut watermelon; freshness preservation.

Practical Application: A newly developed perforated film composed of agar-carrageenan and glycerol—and with the important addition of the antimicrobial agent Nisin—outperformed other film material in preserving fruit freshness based on a number of indicators. Because the film performed equally well at low (4 °C) and high (20 °C) storage temperatures and at different levels of relative humidity, it shows high potential as an application to preserve freshness and extend shelf life of produce packed for retail consumption.

1 Introduction

Fresh-cut fruits and vegetables provide consumers with readily available nutritous foods; however, due to the multiple steps in handling and delivery of the perishable product—packaging, transportation, and storage—fresh-cut produce is highly susceptible to spoilage (Giannakourou & Tsironi, 2021). Microbial food spoilage is a global problem (Snyder & Worobo, 2018), a significant portion of the total fruit and vegetables produced in the world each year go waste (Jeswani et al., 2021). Considering fresh-cut watermelon, the quality of fresh-cut fruit, when stored, is characterized by loss of water, discoloration, softening, reduction of the sweetness value, and mildew and odor. McGlynn et al. (2003) reported a significant decrease in flesh freshness and hardness values of fresh-cut watermelon after 7 to 10 days of storage. Mao et al. (2006) found that the soluble solids content of fresh-cut watermelon decreased significantly when stored at 10 °C for 7 days, while the microbial content increased. Preservation of fresh-cut fruits and vegetables can be divided into physical and chemical preservation. Physical preservation includes traditional packaging film (bag) technology, low temperature cold chain technology, and ultrasonic, ozone, and radiation methods (Artés-Hernández et al., 2021) as well as controlled atmosphere storage (Mendoza-Enano et al., 2019a). Chemical preservation involves chemical preservatives, as well as those derived from natural plant extracts (Suzuki et al., 2021) and biological preservation methods. From the viewpoint of food handling operations, economy, and convenience, a combination of physical and chemical methods—notably, the addition of antimicrobial agents to the packaging material—were assessed in this study, with the goal of extending the shelf life of fresh-cut watermelon.

The development of biodegradable packaging films is extremely important, given the increasing environmental pollution caused by plastics (Sedayu et al., 2019). The main biopolymers of biodegradable films include polysaccharides, proteins and lipids (Lian et al., 2022; Fernandes et al., 2020), among which polysaccharides have been recognized for their diversity of sources, low cost, and simple process for film formation (Mostafavi, 2019). Agar (AG), a polysaccharide extracted from marine red algae, has strong gelation properties, and can form a gel at concentrations as low as 0.004%. Factors such as the degree

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of ionization and pH can affect the gel strength. In addition, agar has good thickening, gelling, and film-forming properties (Atef et al., 2015; Mohajer et al., 2017). Agar is a hydrophilic polysaccharide mixture of agarin and agarose, containing alternating β-(1,3) and α-(1,4) linked galactose residues with sulfated functional groups (Kanmani & Rhim, 2014; Rhim et al., 2011; Rocha et al., 2018). The agar film has the function of protecting the packaged material from the mutual transfer of components, such as water, oxygen, flavor substances, with the surrounding medium (Phan et al., 2005). However, agar film alone is not suitable as a packaging film material due to its low flexibility and hard texture (Atef et al., 2014). To overcome these disadvantages, other components have been introduced to modify the properties of agar (Du et al., 2019; Wang et al., 2017). Nieto (2009) reported that an agar film with a thickness of 508 μm could be produced by using 4% (w/w) agar solutions. The properties of agar films can be improved by adding glycerol (Gl), chitosan, carrageenan (CA), and gelatin to the agar film (Jridi et al., 2019).

Streptococcus lactis (Nisin) is a highly effective, non-toxic and safe antibacterial agent that inhibits Gram-positive bacteria, such as *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp., and *Mycobacterium tuberculosis* via cell membrane perforation. Factors such as pH and temperature affect the activity of Nisin; usually, under conditions of low temperature and low pH, Nisin remains highly stable and exerts antibacterial ability over a long period of time. During the spoilage process in fruits and vegetables, the food is firstly attacked by bacteria and then further infected by fungi at the point of attack. Accordingly, Nisin has been widely used in packaging materials as an anti-bacterial agent; for example, Mauriello et al. (2005) achieved effective inhibition of *Micrococcus luteus* in tryptone soy broth by coating a low-density polyethylene film with Nisin. The N-succinyl chitosan antimicrobial film containing Nisin prepared by Wang et al. (2021) had better physicochemical properties, and the presence of Nisin improved the mechanical water vapor barrier, and optical properties of the film and resulted in extension of the shelf life of strawberries.

Nisin has been approved by the U.S. Food and Drug Administration (FDA) for application in food, and the food supervision department of the Ministry of Health of China has issued standards for the use of Nisin as a preservative in food. Polysaccharides and glycerin are also safe food additives (Aliste et al., 2000). Therefore, in the present study, agar/carrageenan (AG/CA) was used as the main film-forming substrate, glycerol was used as a plasticizer, and Nisin as the antimicrobial agent to prepare an environmentally friendly polysaccharide-based antimicrobial film, to preserve fresh-cut watermelon in storage.

2 Materials and methods

2.1 Materials and reagents

Agar powder was purchased from Qingdao HiTech Park Haibo Biotechnology Co.(Nianjing, China). Carrageenan, Xiangning Bioengineering Co., Ltd (Tengzhou, China).Nisin (titer ≥ 1 000 000 IU/g), Sunberga Biotechnology Co., Ltd (Nanjing, China). Glycerol, Quanrui Reagent Co., Ltd (Liaoning, China). Brain Heart Infusion Agar (BHI), Hi-Tech Park Haibo Biotechnology Co., Ltd (Qingdao, China). Watermelon, purchased from Beijing Hualian Supermarket Miaojie compact bag (PE), Baima Packaging Co., Ltd (Yantai, China).

2.2 Preparation of AG/CA antibacterial film

The methodology in this study is primarily based on the research methods of Wang et al. (2016). AG/CA and glycerol were dissolved in 220 mL of distilled water at 100 °C, according to the component settings in Table 1, and, after homogeneous co-mixing, 30 mL of distilled water containing different masses of Nisin was added to the film-forming solution and co-mixed for 1 to 2 min. The pH of the solution was adjusted to 6.0 (Fael & Demirel, 2020). The film was cast on a 20 cm × 29 cm glass plate, dried at 35 °C for 8 h, and stored at 25 °C and relative humidity (RH) of 20%, 50%, and 90%.

2.3 Performance characteristics of AG/CA antimicrobial films

The following physical and mechanical characteristics of the different films were measured: thickness, tensile strength and elongation-at-break, heat-sealing strength, and the oxygen permeability coefficient and moisture permeability under different relative humidity conditions.

The thickness of the antimicrobial film was determined by referring to the method of Yu et al. (2021), with modifications. The tensile strength and elongation at break of the antimicrobial films were tested by according to standard method ASTM D882/12(TA-XT2i, Stable Micro Systems Ltd., UK). The size of the film was 2 cm × 15 cm, the initial clamping distance was 5 cm, and the pulling rate was set at 2.5 cm/min.

The thermal sealing strength test was carried out refer to the Hernandez-Izquierdo & Krochta (2009) (FMJ-450 seal machine, Jinan LAN Electromechanical Technology Co., LTD). The heat-sealing strength of the film was measured by cutting the film into long strips, 1.5 cm in length and 1.5 mm in width. One end of the film was sealed with a sealing machine. The initial clamping distance of the tensile tester was set to 5 cm, and the pulling and drawing rate was 5 cm/min. The antimicrobial film was opened at 180° with the heat sealing part as the center and tested. The test results were expressed in N/15 mm.

Table 1. Table of antimicrobial membrane composition content.

Film forming substrates	Weight of a single component as a percentage of water / %					
AG	1.6	1.6	1.6	1.6	1.6	1.6
CA	0.2	0.2	0.2	0.2	0.2	0.2
Gl	0.6	0.6	0.6	0.6	0.6	0.6
Nisin	0.00	0.20	0.24	0.28	0.32	0.36

The Water vapor permeability of the antibacterial film was measured according to the Pérez-Córdoba et al. (2018). The area of the test membrane was 0.0032 m^2.

The oxygen permeability of the films was determined by Wang et al. (2022) (BTY-B1, Labthink, Jinan, China). The oxygen permeability coefficient of antibacterial film was tested by differential pressure method. The diameter of the film was 8.5 cm.

In terms of the antibacterial film inhibition function, the two species of bacteria that typically cause spoilage in watermelon, *Listeria monocytogenes* and *Staphylococcus aureus*, were selected for the antibacterial activity tests (Ramos-Villarroel et al., 2012).The preserved strain was activated for three generations at 37 ± 1 °C, and the gradient dilution was performed with 0.9% NaCl to reach a concentration of 108 CFU/mL. The antimicrobial film was then cut into 6-mm circles and placed in BHI medium containing 100 μL of bacterial solution and incubated at 37 ± 1 °C for 48 h. The width of the inhibition circle was calculated using Equation 1.

$$D = \frac{D_1 - D_2}{2} \quad (1)$$

where D is the width of the inhibition circle, D_1 is the outer diameter of the inhibition circle, and D_2 is the diameter of the antibacterial film. The unit of measure is mm.

2.4 Study of antibacterial film on fresh-cut watermelon

Theoretical basis of packaging design for fresh-cut watermelon

To determine optimal packaging conditions for preservation of cut watermelon, the performance of the antimicrobial film was studied under different storage conditions. The optimal concentrations of O_2 and CO_2 for fresh cut watermelon was previously determined to be 5% and 10%, respectively (Mendoza-Enano et al., 2019b). In the curent study, the diffusion coefficient of O_2 was 0.063 $m^2 \cdot h^{-1}$ at 4 °C, and 0.069 $m^2 \cdot h^{-1}$ at 20 °C.

The oxygen consumption rate of fresh-cut watermelon was determined by using an overhead gas analyzer (Jinan LAN Optical Electromechanical Technology Co., LTD) set at a relative humidity of 90% and temperatures of 4 °C and 20 °C. The silica gel gasket was pierced with a sampling needle, and the sampling interval was set to 5 s and the analysis time to 15 s. The average value was taken for 10 times measurements. The experiment was designed according to the gas transfer model Equation 2 of the gas conditioning packaging method of the antibacterial film according to the gas conditioning packaging (MAP).

$$\frac{dno_2}{dt} = \frac{NDo_2 A_p \left(0.21P_a - Po_2\right)}{L_d}\left(\frac{1}{RT)}\right) + \frac{Po_2 S\left(0.21P_a - Po_2\right)}{L} - WRo_2 \quad (2)$$

where N is the number of perforations of the antimicrobial film, D is the diffusion coefficient of the gas ($m^2 \cdot h^{-1}$), Ap is the area of the perforated holes (m^2), P is the partial pressure (atm), Pa is the atmospheric pressure (atm), L is the thickness of the antimicrobial film (μm), Ld is the corrected perforation length (m) for the diffusion resistance of the gas at a perforation depth of 1.1 times the perforation diameter, R is the gas constant ($m^3 \cdot atm \cdot k^{-1} \cdot mol^{-1}$), P is the gas permeability of the antimicrobial film ($mol \cdot \mu m \cdot m^{-2} \cdot h^{-1} \cdot atm^{-1}$), S is the surface area of the antimicrobial film (m^2), W is the mass of the packaged material (kg), and RO2 is the rate of O2 consumption ($mol \cdot kg^{-1} \cdot h^{-1}$).

Storage methods for fresh-cut watermelon

The watermelon was cut into 2 cm × 2 cm squares. An amount of 200 g of these squares were placed in plastic bowls and sealed with the AG/CA-N film containing Nisin, film AG/CA without Nisin, and PE preservation bag (PE) as lids. A control group (no lid, CK) was set up and placed at 4 °C and 20 °C, with relative humidity of 90%. The sampling interval of fresh-cut watermelon during the storage period was 3 d at 4 °C and 1 d at 20 °C.

Indicators of fruit freshness

The following indicators of fruit freshness were measured: weight loss rate, soluble solids content, hardness, titratable acid content, Vc content, and number of bacterial colonies.

Water loss of fresh-cut watermelon was measured using an electronic balance (FA2004N, Shanghai Minqiao Precision Scientific Instrument Co., China) (Equation 3).

$$Weight\ loss\ rate(\%)\ was\ calculated\ as\ follows: \left(initial\ weight - water\ loss\ weight\right) / \left(initial\ weight\right) \times 100\% \quad (3)$$

The hardness value was tested with a GY-4 fruit hardness tester (Beijing Sunshine billion Star Technology Co., LTD). The soluble solids test was conducted according to a previously reported procedure (Wu et al., 2011). The middle part of freshly cut watermelon was selected, and 1–2 mL of juice was applied evenly on the Abbe refractometer (Shanghai Electronic physical Optical Instrument Co., LTD). The amount of titratable acid in fresh-cut watermelon was determined by titration with NaOH solution (Turhan et al., 2012). Determination of Vc in fresh-cut watermelon was via the 2,6-dichloroindophenol method (Tlili et al., 2011).

To determine the effectiveness of the antibacterial film in inhibiting decay of the fruit, the total number of mold and yeast colonies was determined with reference to published methods (Perdones et al., 2014).

2.5 Data processing

Each group of experiments was repeated three times, and the data obtained from the experiments were analyzed through ANOVA and Duncan's multiple comparisons using SPSS version 2.0. (Duncan's Multiple Range Test,P<0.05) (SPSS Inc., Chicago, IL, USA).

3 Results and discussion

3.1 Performance characterization of AG/CA antimicrobial film

Thickness analysis

The greater the dry matter content of the formed film, the greater the thickness of the film. As shown in Table 2, as the

content of Nisin increased, the thickness of the antimicrobial film also increased, from 0.065 ± 0.004mm to 0.103 ± 0.008 mm, which may be due to the better complexation of agar molecules with Nisin molecules and the repulsion between carrageenan and agar molecules, which prevents all molecules from being uniformly distributed in one plane, thus leading to the increased thickness of the film. In addition, the antibacterial films with Nisin addition were significantly different ($P < 0.05$) compared to those without Nisin addition, and the difference between adjacent gradients was not significant ($P > 0.05$), due to the relatively low amount of Nisin added (Table 2).

Bacteriostasis analysis

The spoilage of fresh-cut watermelon is mainly caused by the growth of microorganisms. The antibacterial film prepared in this study effectively inhibited the growth of *Staphylococcus aureus* and *Listeria monocytogenes*, the species of bacteria that are associated with watermlon spoilage. Nisin was the first commercially important bacteriocin (Zhang et al., 2018). It has been widely used in food preservation (Chandrasekar et al., 2017). As shown in Table 2, with the increase of Nisin content, the antimicrobial films showed significant inhibition effect on *S. aureus* ($P<0.05$) and *L. monocytogenes* over a large gradient range ($P<0.05$). The results show that the antimicrobial film can be used as antimicrobial active food packaging to extend the shelf life of food products. The results of our study are similar to the findings of Krivorotova et al. (2016), in which the anti-bacterial properties of Nisin-loaded pectin particles were documented. The antimicrobial film prepared by Bhatia & Bharti (2014) using 10.4% starch, 20% ethanol, and 4% glycerol co-blended with Nisin, lysozyme, and EDTA, effectively inhibited bacterial growth. The results were consistent with the selection of only one antimicrobial agent in the preparation of the composite film, demonstrating that Nisin has good compatibility with the film-forming substrate. It is also evident that the film-forming process maintains the antibacterial activity of Nisin (as expected in the original concept of the study), which was further investigated in the next step.

Mechanical property analysis

Tensile strength bears the larger nominal tensile stress that a packaging material undergoes before being pulled off, and elongation-at-break is the critical sign of the transition to locally concentrated plastic deformation, and determines packaging capability of the; this is usually related to the intermolecular forces and microstructure of the antimicrobial film (Atarés et al., 2010). As shown in Figure 1A, the antibacterial film prepared in this study has good mechanical properties. When the concentration of Nisin was 0.28%, the tensile strength was 23.08 ± 0.65 and 16.09 ± 1.73 MPa under the conditions of RH=20% and 90%, respectively, which is close to that of the composite film (tensile strength of 19.3 ± 1.1 MPa) prepared by Harnkarnsujarit & Li (2017). When the concentration of Nisin increased, the tensile strength of the antimicrobial film gradually decreased and showed significant differences ($P<0.05$) at RH=50% and RH=90%, indicating that the presence of Nisin changed the intermolecular interaction forces between the film-forming substrates of the film, causing the appearance of holes or cavities, which would affect the barrier properties.

The greater the relative humidity, the greater the effect on the mechanical properties of the antimicrobial film. The greater the humidity and the lower the intermolecular hydrogen bonding force, the greater the likelihood for the film fracture. Nisin also has an effect on the elongation-atbreak of the antimicrobial film; within a certain range, the change pattern of elongation-at-break coincides is the opposite of the trend in tensile strength. Although the presence of water molecules also affects the elongation-at-break, especially under high humidity conditions, the value of elongation-at-break of the antimicrobial film is lower. Therefore, with the increase of relative humidity, a swelling phenomenon is evident, in which the water molecules are continuously absorbed by the antimicrobial film, which leads to the flexibility of the molecules of the film-forming substrate. As a result, both the tensile strength and elastic modulus are reduced. Shiroodi et al. (2016) selected protein powders with protein quantity fractions of 93.4% and 90%. Biofilms were prepared using the protein powder and deionized water in a 10% (w/v) ratio, followed

Table 2. Thickness of antimicrobial film and width of antibacterial circle.

Nisin concentration/%		0.00	0.20	0.24	0.28	0.32	0.36
Thickness/mm		0.065 ± 0.004^{d}	0.083 ± 0.002 ^{c}	0.090 ± 0.007^{bc}	0.093 ± 0.004^{b}	0.097 ± 0.005^{ab}	0.103 ± 0.008^{a}
Inhibition circle width/mm	Staphylococcus aureus	0.000^{e}	1.533 ± 0.028^{d}	2.042 ± 0.037^{c}	3.326 ± 0.175^{b}	3.622 ± 0.106^{b}	4.133 ± 0.058^{a}
	Listeria monocytogenes	0.000^{d}	1.131 ± 0.081^{c}	1.195 ± 0.106^{c}	1.812 ± 0.157^{b}	1.913 ± 0.065^{b}	2.197 ± 0.087^{a}

Note: Lowercase letters a~e indicate significant differences, the same letters indicate insignificant differences, and different letters indicate significant differences.

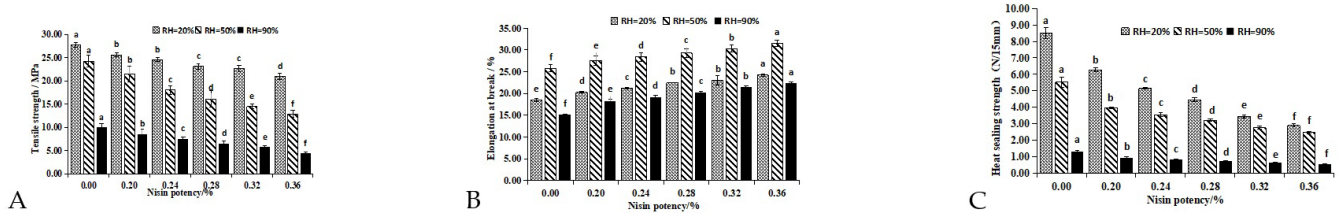

Figure 1. Effect of Nisin content against bacterial film mechanical properties under different relative humidity conditions. Note: (A) is the tensile strength map; (B) is the fracture elongation map; and (C) is the thermal seal strength map.

by the addition of glycerol in a 30:70 (w/w) ratio and 30 mg of Nisin. The resulting film was shown to have a significant inhibitory effect on *L. monocytogenes*; however, the addition of Nisin significantly reduced the mechanical properties of the biofilm, consistent with the results of our study. Under the same humidity conditions, there was a significant difference ($P<0.05$) in the heat-seal strength of the antimicrobial films with the increase in Nisin content, as shown in Figure 1C. When the Nisin content was the same, the relative humidity had a greater effect on the heat-seal strength of the films. When the Nisin content was 0.28%, the heat seal strengths were 4.45 ± 0.12 N/15 mm, 3.21 ± 0.08 N/15 mm, 0.74 ± 0.01 N/15 mm at different relative humidity conditions, respectively. The heat-seal strength of the antimicrobial films in this study was somewhat better than that of the carrageenan-based films studied by Farhan & Hani (2017).

Water vapor transmission analysis

The water vapor transmission rate of packaging materials directly affects the shelf life of the packaged food and is an important parameter for evaluating the performance of food films (Li et al., 2021). The moisture exchange between the packaging film and the food should be minimized as much as possible (Ciannamea et al., 2014). We found in the current study that, at low relative humidity, the moisture permeability of the antimicrobial films increased gradually and significantly ($P<0.05$) as the concentration of Nisin increased, indicating that there was a phase separation between the film-forming substrate and Nisin molecules, which indicates the reason for the decrease in mechanical properties, and also suggests that there might be an effect on the oxygen permeability coefficient. The moisture permeability of the films reached a maximum at RH=90%, indicating that the water molecules also had an effect on the moisture permeability of the films (Figure 2).

Oxygen permeability coefficient analysis

Oxygen in the packaging space can lead to various oxidation reactions in food products, which can result in odor generation, loss of nutritional value, and color changes; accordingly, control of oxygen is extremely important (Kerry & Tyuftin, 2017). The oxygen permeability coefficient is one of the important parameters in assessing the air permeability of antimicrobial films: the larger the value of this coefficient, the higher the air permeability. In the current study, we found a significant, positive correlation ($P<0.05$) between Nisin concentration and oxygen permeability coefficient of the antimicrobial films. At RH=50%, the oxygen permeability coefficient was 13.121 ± 0.127 $\times e^{-14}$ $cm^3 \cdot cm/cm^2 \cdot s \cdot pa$ at 0.28% Nisin. The oxygen permeability coefficient of the antimicrobial films gradually increased with increasing humidity, which explains why the mechanical properties and moisture permeability decreased with increasing Nisin concentration and relative humidity. This finding is in agreement with the results of the study by Ciannamea et al. (2018), in which 40% glycerol was added to gelatin films, and the oxygen permeability coefficient of the films tested at 50%, 70%, and 90% RH (Figure 3).

3.2 Analysis of antibacterial film for fresh-cut watermelon

Packaging design

Integrating the above results, we tested the performance of the antibacterial form with respect to the performance index, cost analysis, and application value. The concentration of Nisin in the film was 0.28%. Because of the relatively high moisture content of fresh-cut watermelon and relatively wet storage environment, the packaging design was selected for high humidity conditions. The aim of the packaging design is to balance the respiration of the fresh-cut fruit with the permeability of the antimicrobial film, thus preventing anaerobic damage or high oxygen concentration in the melon. In this way, the shelf life of the produce can be extended. Because the permeability coefficient of the film is low and cannot meet the suitable storage conditions of the fruit, the film in all treatment groups was perforated (hole diameter of 0.302 mm) (Figure 4; Table 3).

Weight loss rate

Fresh-cut watermelon often loses weight during storage due to respiration and loss of nutrients, and the loss of water causes changes in structural properties, due to the decrease

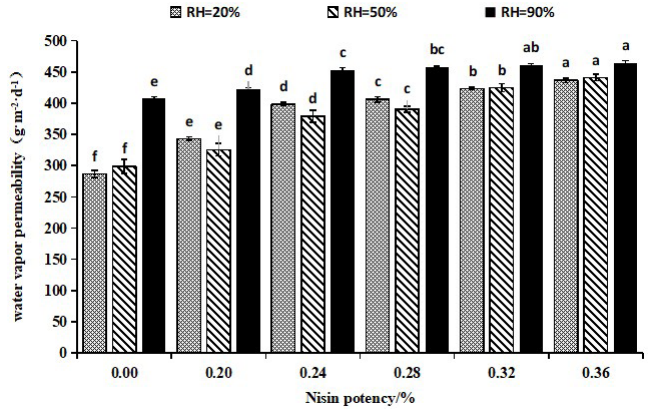

Figure 2. Effect of Nisin content against bacterial film permeability under different relative humidity conditions.

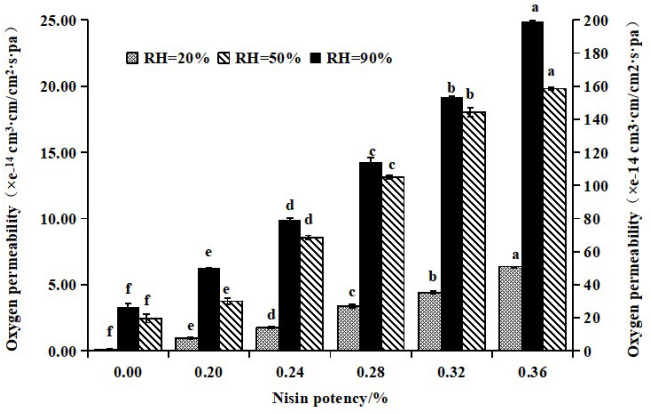

Figure 3. Effect of Nisin content against the bacterial film oxygen transmission coefficient under different relative humidity conditions. Note: RH = 20% and 50% refer to the primary ordinate axis, and RH = 90% refer to the secondary ordinate axis.

in cell expansion pressure. These changes result in reduced storage tolerance and disease resistance; therefore, it is of utmost importance to control water loss. As shown in Figure 5, the weight loss rate increased gradually with the extension of storage time. After 15 d at 4 °C, the weight loss rate was 5.37 ± 0.33% in the CK group, 3.22 ± 0.21% in the AG group, 2.40 ± 0.23% in the AG-N group, and 1.97 ± 0.32% in the PE group. After 5 d of storage at 20 °C, the weight loss rate was 10.02 ± 0.36% in the CK group, 5.54 ± 0.22% in the AG group, 2.03 ± 0.39% in the AG-N group, and 2.43 ± 0.31% in the PE group. In terms of weight loss, it is clear that the PE group performed better than the other groups mainly because the water molecules produced by the respiration of the melon pieces pass with difficulty through the PE preservation film, where they coalesce into water droplets or water mist on the surface of the PE bag; many of these droplets would then fall back to the surface of the fruit, with the result that the rate of weight loss is slowed but the growth of microorganisms is promoted. The weight loss rate of AG-N group was lower than that of AG group, most likely because Nisin in AG-N group had certain inhibitory function on microorganisms, which led to relatively slow loss of water and nutrients from watermelon. The large degree of water loss in the control group (CK) was expected, given that this film did not have any barrier properties. In addition, the weight loss rate of the fruit under low temperature conditions is lower than the corresponding values under the higher temperature treatment because water molecules are easily volatilized, and microorganisms multiply faster under high temperature conditions. When the weight loss rate of fresh-cut fruits and vegetables reaches 4% to 6% of the total weight, the produce begins to wrinkle and lose firmness and freshness (Karakurt & Huber, 2003). Taken together, these results demonstrate that the experimentally prepared antimicrobial film has a beneficial effect on preserving fruit freshness by slowing down the rate of weight loss.

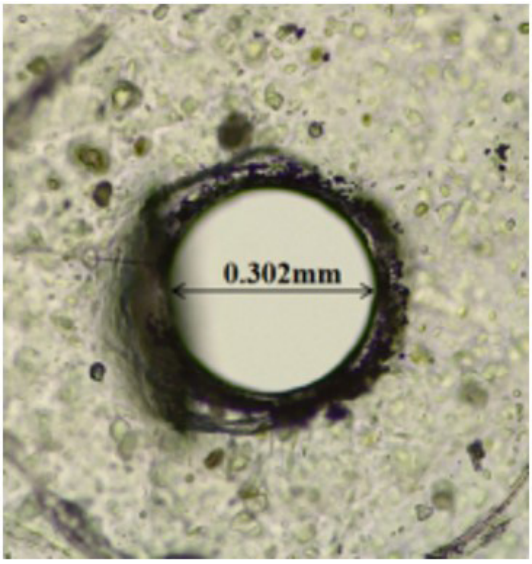

Figure 4. The pore of the antimicrobial film.

Growth of bacterial colonies

The spoilage of fresh-cut fruits and vegetables generally involves bacterial attack, leading to lesions, which then results in further infected by mold and other microorganisms. Therefore, the use of appropriate packaging can effectively reduce the breeding of bacteria, thus protecting the quality of fruit, extending the shelf life. As shown in Figure 6, the bacterial growth trend, as indicated by the total number of colonies, showed a "J" pattern under either storage treatment. Bacterial growth was significantly

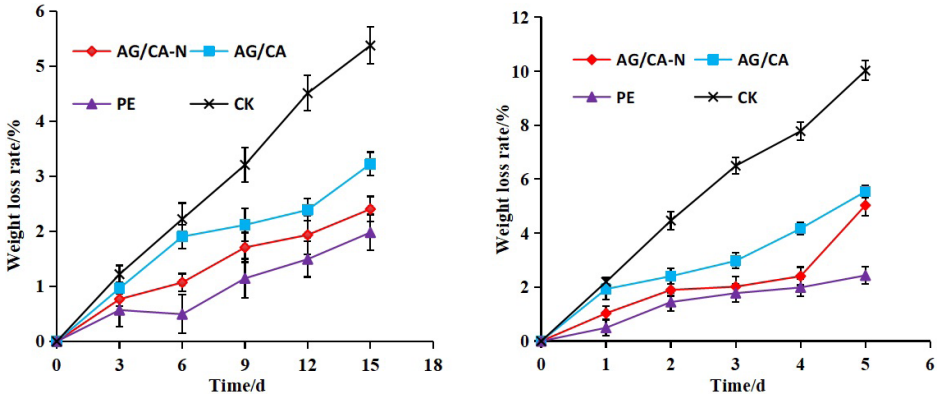

Figure 5. Effect of different packaging materials on the weight loss rate of freshly cut watermelon. Note: The left picture shows the preservation study under 4 °C, and the right picture shows the preservation study at 20 °C, the same below.

Table 3. Packaging method of fresh-cut watermelon.

Weight (g)	Storage temperature (°C)	Packaging film area (m^2)	Respiratory rate RO_2 ($mol \cdot kg^{-1} \cdot h^{-1}$)	Number of holes punched (pcs)
200	4	0.0095	0.0011012	8
	20		0.0023152	17

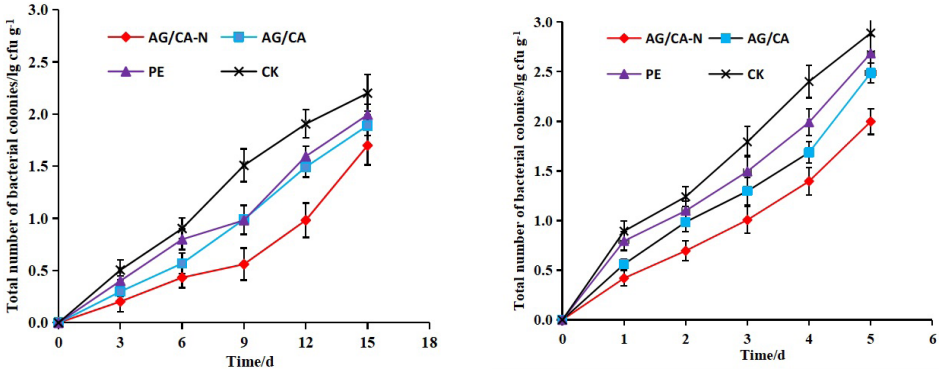

Figure 6. Effect of different packaging materials on the total number of freshly cut watermelon colonies.

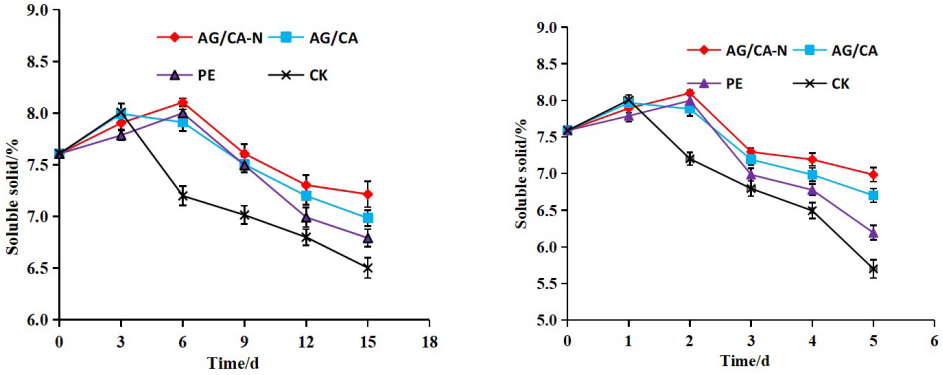

Figure 7. Effects of different packaging materials on the soluble solids of freshly cut watermelon.

lower inhe AG/CA-N group compared to the other groups. The Nisin antibacterial film is an effective barrier against the infestation of external microorganisms and also inhibit the growth of microorganisms inside the packaging environment. The total number of colonies in the AG/CA group was lower than that in the PE bag group, mainly because the PE bag group lacked antibacterial properties. In addition, the water vapor generated by respiration of the fruit could not be discharged in time but, instead, gathered on the surface of the PE bag and would be expected to fall back into the fruit pulp, which provided conditions for microbial growth. This would expain the results reported here. In the AG/CA-N film, the moisture generated by respiration flows smoothly to the external environment, and the AG/CA film can also "lock" some water vapor and prevent it from condensing into droplets, which can reduce the growth of microorganisms, thus extending the shelf life of the cut fruit to a certain extent.

Soluble solids

Soluble solids, which refers to the content of sugar, acids, vitamins and other substances soluble in water, is one of the important reference indicators for evaluating the maturity, internal quality, and edible processing characteristics of fresh-cut fruit. As can be seen from Figure 7, with the extension of storage time, the soluble solids content trended slightly upward and then decreased. At the beginning of storage, the flesh of fresh-cut watermelon would be expected to soften and ripen further, which would cause a small increase in soluble solids content. Due to the influence of respiration and microorganisms, the sugar, acid, vitamins, and other components in the flesh then begin to decrease, and nutrients are gradually lost, resulting in a decrease in content (Mao et al., 2006). This process may also affect the reduction of flesh hardness values. In the present study, the loss of soluble solids was less in the AG/CA-N film under different storage conditions, compared to other treatment groups. Evidently, the AG/CA-N film effectively inhibits the growth of microorganisms in fresh-cut watermelon, while maintaining a stable relative respiration rate through an appropriately selected package design that keeps the ambient gas inside the package at a suitable concentration.

Hardness

The quality of hardness is usually used to determine the measure of maturity of fresh-cut watermelon, which is one of the important indicators of quality and storability, and can also provide an important reference basis for developing storage, packaging and transportation of the produce. As can be seen in Figure 8, the reduction effect of controlling hardness under different storage conditions, the results were in the order of AG/CA-N > AG/CA > PE > CK group. The hardness values decreased gradually with the extension of storage time, and the decrease was greater under high rather than low temperature, which verified the above speculation.

Titratable acids

Titratable acid content (TAC) refers to the free state acid in fresh-cut watermelon and is an important factor affecting its pulp flavor and quality (Dvoracek et al., 2010). As can be seen in Figure 9, the titratable acid content in fresh-cut watermelon under different conditions gradually decreased with the extension of storage time, and TAC reached the lowest value at 15 d of storage at 4 °C: 0.10% in the CK group, 0.12% in PE, 0.14% in AG/CA, and 0.15% in the AG/CA-N group. The corresponding lowest values in the 5 day-20 °C treatment were 0.11%, 0.13%, 0.141%, and 0.146%. The loss of titratable acid was lowest in the AG/CA-N group; the addition of Nisin to the film effectively inhibited the growth of microorganisms and slowed the rate of oxidative decomposition of the fruit tissue and the consumption of titratable acid.

Vc content

Vc, a class of water-soluble vitamins in fresh-cut watermelon, can be oxidized through the enzymtic action of ascorbate peroxidase and ascorbate oxidase. As shown in Figure 10, the Vc content of the AG/CA-N group decreased the slowest among the four groups, followed by the AG/CA and PE groups. The CK group showed the most rapid loss in Vc content. Because the Vc within the fruit pieces is continuously consumed over time, mainly by microorganisms, Nisin combined with the appropriate package design can effectively protect the fresh-cut fruit by slowing down the decrease of Vc content. As well, the loss of Vc was lower under low temperature, indicating that low temperature can inhibit the activity of the above two enzymes.

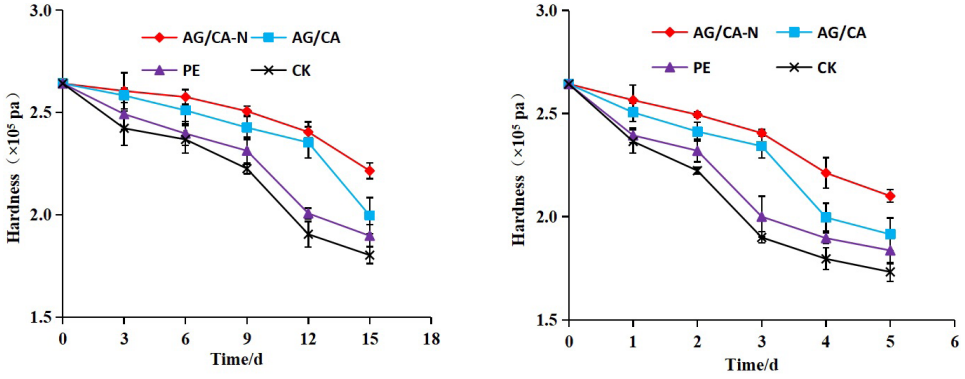

Figure 8. Effect of different packaging materials on the hardness of freshly cut watermelon.

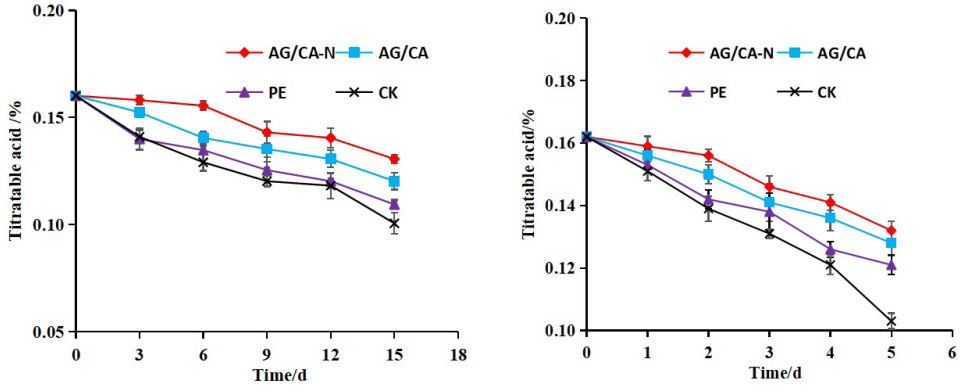

Figure 9. Effects of different packaging materials on titrating acid in freshly cut watermelon.

Figure 10. Effect of different packaging materials on the Vc content of freshly cut watermelon.

4 Conclusion

With the goal of producing an improved, environmentally friendly, antimicrobial packaging material to preserve fresh-cut fruit, agar and carrageenan were used as the main film-forming substrates, glycerol was used as a plasticizer, and Nisin was used as an antimicrobial agent. The performance indexes and the preservation effect of the film on fresh-cut watermelon were assessed. Based on the results of this comprehensive study, the following conclusions were drawn. AG/CA-N antimicrobial film effectively inhibits the growth of *Staphylococcus aureus* and *Listeria monocytogenes*, with inhibition circle width of Nisin at 0.28% is 3.326 ± 0.175 and 1.812 ± 0.157 mm, respectively. The level of relative humidity influence the mechanical properties and barrier performance of the antibacterial film. The antibacterial film with 0.28% Nisin was used for the optimal packaging design of fresh-cut watermelon to study its preservation effect. The results showed that the antibacterial film effectively inhibited the increase in colony number, decreased the rate of weight loss as well as the loss of soluble solids, hardness, Vc, and titratable acid. The improved film developed in this study can serve the purpose of extending the shelf life of fresh-cut watermelon.

In conclusion, the film raw materials used in the study are in line with food safety, such as agar and Nisin. The biodegradable antibacterial film developed and tested in this study has strong potential as an environmentally friendly food packaging material to be applied broadly to extend the shelf life of perishable food products—provided further research produces similar results for different types of fresh fruits and vegetables.

In terms of basic research on the underlying science of antimicrobial films, the migration mechanism of Nisin from both AG/CA-N film as well as the microstructure of antimicrobial film are topics worth investigating in the future.

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