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Effect of antibacterial nanocomposite film on the preservation of cheese

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Abstract

High density polyethylene (HDPE) and nano-ZnO were used to prepare nano-ZnO/HDPE composite film with a nano-ZnO content of 0.5wt%. The morphology, mechanical, barrier and antibacterial properties, as well as the preservation to cheese of the films were studied. The results showed that the ZnO nanoparticles had a good dispersion in HDPE matrix so that the improvement of the mechanical, barrier and antibacterial performances of the film was achieved after the addition of nano-ZnO to HDPE. In comparison to cheese packaged in HDPE bags, it was found that the sensory score of the cheese in nano-ZnO/HDPE bags increased from 66.6 to 73.7 and the pH of cheese was closer to the standard sample at storage time of 7d. Furthermore, nano-ZnO/HDPE inhibited effectively the increase of the total bacterial count (TBC) on cheese contrast of HDPE. That indicates the prepared nano-ZnO/HDPE is potential in cheese packaging to extend the shelf life.

Keywords: ZnO nanoparticles; cheese; antibacterial; packaging.

Practical Application: Antibacterial film with excellent mechanical and barrier properties based on HDPE and nano-ZnO for application of cheese products packaging.

1 Introduction

Cheese is a concentrated, fermented milk product that is produced by removing a large amount of water from milk and retaining the nutritional component. Its high protein content, however, makes it highly susceptible to bacterial attack and spoilage (Feeney et al., 2021; Woraratphoka et al., 2021). Currently, cheese preservation is mainly based on traditional refrigeration techniques, but this method requires equipment support and lacks flexibility (Appendini & Hotchkiss 2002; Quintavalla & Vicini, 2002).

In this regard, several attempts have been made to develop various physical and chemical preservation methods to reduce microbial contamination and improve the shelf life of these food products (Amjadi et al., 2019; Medeiros et al., 2014).

ZnO nanoparticles are inexpensive, naturally white, non-decomposable, non-discoloring and thermally stabl (Fang et al., 2006; Imazato, 2003). After Sawai et al. (1995) discovered in 1995 that ZnO powders have antibacterial properties against certain bacteria, an increasing number of scholars began to devote their research to the antibacterial properties of ZnO. Moreover, these ZnO nanoparticles are approved by the Food and Drug Administration (FDA) and generally recognized as safe (GRAS) (Noshirvani et al., 2017).

The preliminary work of this experiment found that the ZnO nanoparticles/polymer composites not only had impressive mechanical properties and UV absorption, but also their antibacterial properties were excellent (Li & Li, 2010; Li et al., 2015). In this study, a nano-ZnO/HDPE composite film with a nano-ZnO content of 0.5 wt % (Optimum concentrations of ZnO nanoparticles was determined according to our pre-tests to achieving the highest film properties without obvious aggregation of nanoparticles.) was used for packaging cheese and subsequently the preservation performance of the film on cheese was investigated. This was intending to expand the application of this film in food packaging, leading to a new technological approach being pursued for cheese preservation.

2 Materials and methods

2.1 Materials

High density polyethylene (HDPE) with Brand No. 5021d was provided from CNOOC and Shell Petrochemicals company Limited. Nano-ZnO (average particle size 50 nm) was purchased from Shanxi Wenxiyaoxing Zinc Products Factory in China. γ-aminopropyltriethoxy silane (KH550) as a surface modifier was brought from Nanjing Shuguang Chemical Factory. Whole fat reconstituted cheese was from Inner Mongolia Yili Industrial Group. The other reagents were obtained from Tianjin North Tianyi Chemical Reagent Factory.

2.2 Preparation of nano-ZnO/HDPE composite film

The nano-ZnO was first pretreated with KH550, subsequently, the composite film (nano ZnO content 0.5 wt %, thickness about 120 um) was generated using the melt blending method via a Haark mixing machine (Haark, Thremo Electro Instument, Germany), an extruder (Brabender Instument, Germany), a two rollers (SK-160B, Shanghai Rubber Machinery Factory, China),

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and a pressing machine (SL-45, Shanghai Rubber Machinery Factory, China). The preparation process is shown in Figure 1.

2.3 Characterization of nano-ZnO/HDPE composite films

Cross sections of nano-ZnO/HDPE composite films fractured in liquid nitrogen were observed with

a scanning electron microscope (SEM; JSM-6380, JEOL Inc., Japan) to observe morphology of the film.

An electronic universal testing machine (CMT4503, New Sans instrument, Shenzhen, China) was used to determine the mechanical properties of the nano-ZnO/HDPE composite films at a crosshead speed of 200 mm/min according to the standard of GB/T 16421-1996 and QB/T 1130-91. Six replicates were measured for each sample (Li et al., 2020; Li et al. 2021).

The moisture permeability of film was measured according to the standard of GB 1037-88. The oxygen permeability was determined by using a Gas Permeameter (GDP-C, Brugger Feinmechanik GmbH, Germany). Three replicates were measured for each sample.

The antibacterial activities against *Escherichia coli* (*E. coli*) were determined by plate counting (Li & Li, 2010). A 0.2 mL solution of bacteria (2.0–5.0 × 10^6 cell/mL) was added onto the film surface and incubated for 24h. Then the bacteria suspension washed off from the film surface was plated onto nutrient broth agar to observe the colony forming units (CFU) of bacteria.

2.4 Application of nano-ZnO/HDPE composite films in Cheese packaging

Cheese packaging

Approximately 5g of cheese was soaked in alcohol for 2 minutes. It was then transferred into bags (8cm × 8 cm) made of HDPE or nano-ZnO/HDPE films. The bags shown in Figure 2 were thereafter sealed and stored at 23 °C for 7 days before being tested for cheese quality. The cheese in original packaging stored at 5 °C was as the standard sample.

Sensory evaluation

The sensory evaluation was assessed by 20 semi-trained panelists (10 males and 10 females, 20-25 years old), which were not awarded of the experimental procedure. The cheese was scored according to Table 1 (Cattaneo et al., 2008), and the average score from twenty participants was taken as the final result (Ali et al., 2021; Delorme et al., 2021).

pH value measurement

An amount of cheese (1.5 g) was dispersed in deionized water (40 mL) with an ultrasonator (KQ-300DV, Kunshan, China) for 30 minutes. The pH value of the supernatant was measured with an acidity meter (PHS-25B, Dapu Instrument, Shanghai, China). Three replicates were measured for each sample.

Microbiological analysis

For assay the total bacterial count (TBC) in the samples, the cheese samples (2 g) were brought from the packaging bags with 50 ml of saline and homogenized using a water bath oscillator (HZS-H, Harbin Donglian, China) at 150 r/min for 15 min. The 1mL of diluted homogenates solution (1:10) were spread for the total bacterial counts by plate count agar and incubated at 37 °C for 24h (Sani et al., 2017).

3 Results and discussion

3.1 Morphology of nano-ZnO/HDPE composite film

Figure 3 shows that after adding nano-ZnO (0.5 wt %) to HDPE, the ZnO particles were uniformly dispersed in matrix at the nanometer level with no large-area agglomeration.

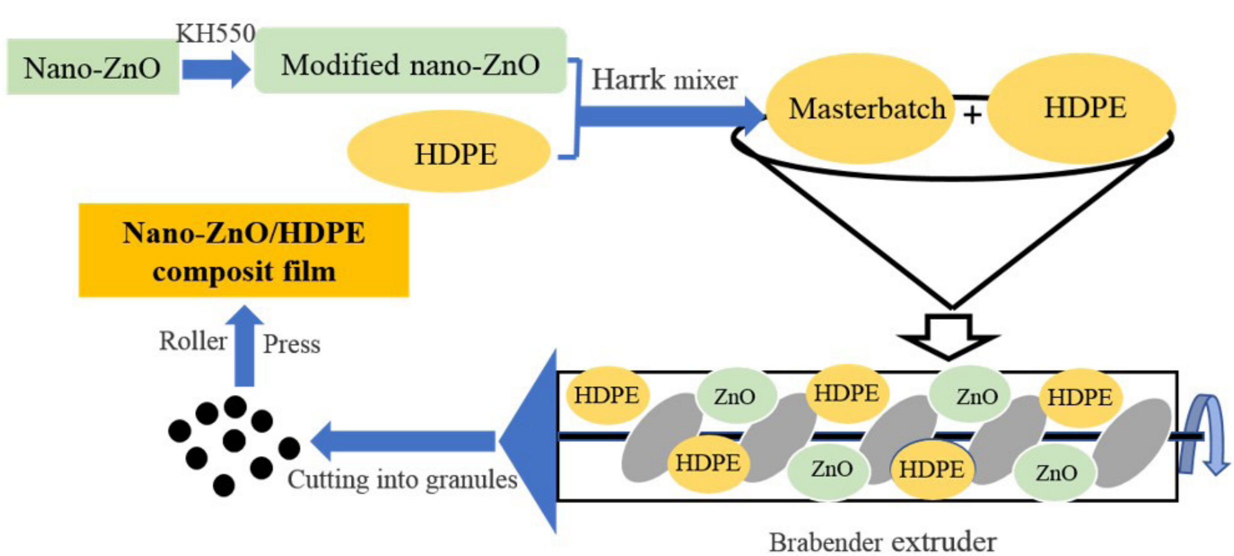

Figure 1. Preparation of nano-ZnO/HDPE composites.

Table 1. Quality evaluation of cheese.

Rating criteria	Rating range	
	0 points	20 points
Smell and taste	Taste musty, sour, bitter and other undesirable odors	Tastes cheesy with a characteristic flavor, no unpleasant smells
Color and appearance	Visible variation in appearance, uneven or indistinct colors	White or pale yellow in color
Histological architecture	Loose condition with cracks in the outer skin and sandy sections	Uniform and consistent texture, moderate softness, moist, fine condition
Elasticity and hardness	Too small or too large, broken after squeezing and cannot be recover	Good elasticity, does not break after squeezing and can be recovered
Melting and stringy	Poor melting properties, more grease precipitation, not stringy	Good melting fluidity, no obvious grease precipitation and stringy

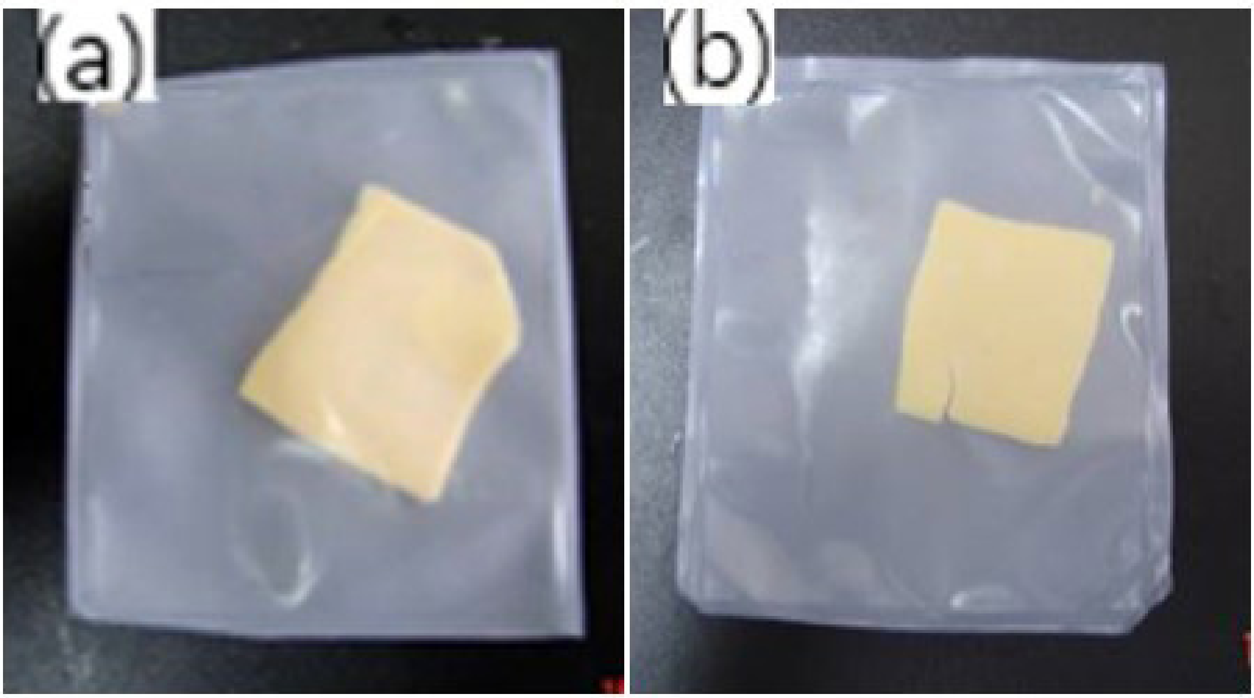

Figure 2. Cheese packaging, (a) HDPE film; (b) nano-ZnO/HDPE composite film.

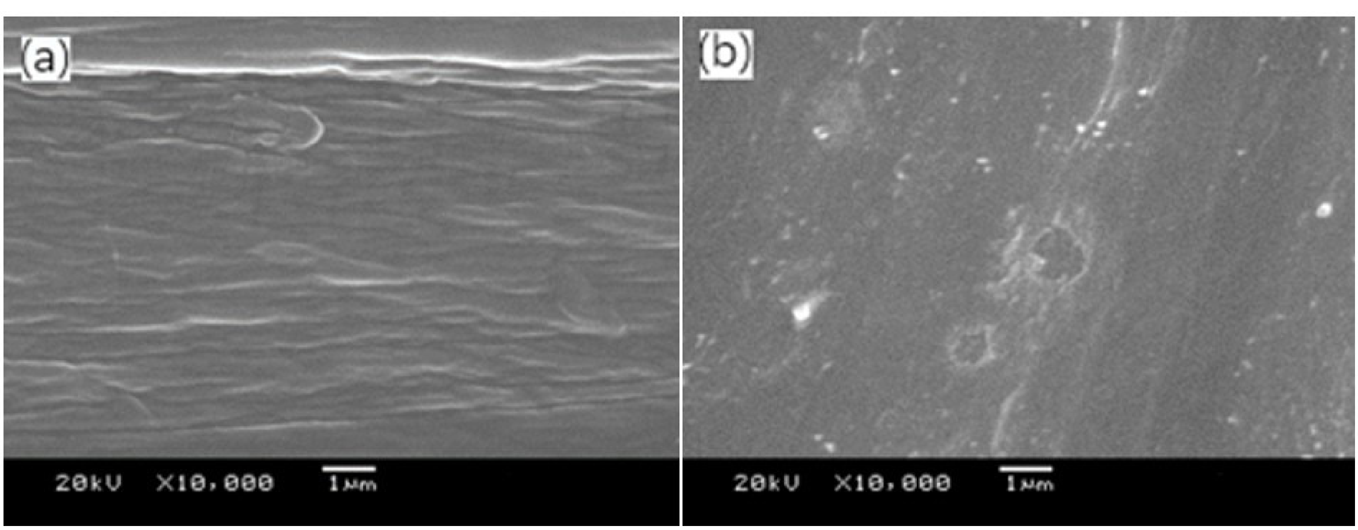

Figure 3. Cross-section morphology of HDPE (a) and nano-ZnO/HDPE composite film (b).

3.2 Mechanical and barrier properties of nano-ZnO/HDPE composite film

In Table 2, it can be deciphered that the mechanical properties and barrier properties of the nano-ZnO/HDPE composite film were improved when the nano-ZnO content was only at 0.5%. This was due to the fact that when a small amount of nano-ZnO was added, the nano-ZnO was better dispersed in the matrix. The small particle size and large specific surface area of the nanoparticles made it easier for strong interfacial interactions with the HDPE matrix. This effectively improved the fracture work required for crack extension and stop crack growth. In addition, as rigid particles, inorganic nanoparticles had the balancing effect of stress concentration and stress radiation. By absorbing the impact and radiation energy levels, the polymer had no obvious stress concentration phenomenon and achieved the mechanical equilibrium state of the composite material (Li & Li, 2010). Liu & Jia (2011) reported that the tensile strength was the highest when nano-ZnO amount was 3% under the two-step, the tensile strength was increased up to about 22%, and the fracture elongation rate was increased up to about 11%. Chen et al. (Chen et al., 2021) reported that with the addition of 1.0 wt% Cu-MOF, the tensile strength and elongation at break are increased to 45.7 MPa and 2.4%, respectively. On increasing the amount of Cu-MOF, the mechanical properties of the CA/Cu-MOF nanocomposite are further improved. In addition, the improved barrier performance of the nanocomposite film may have been due to the refinement of the HDPE grains because of the addition of ZnO nanoparticles (Li et al., 2015).

3.3 Antibacterial acitivity of nano-ZnO/HDPE composite film

Figure 4 shows the colonies recovered on the films incubating with *E. coli* for 24 h。It was found that CFU amount of *E. coli* was dramatically reduced for nano-ZnO/HDPE composite films in contrast with the HDPE films as control from Figure 4. That reveals that the addition of ZnO to HDPE endows favorable antibacterial activity of nano-ZnO/HDPE composite film. Research affirms (Jahed et al., 2017; Li & Li, 2010) that nano-ZnO can produce antimicrobial properties through both zinc ion dissolution and photocatalytic generation of reactive oxygen species.

3.4 Sensory evaluation of packaged cheese

The sensory scores of cheese stored at 7d were shown in Table 3. It can be seen that the total sensory scores of the cheeses packed with nano-ZnO/HDPE film were higher than those of the cheeses packed with HDPE film. That indicated that the nano-ZnO/HDPE composite film had a effectively effect on the freshness of the cheeses. The sensory properties of the cheeses were notably improved after being packaged and stored for

Table 2. Properties of nano-ZnO/HDPE composite films.

	Mechanical properties			Barrier properties	
Nano-ZnO/%	Tensile strength /MPa	Elongation at break /%	Tear strength /MPa	Oxygen permeability coefficient $\times10^{-14}$/(cm^3.cm/cm^2.s.Pa)	Moisture permeability coefficient $\times10^{-13}$ /(g.cm/cm^2.s.Pa)
0	20.01 ± 1.2	746.63 ± 7.2	151.36 ± 10.1	8.80 ± 0.7	6.32 ± 0.2
0.5	37.50 ± 2.3	854.67 ± 5.6	160.16 ± 12.2	8.08 ± 0.8	5.29 ± 0.5

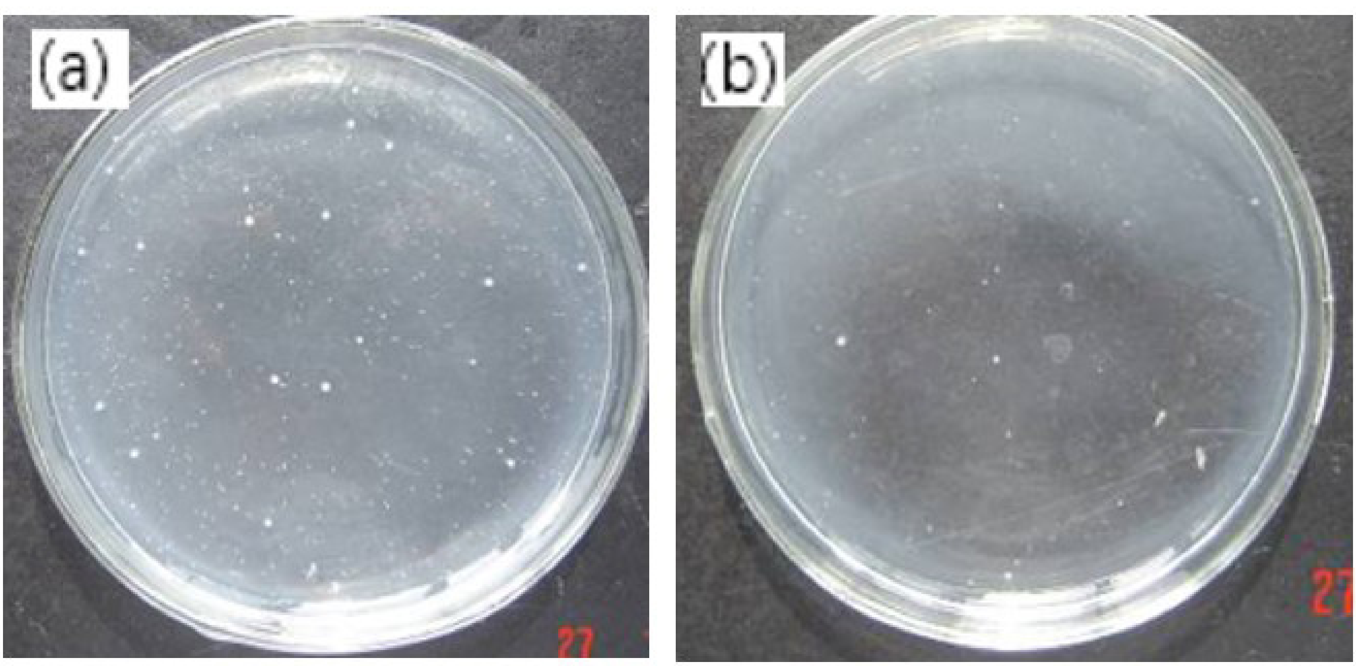

Figure 4. CFU of *E. coli* on the HDPE (a) and nano-ZnO/HDPE composite films (b).

Table 3. Cheese quality on storage of 7d.

samples	sensory scores	pH	TBC($\times 10^4$CFU/mL)
Standard	81.5 ± 2.3	6.212 ± 0.1	0 ± 0.1
HDPE	66.6 ± 6.2	6.140 ± 0.1	8.2 ± 0.1
nano-ZnO/HDPE	73.7 ± 3.4	6.167 ± 0.1	3.2 ± 0.1

several days, in comparison to the film without the addition of ZnO nanoparticles.

3.5 pH value of packaged cheese

Table 3 presents the pH of cheese samples on the storage time of 7d. In general, the pH of cheese decreases slightly at the beginning of spoilage, which might be attributed to the production of CO_2 by microorganisms due to degradation of lactate and decarboxylation of amino acids at the cheese surface (Youssef et al., 2015, 2018). Moreover, reduction of the cheese pH reveals the presence of lipolysis, which is unfit for consumers (Singh et al., 2018).

As shown in Table 3, when the cheese was packed with the nano-ZnO/HDPE film and left for 7 days, the pH of the cheese decreased relative to that of the standard cheese. Nevertheless, the decrease was less than that of the cheese wrapped in the pure HDPE film. This indicated that the nano-ZnO/HDPE composite film was more effective in preserving the cheese than the pure HDPE film.

3.6 Microbial growth of packaged cheeses

Table 3 illustrates that the packaged cheese was contaminated with microorganisms after being stored for 7 days, comparing with the standard sample. Furthermore, the bacteria for cheese packaged in the nano-ZnO/HDPE composite film was 3.2×10^4 CFU/mL, significantly less than that of the HDPE film (8.2×10^4 CFU/mL) attributed to the antibacterial property of nano-ZnO/HDPE. That suggests that the nano-ZnO/HDPE composite film had a preservative effect on cheese.

4 Conclusion

The nano-ZnO/HDPE composite film with a nano ZnO content of 0.5% was prepared by the melt blending method. The ZnO particles were uniformly dispersed in HDPE matrix. The mechanical, barrier and antibacterial properties of the composite film was dramatically improved compared to the HDPE film. The decrease of sensory and pH of cheese packaged with nano-ZnO/HDPE was slower than that packaged with HDPE. The nano-ZnO/HDPE composite film also inhibited effectively the microbial growth of the cheese stored at 7d. These results indicate that the prepared nano-ZnO/HDPE composite film has potential application in preservative packaging for prolong the shelf life of cheese.

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