**Zein/chitosan Janus film incorporated with tannic acid and cinnamon essential oil co-loaded Pickering emulsion for sustained controlled release and pork preservation**

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**Abstract**

The development of active packaging offers a promising approach to reducing food waste. However, challenges remain, particularly in achieving efficient release dynamics of active compounds and balancing the barrier properties. Herein, a Janus structure zein/chitosan film is custom designed by layer-by-layer casting method to achieve sustainable and unidirectional release performance of antimicrobial agent, which comprises an inner loading layer of tannic acid (TA) and cinnamon essential oil (CEO) co-loaded Pickering emulsion incorporated with chitosan and an outer barrier layer of zein. The good interfacial compatibility between the entities of Pickering emulsion/chitosan loading layer and zein barrier layer had be confirmed via physicochemical structure characterization. The lower swelling rate of Pickering emulsion/chitosan film (47.61% ~ 51.71%) indicated the sustained and stable release rate of substances from the inner loading layer, while the zein barrier layer restricted the diffusion of active molecules due to the high swelling rate (162.52%). In addition, the films showed excellent antimicrobial activity (> 99% against key foodborne pathogens) and radical scavenging activity (2.5-fold enhancement). Moreover, the film loading layer showed predominantly controlled by a quasi-Fickian diffusion, and prolonged the shelf life of pork by 6 days under the unidirectional sustained release. Our work presents a promising fabrication strategy of antimicrobial packaging film with sustainable release performance for food preservation.

**Keywords**: antimicrobial packaging; Pickering emulsions; sustained release properties; food preservation

**1. Introduction**

Annually, approximately 1.3 billion tons of food are wasted or lost at various stages of the supply chain, especially manifest in perishable items such as fruits, vegetables and meat. This phenomenon has raised widespread concerns about the food safety and environmental sustainability[1, 2]. Hence, it is imperative to adopt resolute measures in enhancing global endeavors aimed at mitigating food waste [3, 4]. Package plays a crucial role in safeguarding food items, shielding them from potential damage and effectively prolonging their shelf life. Conventional packaging made from petroleum-based plastics has presented significant challenges in terms of resource consumption and environmental pollution, which persist throughout the entire life cycle of petroleum-based packaging materials [5, 6]. Currently, researcher engaged in active investigation on modern packaging technologies that exhibit eco-environmental attributes and possess the potential to enhance the shelf life of food [3, 7-10].

Recent studies have shown that cinnamon essential oil (CEO), natural substances extracted from the bark or leaves of the *Cinnamomum zeylanicum*, has utilized as an antimicrobial agent due to its significant inhibitory effect on foodborne microorganisms and inherent naturalness, which has great development in food active packaging[11, 12]. The chemical composition of CEO comprises over 20 compounds, with cinnamaldehyde being the most predominant (>70%), other constituents include eugenol, cinnamyl acetate, and α-pinene[13, 14]. The antimicrobial activity of CEO is attributed to the strong interact between hydrophobic molecules mentioned above and the microbial cell membrane. Antimicrobial packaging films containing CEO have been successfully fabricated and employed in reducing the waste of food items[14]. However, current packaging films containing CEO face challenges related to inefficient release dynamics, involves three stages: (a) the hydrophobicity of CEO renders it incompatible with bio-polymeric materials and the thermal sensitivity of CEO results in the inactivation of components during the film-forming process[11]; (b) blending CEO in bio-polymeric materials directly may result in a reduction of availability within the packaging cause of ‘burst effect’[15]; (c) the CEO is released along both sides of packaging film, nearly half of the CEO was released into the surroundings[16]. It is crucial to explore ways to develop a packaging film that solves both issues at once.

Pickering emulsion is a promising method to encapsulate and deliver bioactive compounds. It can maximize the retention of essential oils during the film-forming process through the barrier effect, and control the release of bioactive compounds [17, 18]. Additionally, Pickering emulsion is regarded as safer than conventional emulsion because the elimination in molecular surfactants. In our previous studies, we developed a CEO loaded Pickering emulsion enabled by zein-TA hybrid particles [21], which had regulated for good physical stability and enhanced antimicrobial activity against spoilage bacteria. However, whether this TA and CEO co-loaded Pickering emulsion strategy is suitable for food active packaging has not been confirmed.

In addition, to further achieve sustained release of CEO from packaging film, it is an ideal method to design the structure of films. A Janus structure based on multilayer film crosslinking, involves creating a bifunctional material with distinct properties on each side of the film, has been proposed in some studies due to their asymmetric structure and multifunctional properties [22, 23]. Natural polymeric materials (i.e., starch, carrageenan, pectin, gelatine, zein and so on) are potential alternatives for the production of packaging materials owing to their excellent film-forming properties and environmentally friendly[19]. In which, chitosan (CS) is one of the most renewable polymers in nature, mainly extracted from crustaceans. CS is a positively charged polyelectrolyte in solution, which is suitable for forming a loading layer with negatively charged TA and CEO co-loaded Pickering emulsion via electrostatic interactions. Zein is a prolamin found in corn gluten meal waste. Compared with CS, zein exhibits batter water and gas barrier properties, thereby presenting an opportunity to preventing the excessive diffusion of CEO. It is hypothesized that enhanced physical stability of Pickering emulsion and tightly cross-linking of chitosan with zein are benefit for sustained release of CEO, and Janus structure multilayer can maximize the utilization efficiency of CEO in antimicrobial package. Moreover, the proposed film is composed of biodegradable and renewable materials, including CS and zein, which enhance its environmentally friendly properties.

In this work, a Janus structure multilayer antimicrobial packaging film is custom designed by layer-by-layer casting method to achieve an improvement in antimicrobial properties of films. The TA and CEO co-loaded Pickering emulsion incorporated with CS served as inner loading layer of multilayer film, while zein film functioned as the outer barrier layer. We comprehensively assessed the structural, mechanical, barrier, and functional properties of developed films, meanwhile, the sustainable release properties and release behavior were researched in detail. Furthermore, the potential of result films as high-performance antimicrobial film was investigated in preserving meat. This work provides an effective strategy for antimicrobial packaging through the controlled release of cinnamon essential oil, aiming to improve food preservation.

**2. Materials and methods**

**2.1. Materials**

Zein (CAS#9010-66-6, from corn) and trichloroacetic acid (TCA) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). CS (CAS#9012-76-4, deacetylation degree > 90% viscosity 0.2 pa s), gelatine (CAS#9000-70-8, type A, bloom value of 300), and glycerol were procured from Macklin Bio-Tech Ltd. (Shanghai, China). TA was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). CEO was purchased from Jiangxi Taicheng natural perfume Co., Ltd. (Ji’an, China). Plate count agar, yeast extract, and tryptone were procured from Beijing Land Bridge Technology Co., Ltd (Beijing, China). Other reagents (analytically pure) were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). *Pseudomonas paralactis* MN10, *Acinetobacter pullicarnis* MN21, and *Lactobacillus sakei* VMR17 were kindly supplied by Prof. Zhang’s lab (Institute of Food Science and Technology, Beijing, China).

**2.2. Preparation of** **co-loaded TA and CEO Pickering emulsion**

The co-loaded TA and CEO Pickering emulsion was prepared according our previous procedure[20] . Briefly, zein (10 mg/mL) and TA (2 mg/mL) were dissolved in 70% (v/v) ethanol in water to form the zein and TA hybrid particle dispersion solution. Subsequently, CEO was added dropwise to the zein and TA hybrid particle dispersion solution at a ratio of 1:9 (v/v) and sheared with a high shear homogenizing emulsifier (HR-500, Shanghai Huxi Industry Co., Ltd, China) at 8000 rpm for 2 min. Finally, the co-loaded TA and CEO Pickering emulsion was obtained by adding an equal volume of ultrapure water to the CEO-zein and TA hybrid particle dispersion solution.

**2.3. Fabrication of the zein/chitosan films with Janus structure (ZCPE) films**

The ZCPE films were prepared with layer-by-layer assembly. Briefly, 5 wt% zein solution was prepared by dispersing zein power in 70% (v/v) acetic acid in water. Then, 0.3 wt% gelatine and 30% (w/w, based on the zein powder mass) were added to above zein solution to form the Z layer film forming solution. After stirring, the above solution (10 mL) was cast into a 90 mm diameter plastic plate and dried at 50℃ until a sticky film had formed (Z layer).

CS (1.0 g), gelatine (0.3 g), and glycerol (0.3 g) were mixed in 100 mL of 2% (v/v) acetic acid in water. Different concentrations of zein and TA hybrid particle stabilized Pickering emulsion (0%, 3%, 6%, and 9%, v/v) were added to the mixture and designed as CPEX film-forming solutions, where X is the concentration of the co-loaded TA and CEO Pickering emulsion. Twenty milliliters of CPEX film-forming solution were poured onto the Z layer and then dried 50℃ for 12 h. The prepared bilayer films were named as ZCPE0, ZCPE3, ZCPE6, and ZCPE9. Before further measurement, all films were conditioned at 55% relative humidity for 72 h.

**2.4. Characterization of the ZCPE films**

A detailed description of the characterization methods of ZCPE films in morphology, structure, thermal stability, optical properties, mechanical properties, water content, swelling, solubility and barrier properties were reported in the supporting information.

**2.5. Antimicrobial activity of the ZCPE films**

The antimicrobial activity of the films against *P. paralactis* MN10, *A. pullicarnis* MN21, and *L. sakei* VMR17 was evaluated based on the method described by literature[21]. In brief, the films was cut into 20 × 20 mm squares and placed in 6-well plates. One hundred microliters of bacterial solution (approximately 106 colony-forming units [CFU]/mL) were added to the loading side of the films and cultured at 30℃ for 2 h. Then, 9.9 mL of lysogeny broth (LB) medium was added to each well, followed by further culturing under the same conditions. One hundred microliters of the bacterial suspension were spread uniformly on an LB agar plate and uniformly and incubated at 30 ℃ for 24 h. Sterile filter paper was used as the control group. The antimicrobial ratio was calculated with the following equation:

Antimicrobial ratio (%) = ,

where S1 and S2 are the bacterial colony counting for the control and film groups, respectively.

**2.6. Antioxidant activity of ZCPE films**

The antioxidant activity of films was determined by DPPH (BC4755, Solarbio) and ABTS (BC4775, Solarbio) radical scavenging activity. The measurement and calculation process were carried out in strict accordance with the manufacturer’s instructions.

**2.7. Release behavior of the ZCPE films**

The release behavior of the films was monitored according to the previous studies[16] with slight modifications. ZCPE9 films were cut into 20 × 20 mm squares and securely fixed within a connector. Phosphate-buffered solution (PBS, pH 7.0) was added to both sides of the connector, ensuring that only the portion of the film exposed through the connector cavity was in direct contact with the PBS. This setup allowed independent monitoring of the release behavior from the Z layer and the CPE9 layer of the ZCPE9 film. The tests were conducted under constant temperature (4°C) and humidity conditions (50% relative humidity). At specific time intervals, 200 μL of PBS was withdrawn, and its absorbance at 287 nm was measured using a spectrophotometer. The withdrawn PBS was replaced with fresh PBS to maintain consistent test conditions. The cumulative release of CEO was calculated, and a CEO release kinetic curve was constructed based on these measurements.

The release mechanism of the ZCPE films was evaluated by using Korsmeyer-Peppas model [22], which is expressed as:

Mt/M∞=k tn,

where Mt/M∞ represents the fraction of CEO released at given time t, k is release velocity constant, and n is the diffusional exponent, which indicates the type of release mechanism.

**2.8. Application of the ZCPE films for meat preservation**

Fresh chilled meat was purchased from a local supermarket (Haidian, Beijing, China). The meat was cut into 15.0±0.5 g pieces and wrapped with a commercial polyethylene (PE) film, the ZCPE0 film, or the ZCPE9 film, unwrapped meat served as the control group. All samples were processed in a sterile environment and stored at 4.0 ± 0.5℃ for 15 days.

2.8.1. Color

The color parameters of meat were monitored using a CM-600d colorimeter (Konica Minolta, Tokyo, Japan). L\*, a\*, and b\* were recorded at three different locations on the surface of meat.

2.8.2. Total viable count (TVC)

The TVC of meat was measured according to literature with a slight modification[23]. Briefly, 10.0 g ± 0.5 g of meat was placed in 90 mL of sterile saline (0.9% [v/v] NaCl) solution in aseptic bags. It was subjected to extraction with a JN-400i sterile equaliser mixer (Ningbo Jiangnan Instrument Factory, Ningbo, China). The extract was diluted 10-fold with sterile 0.9% (v/v) NaCl solution. Finally, 100 μL of the diluted extract was spread on plate count agar and incubated at 37 °C for 48 h.

2.8.3. Total volatile basic nitrogen (TVB-N)

The TVB-N of meat was measured by using an auto-Kjeldahl device (K9840, Hanon Instruments, Jinan, China). Before measurements, 6.0 ± 0.2 g of meat was added to 30 mL of 2% (w/v) TCA and homogenised. Ten milliliters of the meat-TCA mixture supernatant and 5 mL of 1% (w/v) magnesium oxide solution were added to the distillation tube. After distillation, the receiving bottle of liquid was titrated using 0.01 M HCl. The TVB-N of meat was calculated using the equation:

TVB-N (mg/100 g) =,

where V1 is the volume of consumed 0.01 M HCl solution (mL), V2 is the volume of consumed 0.01 M HCl solution (mL) in the blank group, m is the weight of meat (g), V is the volume of the used filter (mL), and V0 is the total volume of filtrate (mL).

**2.9. Statistical analysis**

The experiments were performed in triplicate with individually prepared films. The results are expressed as the mean ± standard deviation. The data were analyzed using SPSS Statistics 26.0 (IBM Corp., Armonk, New York, USA). Analysis of variance (ANOVA) followed by Duncan’s multiple tests was used to evaluate the significance of the differences in means. *P* < 0.05 was considered to be statistically significant.

**3. Results and discussion**

**3.1. Fabrication and characterization of the ZCPE films**

We fabricated the ZCPE films by using LBL assembly. First, we cast the zein based film forming solution to form the first layer. We uniformly incorporated the co-loaded TA and CEO Pickering emulsion into CS to form the loading layer film forming solution. When we cast above mixture on the first layer, there was weak adhesion due to differences in hydrophobicity between zein and the CS emulsion mixture. To solve this problem, we introduced gelatine into the zein solution and the CS-emulsion mixture as an “adhesive”. The zein–gelatine film formed the Z layer and the CS–gelatine–emulsion film formed the loading layer. Finally, we prepared the ZCPE films by casting the precursor solution of the loading layer onto the Z layer. As anticipated, these two layers showed satisfactory interfacial compatibility.

3.1.1. Morphology of the ZCPE films

We investigated the microstructure of the ZCPE films by using a scanning electron microscope. As presented in Fig. 1A, the surface of Z layer appeared smooth for ZCPE9 and other ZCPE films. However, compared with the ZCPE0 film, the surface of the loading layer of the ZCPE9 was rough, perhaps due to the aggregation of co-loaded TA and CEO Pickering emulsion caused by drying stress during the film formation.

The similar aggregation was observed in sodium alginate films containing ginger essential oil-loaded Pickering emulsion[24]. The ZCPE0 and ZCPE9 film cross-sections showed the seamless combination of the Z and loading layers, reflecting the good interfacial compatibility between these two layers. Additionally, we noted porous in the ZCPE9 loading layer, primarily related to CEO evaporation during film formation.

3.1.2. Structure and thermal properties of the ZCPE films

The intermolecular interactions between co-loaded TA and CEO Pickering emulsion embedded in the loading layer was evaluated by using FT-IR spectry. As presented in Fig.1B1, the broad peaks in the range of 3300-3000 cm-1 are attributable to the O-H and N-H stretching vibrations[25]. The characteristic band was shifted from 3230 to 3236 cm-1 as the amount of co-loaded TA and CEO Pickering emulsion increased, implying weaker hydrogen bonds between co-loaded TA and CEO Pickering emulsion and CS. In addition, the intermolecular interactions of the layer to layer were also characterized (Fig. 1B2). Compared with the CPEX layer, the characteristic peak at 3300-3000 cm-1 showed a significant shifted when combined with the Z layer. These results indicate the strong hydrogen bonding between the loading layer and Z layers, providing compelling evidence of the interfacial compatibility of the ZCPE films. Additionally, the others distinctive peaks were observed at 2900-2800 cm-1, near 1700 cm-1, and near 1550 cm-1, belonging to the stretching of =C-H, amide Ⅰ, and amide Ⅱ, respectively. Although there were some degrees of strengthening, weakening, and shifting in the ZCPE films, these changes only involved non-covalent interactions, such as hydrogen bonds and electrostatic interaction, demonstrating the fabrication process did not significantly change the chemical structure of these films. After assessing the interactions present in the ZCPE films, we investigated the crystal structure using XRD. As shown in Fig. 1C1, we recorded two typical characteristic peaks at 2θ of approximately 9° and 20.0°, assigned to hydrated crystal and regular lattice, respectively. We observed these peaks for all single CPEX layers, suggesting the crystal structure of the loading layer was maintained after incorporating of co-loaded TA and CEO Pickering emulsion. However, the intensity of the corresponding peak at 2θ around 20.0° was decreased as the concentration of co-loaded TA and CEO Pickering emulsion increased, this phenomenon might be attributed to hydrogen bonding between the emulsion and CS. We also investigated the crystallinity of the ZCPE films from the side of Z layer (Fig. 1C2). Notably, the main XRD peaks from this side are wider and weaker than the single CPEX layer, implying that the crystal structure tends to be amorphous due to the strong interaction between the Z and CPEX layer. Generally, materials with lower crystallinity show good elongation in mechanical properties[26], which we confirmed in section 3.1.4.

The thermal stability is critical for potential secondary processing and overall material stability of films, which could be assessed by thermogravimetric analysis. The temperature-dependent thermogravimetric and corresponding differential thermogravimetric curves can be observed in Fig.1D1 and 1D2, respectively. The weight of the ZCPE films decreased as the temperature increased, with three distinct steps. The first stage appeared at 80-120°C, which could be attributed to the evaporation of water and volatile substances in the films. In this stage, all ZCPE films behaved similarly. The second stage occurred from 120 to 250°C; it may be related to the degradation of CS and glycerol. The third stage was at 250-380°C, corresponding to the decomposition of the polymeric backbone alongside carbon. The films containing co-loaded TA and CEO Pickering emulsion had a higher degradation temperature, indicating an improvement in their thermal stability. This result could be explained by the interaction and the higher compatibility between the film components. Collectively, these results imply enhanced thermal stability of the ZCPE films due to the addition of co-loaded TA and CEO Pickering emulsion.



**Fig. 1** (A) SEM images of ZCPE films, including surface and cross-sectional of films. (B1, B2) FTIR spectra of loading layer and ZCPE films. (C1, C2) XRD patterns of ZCPE films, measured from loading layer and Z layer, respectively. (D1) TG curves and (D2) DTG curves of ZCPE films.

3.1.3. Optical properties of the ZCPE films

The color parameter of the ZCPE films is summarized in Table 1. L\* decreased significantly with the addition of co-loaded TA and CEO Pickering emulsion (*P* < 0.05). a\* and b\* increased from 0.58 to 6.63 and from 40.03 to 56.62, respectively. As the content of co-loaded TA and CEO Pickering emulsion increased, the color saturation deepened. We measured the visual chromatic aberration as ΔE, which increased. The higher ΔE and the lower WI of the ZCPE films are consistent with the appearance of the ZCPE films. To clarify the optical properties of the films, we measured their UV–Vis transmittance spectra (Fig. 2A). Light transmittance decreased gradually as the amount of co-loaded TA and CEO Pickering emulsion increased, this phenomenon that could be attributed to the abundant unsaturated chromophile bonds in CEO. In particular, the ZCPE films blocked almost 100% of UV light (200–400 nm). The opacity of the films (Table 1) correlated positively with the emulsion content. Compared with the ZCPE0 film, the opacity of the ZCPE9 film increased by 73.31%. These results indicate an enhanced UV barrier capability of the ZCPE films.

3.1.4. Mechanical properties of the ZCPE films

We determined the TS and EAB to evaluate the mechanical properties of the ZCPE films (Fig. 2B). The TS and EAB of the ZCPE0 films were 21.32 MPa and 3.34%, respectively. After loading the emulsion, the TS of the ZCPE9 film decreased slightly to 19.05 MPa, which might be due to the fact that emulsion droplets disturbed the continuity of the film’s interior[27]. This is similar to the results reported by Xu et al.[28], who prepared konjac glucomannan films with oregano essential oil- loaded Pickering emulsion. The EAB of the ZCPE films increased, especially for the ZCPE9 film. This change could be explained by the replacement of strong interactions between polymers with weak interactions between oil and polymer[29], suggesting the plasticization of co-loaded TA and CEO Pickering emulsion increased the flexibility and deformability of films. Specifically, as the content of the Pickering emulsion increases, there is a replacement of strong polymer-polymer interactions with weaker oil-polymer interfacial interactions. This leads to a reduction in the cohesive strength of the matrix, resulting in a slight decrease in TS. At the same time, the presence of the emulsion droplets acts as stress concentrators, which improve the film's ability to deform under applied stress, thereby increasing EAB. Some researchers have reported that the addition of essential oil loaded emulsion can negatively impact the mechanical properties of films[24, 30]. Therefore, future research on emulsion-containing films could focus on strategies to enhance their mechanical performance while maintaining other functional properties. However, we recognize that the balance between strength and flexibility will depend on the specific requirements of the application.

3.1.5. Barrier properties of the ZCPE films

The barrier properties of food packaging materials are critical for maintaining the food’s quality. Here, we evaluated the barrier properties of the ZCPE films to water and oxygen. As shown in Fig. 2C, the WVP of ZCPE0 film was 8.34 × 10−11 g/ (Pa s m), it decreased to 7.85 × 10−11 g/ (Pa s m) for the ZCPE9 film, suggesting that the addition of co-loaded TA and CEO Pickering emulsion improved the water vapor barrier performance. The essential oil loaded emulsion increases the tortuosity of the water molecule diffusion path, which could enhance the water barrier properties of films[25]. However, OP of the ZCPE films increased from 6.76 to 11.02 g/ (Pa s m) (Fig. 2D), indicating decreased oxygen barrier performance. Liu et al. [31]attributed this negative effect in oxygen barrier performance to the evaporation of essential oil during the film-forming drying process. These results show that the presence of essential oil loaded emulsion may alter a film’s barrier performance.



**Fig. 2** (A) UV-vis transmission spectra of ZCPE films, (B) mechanical properties, (C) water barrier property and (D) oxygen barrier property of ZCPE films.

**Table 1 Colorimetric parameters of ZCPEX films.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Film** | ***L\**** | ***a\**** | ***b\**** | **ΔE** | **WI (%)** | **Opacity** |
| **ZCPE0** | 78.03±0.52a | 0.58±0.24d | 40.03±1.69c | 53.12±1.45d | 54.33±1.67 | 2.66±0.01d |
| **ZCPE3** | 77.01±0.22a | 1.92±0.03c | 51.16±0.62b | 61.94±0.34c | 43.88±0.48 | 3.00±0.04c |
| **ZCPE6** | 74.50±0.78b | 3.83±0.92b | 53.72±1.18b | 65.61±1.42b | 40.40±1.42 | 3.75±0.01b |
| **ZCPE9** | 71.40±0.72c | 6.63±0.29a | 56.62±0.74a | 69.85±0.12a | 36.21±0.40 | 4.61±0.03a |

The values were shown as mean±standard deviation (SD). Different letters represent significant differences

3.1.6. Water sensitivity of the ZCPE films

The water sensitivity of films (especially those biobased polymers films) is intended to evaluate the material's susceptibility to water interaction, which is relevant to its potential application in food packaging where exposure to moisture can affect material performance[32] . The water content of the ZCPE films ranged from 23.60% to ~24.01% (Fig. 3A), with no significant difference between the films (*P* > 0.05). Noticeably, the Z layer had a lower water content than the loading layer, indicating that the Z layer is responsible for the low water permeability of the ZCPE films. A high-water content of a film could lead to a negative effect on TS and water barrier properties[33]. The water solubility could predict the stability of films in application. As shown in Fig. 3B, the water solubility of the ZCPE films decreased with the addition of co-loaded TA and CEO Pickering emulsion. For practical applications, reducing the water solubility of films could expand their application range in foods with a high moisture content[34]. Fig. 3C shows the swelling rate of the ZCPE films. The swelling rate of CPEX layer (47.61% ~ 51.71%) was lower than the swelling rate of the Z layer (162.52%), indicating the sustained and stable release rate of active substances from the CPEX layer. The lower swelling rate of the CPEX layer provides a more controlled and gradual release of antimicrobial agents, even as humidity fluctuates. This stability is advantageous in real-world applications, where humidity levels can vary during storage and transport. Of note, the swelling rate was not significantly different between the ZCPE films (*P* > 0.05). The swelling rate of ZCPE films was about 65%, which is close to the swelling rate of CPEX layer. This phenomenon could be attributed to a large number of hydroxyl group are involved into the assembly of ZCPE films, resulting in the reduction of binding sites for water molecules[35]. To further evaluated the water sensitivity of ZCPE films, we measured the WCA of each side of the films (Fig. 3D). The WCA of the Z layer barely changed (about 45°), while the WCA of the loading layer decreased as the proportion of emulsion increased. Surprisingly, the WCA of the Z layer was lower than the predicted WCA, perhaps due to hydrophilic groups of zein that face outward during the film drying process, as has been observed in gelatine – zein bilayer film[36].



**Fig. 3** (A) Water content, (B) water solubility, (C) swelling rate, and (D) water contact angle of ZCPE films.

**3.2. The antimicrobial and antioxidant properties of the ZCPE films**

3.2.1. Antimicrobial activity

Microorganism proliferation accelerates the spoilage of meat[37]. We evaluated the antimicrobial activity of the ZCPE films against bacteria related to meat spoilage: *P.paralactis* MN10, *A.pullicarnis* MN21, and *L.sakei* VMR17 (Fig. 4A). Visible dense colony units formed on the agar plates of control group, whereas there were scarcely any colony units on the agar plates of the ZCPE film groups, except for the ZCPE0 film. The antimicrobial ratio of ZCPE9 against *P.paralactis* MN10, *A.pullicarnis* MN21, and *L.sakei* VMR17 was > 99% (Fig. 4B). The *P. paralactis* MN10 and *A. pullicarnis* MN21 were the main genera found in our previous work of screening bacteria in meat, and these two strain with the highest spoilage ability in vitro respectively [38]. *L. sakei* VMR17 serves as a representative of gram-positive bacteria with spoilage potential in meat products. By focusing on these representative strains, we aimed to demonstrate the potential applicability of ZCPE films in mitigating spoilage and extending the shelf life of meat products. The excellent antimicrobial activity observed can be attributed to the release of CEO from the ZCPE films. CEO demonstrates strong antibacterial properties due to its rich composition of bioactive compounds, particularly cinnamaldehyde, which serves as its primary active component. Cinnamaldehyde interacts with the lipid bilayer of bacterial cell membranes, increasing their permeability. This disruption results in the leakage of intracellular contents, such as ions and proteins, ultimately leading to bacterial cell death[11, 25]. These results indicate that the ZCPE films have satisfactory antimicrobial properties and potential to maintain the quality of packaged food.

3.2.2. Antioxidant activity

The antioxidant activity of films is another critical factor for functional food packaging. The DPPH and ABTS assays are effective methods for assessing the ability of antioxidants to neutralize stable free radicals. These assays align well with the characteristics of essential oils in our film, particularly due to their effectiveness in non-polar systems. Moreover, the combination of DPPH and ABTS assays provides a comprehensive evaluation of the antioxidant capacity of natural compounds within film matrices, ensuring reliable and robust analysis[39, 40]. Both the DPPH and ABTS radical scavenging activity of the ZCPE films was increased significantly (*P* < 0.05), from 28.01% to 74.30% for DPPH radical scavenging activity, and from 17.93% to 93.80% for ABTS radical scavenging activity (Fig. 4C). In addition, we noted an emulsion concentration dependent effect of antioxidant activity. The antioxidant activity of the ZCPE films is due to the TA and CEO. TA has abundant hydroxyl groups, that provide good hydrogen donating capacity and promote DPPH and ABTS radical scavenging (Fig. 4D)[41]. A large number of terpenoids and aldehydes endow CEO with good antioxidant activity. Hence, the co-loaded TA and CEO Pickering emulsion enhances antioxidant activity due to the synergistic effects of TA and CEO[20].



**Fig. 4** (A) Colony forming units of *P.paralactis* MN10, *A.pullicarnis* MN21, *L.sakei* VMR17 after cultivation with ZCPE films. (B) Antimicrobial ratio of ZCPE films against *P.paralactis* MN10, *A.pullicarnis* MN21, *L.sakei* VMR17. (C) Antioxidant activity of ZCPE films and (D) reaction of antioxidants and DPPH, ABTS free radical.

**3.3. Release mechanism of the ZCPE films**

3.3.1. Release kinetics

To investigate the release properties of CEO from ZCPE films, we subjected the ZCPE9 film to migration assays using PBS as a common food simulated medium at 4℃. As shown in Fig. 5A, the ZCPE9 film had obvious controlled release characteristics, showing burst release behavior for initial 1-2 days and followed by sustained release for 3-7 days. It is worth noting that much more CEO was released from loading layer (CPE9 layer) than the Z layer, implying the blocking effect of Z layer regarding active substance release. This characteristic might be related to the high swelling rate of the Z layer. We prepared the blend film by mixing the loading layer (CPE9 layer) and Z layer film-forming solutions together and then casting. The release characteristics of blend film was shown in Fig. S1. The release patterns of CEO from both sides of the blended film were similar. However, the amount of CEO released from the loading layer of the blended film was lower than the amount of CEO released from the loading layer of the ZCPE9 film. The important contribution of the ZCPE9 films in controlling active release was highlighted by contrasting with the blend film. The loading layer functions as the inner layer for sustained release of active substances, while the Z layer serves as a barrier to minimize the release of active substances into the environment. The efficiency of antimicrobial packaging could be improved by reducing the waste of active substances.

3.3.2. Diffusion release mechanism

We further evaluated the release mechanism of CEO from the ZCPE9 film by fitting the release profile with mathematical models. The zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models were used to analyse the release behaviour of CEO from the ZCPE9 loading layer[42]. The related parameters are shown in Fig. 5B. Based on the regression coefficients obtained from models, the Korsmeyer–Peppas model demonstrated the highest reliability to fit the experimental release data of CEO from the loading layer of ZCPE9. Therefore, we applied the Korsmeyer–Peppas model to explore the release mechanism of CEO from the ZCPE9 film (Fig. 5C). The n parameter in the Korsmeyer–Peppas model represents the active substance release type: n < 0.5 for quasi-Fickian diffusion, n = 0.5 for Fickian diffusion, and n > 0.5 for anomalous transport[22]. The n value of the ZCPE9 Korsmeyer–Peppas model was < 0.5, indicating that CEO release from the ZCPE film follows a quasi-Fickian diffusion process.



**Fig. 5** (A) Time-dependent release curve of ZCPE9 film. (B) Analysis of CEO release kinetics from loading layer of ZCPE9 by zero-order, first-order, Higuchi and Korsmeyer-Peppas kinetic model. (C) Kinetic fitted results of CEO release from different side of ZCPE9 under Korsmeyer-Peppas model.

**3.4. Application of ZCPE films in preserving meat**

The ZCPE9 film was selected for practical applications in meat preservation. based on its optimal combination of mechanical properties, barrier performance, and antimicrobial and antioxidant activities.

Meat color, an important quality attribute that influences purchasing decisions, is indeed one of the most critical parameters for freshness evaluation[43, 44]. Studies have demonstrated a direct correlation between meat color and its freshness and quality[45, 46].The brightness and redness result of meat are presented in Fig. 6A and Fig. 6B, respectively. L\* showed a downward trend, meaning that the meat gradually became darker. a\* showed an upward trend, except for the PE film group. The opposite result of the PE film group was due to the mucus and white colonies on the surface of the meat, consistent with a previous study[47].

Meat spoilage is highly correlated with bacterial proliferation. We assessed the spoilage degree of packaged meat by determining the TVC. As shown in the Fig. 6C, the TVC increased in each group during the storage period. On day 6, the TVC of the control group and PE film group was 6.01 and 6.09 lg CFU/g, respectively, exceeding the limit (6.00 lg CFU/g) stipulated by the People’s Republic of China. The TVC of fresh meat was 2.94 lg CFU/g and increased up to 5.89 lg CFU/g in the ZCPE0 group on day 15, consistently remaining below the limit value throughout the preservation process. This outcome could be attributed to the CS in the ZCPE0 film, retarding bacterial proliferation. The TVC of the ZCPE9 group increased slowly, to a maximum of 4.45 lg CFU/g on day 15, suggesting the sustained release of antimicrobial substances.

TVB-N is main product of protein degradation and can reflect the freshness of meat. As displayed in Fig. 6D, the initial TVB-N of fresh meat was 5.45 mg/100 g, slightly lower than previous studies[47]. TVB-N increased in all groups during storage, probably due to bacteria-mediated breakdown. On day 9, TVB-N was 16.03 and 15.04 mg/100 g for the control and PE film groups, exceeding the limit of 15 mg/100g according to Chinese Standard GB 2707-2016, suggesting the inedibility of the meat. By contrast, TVB-N of the ZCPE9 group (15.41 mg/100 g) was much lower than control and PE groups on day 15, but it still which slightly exceeded the limitation. In conclusion, the ZCPE9 film could extend the shelf life of fresh meat by inhibiting the growth of microorganisms and decelerating the bacteria-induced protein degradation.



**Fig. 6** (A) L\*, (B) a\*, (C) total viable counts, and (D) total volatile basic nitrogen value of meat samples wrapped with PE films, ZCPE0 films and ZCPE9 films.

**4. Conclusions**

In this work, we successfully fabricated a zein/CS film with the Janus structure and incorporated co-loaded TA and CEO Pickering emulsion. The constructed films possessed good interfacial compatibility, enhanced thermal stability, and excellent barrier and UV resistance properties. In addition, the ZCPE films provided excellent functional performance in antimicrobial activity against *P. paralactis* MN10, *A. pullicarnis* MN21, and *L. sakei* VMR17; the antimicrobial ratio of the ZCPE9 film was almost 100%. The DPPH and ABTS radical scavenging activities increased from 28.01% to 74.30% and from 17.93% to 93.83%, respectively. The release behavior of CEO from ZCPE9 film showed obvious sustained controlled release characteristics and followed a quasi-Fickian diffusion process. Moreover, for practical meat preservation, compared to control group, the TVC and TVB-N in the ZCPE9 group were decreased by 2.76 lg CFU/g and 35.01%, respectively, during 15 days of storage. Overall, the current work provides a promising alternative for high-performance and eco-friendly antimicrobial packaging, addressing the growing need for sustainable solutions in the food industry.

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