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Incorporating quercetin nanocrystals in chitosan-polyvinyl alcohol composite film with cinnamon essential oil loaded Pickering emulsions for enhanced controlled release properties

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ABSTRACT

Despite extensive efforts in developing bio-based active packaging, most current films still suffer from poor release kinetics of active ingredients. This study presents an innovative strategy to improve the controlled-release performance of antimicrobial packaging by incorporating cinnamon essential oil (CEO)-loaded Pickering emulsions as carriers and quercetin nanocrystals (QNs) as nanofillers into a chitosan-polyvinyl alcohol composite (CS-PVA/PEs/QNs). The resulting CS-PVA/PEs/QNs film exhibited excellent mechanical properties, achieving a tensile strength of 39.09 ± 0.46 MPa. We attribute this enhancement to a strengthened hydrogen-bonding network among QNs, the CEO-loaded Pickering emulsions, and the CS-PVA matrix, as confirmed by FTIR, XRD, and TGA. Furthermore, the film demonstrated remarkable antimicrobial activity (>99.99 % inhibition against food spoilage bacteria) and a significantly enhanced antioxidant capacity (approximately five-fold increase). The incorporation of QNs effectively modulated the CEO release kinetics, which followed a Fickian diffusion mechanism in high-fat food simulants, thereby suggesting a potential for long-term antimicrobial efficacy. Owing to these controlled-release antimicrobial characteristics, the CS-PVA/PEs/QNs film proved highly effective in preserving fresh meat, extending its shelf life to 11 days at 4 °C. This work provides a promising strategy for designing advanced controlled-release systems and contributes to the development of high-performance antimicrobial packaging.

1. Introduction

Microbial spoilage of food poses a dual threat to environmental sustainability and global food security. According to the Food and Agriculture Organization (FAO) of the United Nations, approximately one-third of all food produced globally is lost or wasted annually, with microbial spoilage accounting for an estimated 20–25 % of these losses. (Mather et al., 2024; Parfitt et al., 2021). Fresh meat provides an ideal ecosystem for diverse microorganisms due to its high-water content and nutrient richness. During storage, microbial growth promotes spoilage, undermining both the physicochemical/sensory qualities and the safety of the product by increasing the risk of foodborne illnesses. Consequently, developing effective preservation strategies to inhibit microbial proliferation while maintaining meat freshness has become a critical objective in the food industry.

Antimicrobial packaging, which incorporates antimicrobial agents into packaging materials, has been developed as an effective strategy to inhibit the growth of foodborne pathogens and spoilage microorganisms in fresh meat products (Du et al., 2023; W. Zhang et al., 2023). Cinnamon essential oil (CEO), as a plant-derived active compound, exhibits potent antimicrobial properties and diverse bioactivities. It has been widely explored as an active agent in antimicrobial packaging to effectively inhibit the growth and proliferation of spoilage microorganisms in fresh meat. However, CEO is usually highly volatile and sensitive to various environmental factors, the direct incorporation of CEO into packaging materials often suffers from burst release and poor release

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kinetics, which limits antibacterial persistence and efficacy (Chang et al., 2022; Rojas et al., 2024). To improve the retention of CEO in the CS-PVA composite film, it can be encapsulated in oil-in-water (O/W) Pickering emulsions (PEs) and then incorporated into the film matrix (X. Chen et al., 2024; Y. Cheng et al., 2024; Ji and Wang, 2025). Although the incorporation of CEO-loaded PEs protects the CEO, it also introduces hydrophobic phases and particle-laden interfaces into the film matrix (Fan et al., 2024). These structural disruptions can compromise the film's compactness and continuity, resulting in diminished mechanical performance (D. Wang et al., 2024; J.-D. Wang et al., 2024). Moreover, CEO can migrate rapidly through structural defects in the film, compromising its intended sustained-release function. This premature release, combined with the inherent structural weaknesses, severely limits the practical application of CEO-loaded PEs in food packaging. To overcome these limitations, reinforcing the film matrix with nanofillers emerges as a promising strategy. This approach not only enhances the overall film performance but also provides precise control over the CEO release profile, advancing the development of effective antimicrobial packaging systems. Quercetin nanocrystals (QNs) are formed through the self-assembly of quercetin molecules into nanoscale crystalline particles, which possess an ultrafine size and a high specific surface area. These nanocrystals can be uniformly dispersed within a polymer matrix to establish strong intermolecular interactions with the polymer chains, thereby enhancing the film's structural uniformity and compactness (Li et al., 2023).

Additionally, QNs exhibit potent antioxidant and antimicrobial properties (Guo et al., 2024), making them attractive green multifunctional nanofillers for active food packaging. Despite their promise, the application of QNs in such systems remains rarely reported. Crucially, their potential synergy with PEs for modulating the release of CEO is unexplored. We hypothesize that a uniform dispersion of QNs restricts polymer chain mobility and reinforces the matrix via their rigid crystalline structure and high aspect ratio. This reinforcement is anticipated to stabilize the network of CEO-loaded PE-based films, enhance their mechanical properties, and ultimately facilitate the sustained release of CEO.

This study fabricated a CEO-loaded PEs/chitosan (CS) /polyvinyl alcohol (PVA) composite film reinforced with QNs, designated as CS-PVA/PEs/QNs, using a stepwise assembly method (Scheme 1). The effects of QNs and CEO-loaded PEs on the physicochemical properties of the CS-PVA composite film—including its mechanical, optical, barrier, antimicrobial, and antioxidant properties—were systematically investigated. Furthermore, the efficacy of the CS-PVA/PEs/QNs film in preserving fresh meat was evaluated, and the underlying mechanisms were elucidated by analyzing microbial community dynamics. To our knowledge, this is the first study to utilize green nanofillers (QNs) for simultaneously enhancing the mechanical properties of CEO-loaded PE-based films and achieving sustained CEO release. This work provides a novel strategy for engineering sustainable, high-performance antimicrobial packaging with superior controlled-release capabilities, offering significant potential for extending the shelf life of perishable foods.

2. Experimental sections

2.1. Materials

Quercetin (97 %), 1,1-diphenyl-2-picrylhydrazyl (DPPH, 97 %) and 2,2′-Azinobis- (3-ethylbenzthiazoline-6- sulphonate) (ABTS) were procured from Shanghai yuanye Bio-Technology Co., LTD. PVA (Mw31000–50000, alcoholysis degree 98–99 %), CS (deacetylation degree ≥95 %) and glycerol were obtained from Shanghai Macklin Biochemical Technology Co., LTD. *Pseudomonas paralactis* MN10 (*P. paralactis* MN10), *Acinetobacter pullicarnis* MN21 (*A. pullicarnis* MN21), and *Lactobacillus sakei* VMR17 (*L. sakei* VMR17) were sourced from Prof. Zhang's lab (Institute of Food Science and Technology, Beijing, China). Ethanol absolute, acetic acid and other chemical reagents were supplied by Beijing Sinopharm Chemical Reagent Co., Ltd., the reagents used in this work were all analytical grade unless explicitly stated otherwise.

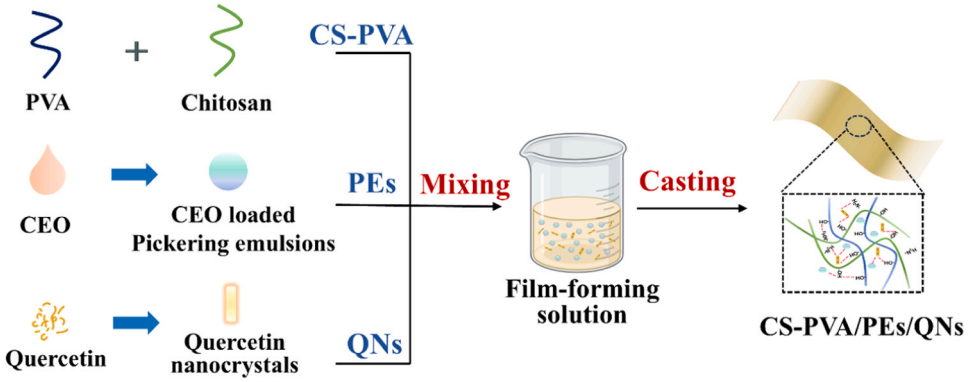
2.2. Preparation and characterization of QNs

QNs were prepared using ultrasonic-assisted antisolvent crystallization method, following the procedure described in literature (Li et al., 2023) with some modifications. The detailed preparation process was provided in the Supplementary Information. QNs subjected to ultrasonic treatment for 30, 60 or 120 min were labeled QNs-30 min, QNs-60 min and QNs-120 min, respectively.

Detailed characterization of QNs, including particle size, Zeta potential, morphology, structure, and bioactivities (antimicrobial and antioxidant), is provided in the Supplementary Information.

2.3. Preparation of QNs-reinforced CEO-loaded PEs/chitosan-polyvinyl alcohol composite films (CS-PVA/PEs/QNs)

The CS-PVA/PEs/QNs were fabricated using a step-by-step assembly method. CS power was dissolved in acetic acid aqueous solution (2 %, v/v) at 50 ℃ and PVA power was dissolved in ultrapure water at 90 ℃, respectively. After complete dissolution, the 1.5 wt% CS solution was mixed with an isovolumetric 2 wt% PVA solution. Then, 30 wt% glycerol was added to form film-forming solution. The PEs loaded with CEO was prepared as our previously described methods (Fan et al., 2024; Fan et al., 2024). Subsequently, 10 % (v/v) of QNs-60 min QNs suspension and 10 % (v/v) CEO-loaded PEs were blended into CS-PVA film-forming solution, respectively. After stirring for 2 h, 20 mL mixture was cast into a 90 mm Petri dish and dried at 50°C for 12 h, resulting in films named



Scheme 1. Schematic illustration for synthesis of quercetin nanocrystals enhanced chitosan/polyvinyl alcohol/CEO-loaded Pickering emulsions composite films (CS-PVA/PEs/QNs) based on stepwise assembly method.

CS-PVA/QNs and CS-PVA/PEs, respectively. To obtain CS-PVA/PEs/QNs, 10 % (v/v) of QNs-60 min QNs suspension and 10 % CEO loaded PEs were blended into CS-PVA film-forming solution, simultaneously. Before further characterization, all films were equilibrated in a 50 % relative humidity environment for 48 h.

2.4. Characterization of CS-PVA/PEs/QNs

2.4.1. Microscopic morphology and structure

The microstructures of the films including surface and cross-sectional were examined using a SEM (SU 1510 Hitachi, Japan). The cross-sectional parts of films were obtained under liquid nitrogen embrittlement. Infrared spectroscopy (Tensor 27, Bruker, Germany) was utilized to record the chemical structure and potential interactions of films. X-ray diffractometer (Ultima IV, Rigaku, Japan) was employed to determine the XRD pattern of the films at 5–60°. Thermogravimetric analyzer (Pyris Diamond, New Castle, USA) was employed to monitoring thermal degradation of films. The film samples were heated at a rate of 10℃/min in the range of 30–500℃ under nitrogen atmosphere.

2.4.2. Physicomechanical properties

The mechanical properties of films including tensile strength (TS) and elongation at break (EAB) were tested according to our previous procedure (Fan et al., 2023). Film samples with uniform thickness and smooth surfaces were selected for measurement.

The water contact angle (WCA) of films was tested using SZ-CAMC32 contact angle analyzer (Shanghai Xuanjun Instrument Co., LTD, China). For the water content, swelling rate and water solubility evaluations, films (20 ×20 mm) were initially weighed (m0) and then dried at 105℃ for 24 h before being weighed again (m_1). The dried films were exposed to 30 mL ultrapure for 24 h and weighed (m_2) without residual water on the surface. Then, the films were completely dried at 105℃ for 24 h and weighed again (m_3). The water content, swelling rate and water solubility were calculated as follows:

$$\text{Water content (\%)} = (m_0\text{-}m_1)/m_0 \times 100$$

$$\text{Swelling rate (\%)} = (m_2\text{-}m_1)/m_1 \times 100$$

$$\text{Water solubility (\%)} = (m_1\text{-}m_3)/m_1 \times 100$$

The water vapor permeability (WVP) of the films was measured according to the methods described by Fan et al. (2023) with slight modifications. In brief, the films were cut into 20 × 20 mm strips and sealed on top of centrifuge tubes (50 mL vertical) with 20.0 ± 0.5 g anhydrous silica gels. Then, the samples were incubated in a desiccator with saturated NaCl solution (75 % ± 2 % relative humidity) at 25℃ for 48 h. WVP was calculated with the following equation:

$$\text{WVP } (10^{-12}\ \text{g/m s Pa}) = (\Delta m \times e)/(t \times A \times \Delta p)$$

where Δm (g) is the gain in weight of the centrifuge tube; e is the thickness of the film (m); t is the incubation time (s); A (m^2) is the area of the centrifuge tube; and Δp (Pa) is the saturation vapor pressure of water. Nine parallels were used for each film.

2.4.3. Optical properties

A UV–visible spectrophotometer (UV-6000PC, Metash, China) was employed to record the UV shielding properties and optical properties through a previously reported method (Miao et al., 2024). The transparency of films was calculated according to the following equation:

$$\text{Transparency} = -(\log T_{600})/x$$

Where T_{600} is the transmittance at 600 nm, and x represents the film thickness (mm).

2.4.4. Antimicrobial and antioxidant properties

The antimicrobial activity of films was evaluated against *P. paralactis* MN10, *A. pullicarnis* MN21, and *L. sakei* VMR17 based on the method described by (Ma et al., 2023).

A 100 μL bacteria solution (approximately 10^5 CFU/mL) was dripped on surface of film (20 ×20 mm) and culture at 30℃ for 2 h, then 9.9 mL Luria-Bertani (LB) liquid medium was added for further 2 h culturation. A 100 μL above suspension was evenly spread onto an LB agar plate and incubated at 30°C for 24 h. The antimicrobial activity was calculated using the following equation

$$\text{Antimicrobial activity (\%)} = (S_1\text{-}S_2)/S_1 \times 100$$

where S_1 and S_2 are the bacterial colony counting for the control and film groups, respectively.

The antioxidant capacity of films was determined according to the produce of literature (Qin et al., 2024) with some modifications. 0.2 g of film sample was soaked in 5 mL of 95 % ethanol solution for 12 h. A 10 μL of above solution was mixed with 190 μL ABTS work solution (Equal proportion of 7.4 mM ABTS-water solution and 2.6 mM $K_2S_2O_8$-water solution were reacted in the dark for 12 h), after incubated in darkness for 30 min, the absorbance of mixture was determined at 734 nm. The ABTS radical scavenging activity was calculated with the following equation:

$$\text{ABTS radical scavenging activity (\%)} = (1\text{-}(A_1\text{-}A_0)/A) \times 100$$

Where A_1 is the absorbance of the film sample group, A_0 is the absorbance of the blank group, A is the absorbance of the control group.

For DPPH radical scavenging, 10 μL of film sample solution was mixed with 190 μL DPPH solution (0.1 mM), and the absorbance of the mixture was determined at 517 nm. The DPPH radical scavenging activity was calculated with the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (1\text{-}(A_1\text{-}A_0)/A) \times 100$$

Where A_1 is the absorbance of the film sample group, A_0 is the absorbance of the blank group, A is the absorbance of the control group.

2.5. Application in meat preservation

2.5.1. Treatment of meat

The fresh longissimus dorsi muscle of pork was used in the preservation experiment, which was purchased from Beijing Ershang Meat Food Group Co., LTD (Beijing, China) and transported to lab within sterile sampling bags. The fresh pork was cut into cuboids (50 ± 5 g) and placed in a Polypropylene package (3 ×3 ×6 cm) and covered with resulted film and Polyethylene film as a control. The packaged meat was storage in a refrigerator at 4℃.

2.5.2. Sensory evaluation

A sensory analysis group containing 8 trained panelists was employed to evaluate the quality of meat. The items of sensory evaluation involved overall acceptability, odor, appearance and texture, which were scored via 10-point scale. The results were presented as the mean values; lower score indicated a decrease in the freshness of the meat.

2.5.3. Total viable count (TVC) and Total volatile basic nitrogen analysis (TVB-N)

The TVC and TVB-N were determined strictly according to the China National Standards, GB4789.2–2016 and GB 5009.228–2016, respectively.

2.5.4. Microbial diversity analysis

The pork samples packaged with different films were taken in a sterile environment for microbial diversity analysis according to the procedure of wen et al. (X. Wen et al., 2022). Total microbial DNA was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instruction and checked by 1 %

agarose gel electrophoresis experiments. The V3-V4 region of the 16S rRNA was employed for PCR amplification. Afterward, the Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) provided sequencing procedures of samples utilizing the Illumina MiSeq platform. The raw sequencing data was processed using UPARSE (version 11) to cluster operational taxonomic units (OTUs) at 97 % similarity level.

2.6. Release kinetics

The release behavior of films was evaluated according to the method described by Zhu et al. (Zhu et al., 2025). A 50 % ethanol and 95 % ethanol solutions were selected as food simulants to represent semi-fatty and fatty foods, respectively. A 5 × 5 cm film was dipped into 20 mL food simulants for 120 h with slight continuous shaking at 25 °C. At the selected time points, 100 μL of the solution was taken to measure the absorbance at 287 nm. The concentration of CEO in the solution system was periodically quantified based on a previously established standard curve. To describe the mass transfer mechanism, the cumulative release profile data were fitted to various kinetic release models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas (Fan et al., 2024).

2.7. Biodegradability test

For the details of the biodegradability test, please refer to the Supplementary Information.

2.8. Statistical analysis

The experiments were conducted in triplicate unless otherwise specified, and the results were expressed as mean ± standard deviation (SD). Statistical analysis of the release data was performed using a linear mixed-effects model, with group and time as fixed effects and replicate samples as random effects. The significance of the group × time interaction was assessed using F-tests. The one-way analysis of variance (ANOVA) and Tukey's test were evaluated using SPSS 26.0 (SPSS Inc, Chicago, USA) under a 95 % confidence interval.

3. Results and discussion

3.1. Preparation and characterization of the QNs

QNs were prepared *via* an ultrasound-assisted antisolvent precipitation method. The resulting QNs exhibited a particle size distribution of 100–1000 nm (Fig. S1a) and a negative surface charge (ζ-potential) in aqueous solution (Fig. S1b), with the size decreasing as ultrasonication time increased. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images confirmed their rod-like morphology, typical of one-dimensional nanofillers (Fig. S1c-d). Spectroscopic analyses (FTIR, XRD, UV–vis, and fluorescence) collectively demonstrated that QNs formed *via* a self-assembly process stabilized by non-covalent interactions, which underpinned their structural stability (Fig. S1e-h). Notably, the QNs displayed pH-responsive optical behavior, suggesting their potential for intelligent packaging applications (Fig. S2a-e).

To determine whether the functionality of QNs extends beyond their structural role, we systematically assessed their antimicrobial and antioxidant activities in comparison with raw quercetin. As shown in Fig. S3a, QNs exhibited significantly stronger antimicrobial activity against P. paralactis MN10, A. pullicarnis MN21, and L. sakei VMR17 than raw quercetin. Furthermore, QNs demonstrated antioxidant efficacy comparable to that of the raw compound (Fig. S3b-d).

3.2. Preparation and characterization of CS-PVA/PEs/QNs

CS, a naturally derived polysaccharide, is widely used in food packaging owing to its excellent film-forming ability and biodegradability. To overcome its inherent brittleness and poor mechanical strength, PVA was incorporated to enhance the film's strength and broaden its potential for packaging applications. The CS-PVA composite film matrix was further functionalized with QNs and CEO-loaded PEs *via* a stepwise assembly method (Scheme 1). The microstructure of the films was examined using SEM (Fig. 1a). The surface of the pure CS-PVA film appeared smooth and uniform. In contrast, the surfaces of films containing QNs (CS-PVA/QNs), CEO-loaded PEs (CS-PVA/PEs), or both (CS-PVA/PEs/QNs) showed varying degrees of particle protrusions. A layered structure was observed in the cross-section of CS-PVA/QNs, likely resulting from the parallel self-assembly of molecules. The addition of QNs also improved molecular ordering, leading to a more compact arrangement. However, some aggregates were visible in the cross-section of CS-PVA/PEs/QNs, which may be attributed to the adsorption of QNs onto the surfaces of the PEs droplets. FTIR spectroscopy was used to investigate structural changes in the films (Fig. 1b). A noticeably strengthened absorption peak appeared near 3300 cm^{-1} in CS-PVA/QNs, attributable to the phenolic hydroxyl groups of QNs. Moreover, this characteristic peak broadened upon the addition of CEO-loaded PEs, suggesting the formation of additional hydrogen bonds. The overlapping vibrational frequencies of both strong and weak hydrogen bonds contributed to the observed peak broadening. X-ray diffraction (XRD) was employed to evaluate the crystalline structure of the films (Fig. 1c). All films showed a distinct characteristic peak at $2\theta = 19.9^{\circ}$, corresponding to semi-crystalline PVA. This peak was enhanced in CS-PVA/QNs, possibly due to an increased preferred orientation following QN incorporation. In contrast, the incorporation of CEO-loaded PEs reduced crystallinity. This decrease can improve the flexibility of molecular chains, which is beneficial for packaging applications (Jiang et al., 2024). The thermal stability of the films was assessed using TG and DTG curves (Fig. 1d–e).

Weight loss occurred in four main stages, with CS-PVA, CS-PVA/QNs, CS-PVA/PEs, and CS–PVA/PEs/QNs all exhibiting similar trends. The initial mass loss (~35–130 °C) and the second stage (~130–245 °C) corresponded to moisture evaporation and glycerol degradation, respectively. Thermal degradation between 245 and 360 °C was primarily associated with the breakdown of CS and PVA molecular chains. Finally, the residual mass at 550 °C indicated the concurrent decomposition of CS, PVA, QNs, and other components.

3.2.1. Physico-mechanical properties

Mechanical property is a critical indicator for evaluating the applicability of food packaging materials. Here, the effects of varying amounts of QNs and CEO-loaded PEs on the mechanical properties of film were investigated. The TS and EAB of neat CS-PVA film were 30.92 ± 0.84 MPa and 113.71 ± 2.17 %, respectively. However, increasing the CEO-loaded PEs content resulted in a decrease in both TS and EAB (Fig. 2a), which could be explained that the integrity of the film was disrupted by higher emulsion additions (Almasi et al., 2020; J.-D. Wang et al., 2024). This negative impact on the mechanical properties could be mitigated by enhancing the high density of the polymer network. With the content of QNs increased, TS values showed a gradual increase trend (Fig. 2b), CS-PVA/PEs_1/QNs_2 film showed the maximum value was 39.09 ± 0.46 MPa, and the TS value was no statistically significant differences with CS-PVA/PEs_1/QNs_3. As nanofillers, QNs are embedded between CS-PVA polymer chains, where they effectively transmit, distribute, and dissipate external stress through their intrinsic properties and interfacial interactions with the polymer matrix (Li et al., 2023). This mechanism compensates for the plastic deformation induced by the CEO-loaded PEs within the CS-PVA matrix. In addition, QNs enhance interfacial interactions among film components through non-covalent interactions, thereby improving the integrity of the composite matrix (Fig. 2c). The improved TS of films emphasized the great value in application of QNs as nanofillers. It is important to highlight that the mechanical properties of the films developed in this study significantly

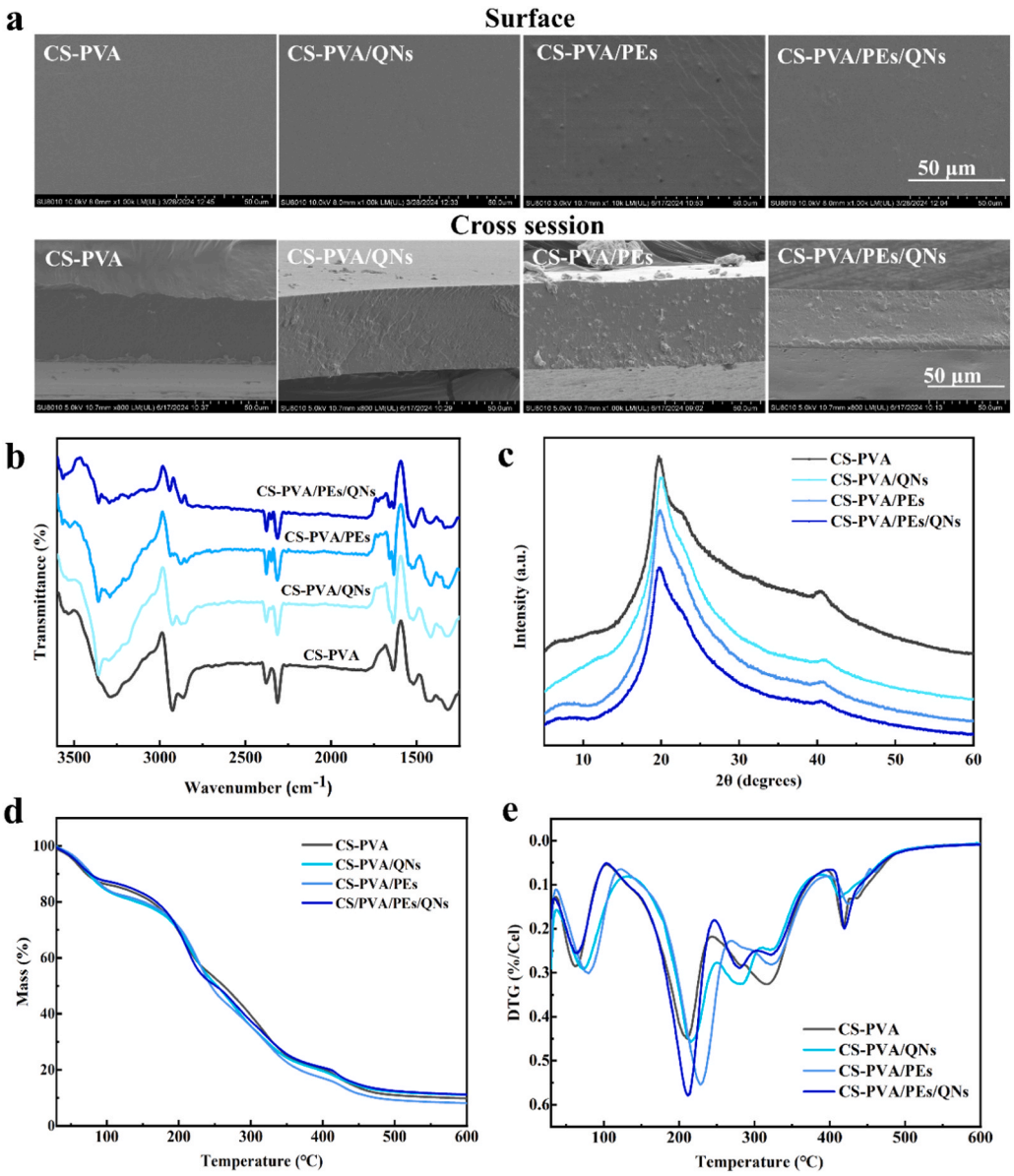


Fig. 1. Characterization of the CS-PVA/PEs/QNs; scanning electron microscopy (SEM) images of films, including surface and cross-section morphologies (a), FTIR spectra (b), XRD spectra (c), thermogravimetric curve (d) and derivative thermogravimetric curve of films (e).

surpass those of other emulsion films loaded with essential oil (X. Chen et al., 2024; Fan et al., 2023; Xia et al., 2023; Xu et al., 2024; Yu et al., 2024) (Fig. 2d).

The assessment of water resistance is crucial for evaluating the stability and reliability of packaging materials in practical applications. The incorporation of CEO-loaded PEs and QNs significantly reduced the WC of PC films (Table 1). This reduction can be attributed to the inherently hydrophobic properties of CEO. Additionally, the presence of QNs contributed to the formation of a denser and more compact polymer network through physical filling and enhanced interfacial interactions, as evidenced by SEM images, thereby further restricting water diffusion and retention within the film matrix. The reduced WS (Table 1) and SR (Fig. 2e) further proved the contribution of QNs in maintaining the structural stability of the film. The WCA was measured to evaluate the hydrophobicity of films (Fig. 2f). The observed increase in WCA supports the aforementioned viewpoint, suggesting that the specific interactions between QNs and CEO-loaded PEs could influence the surface characteristics of PCE@QNs. Overall, the observed improvements in water resistance demonstrated the potential of films for applications requiring enhanced moisture resistance.

3.2.2. *Barrier properties*

Water vapor permeability (WVP) of packaging film was the dominant factor in preventing moisture damage (D. Wang et al., 2024). The WVP of CS-PVA/PEs/QNs film was found to be significantly lower than that of PC, indicating the improvement in water vapor barrier properties of films (Fig. 2g). The CEO-loaded PEs impeded water vapor penetration through the emulsion droplets (Fan et al., 2023). Concurrently, the nanoscale dimensions of QNs enable them to fill the pores within the material, thereby shortening the diffusion path for water vapor. These mechanisms work synergistically to create a dense physical barrier, which significantly improves the water vapor barrier properties of the film. Transparency quantifies how clearly a film is perceived by the human eye, and also serves as an indicator of its light-blocking capability, which is particularly relevant for protecting light-sensitive food products. The transparency value of CS-PVA/PEs/QNs was higher than that of CS-PVA (Table 1), indicating reduced light transmittance. This reduction can be attributed to the incorporation of CEO-loaded PEs and

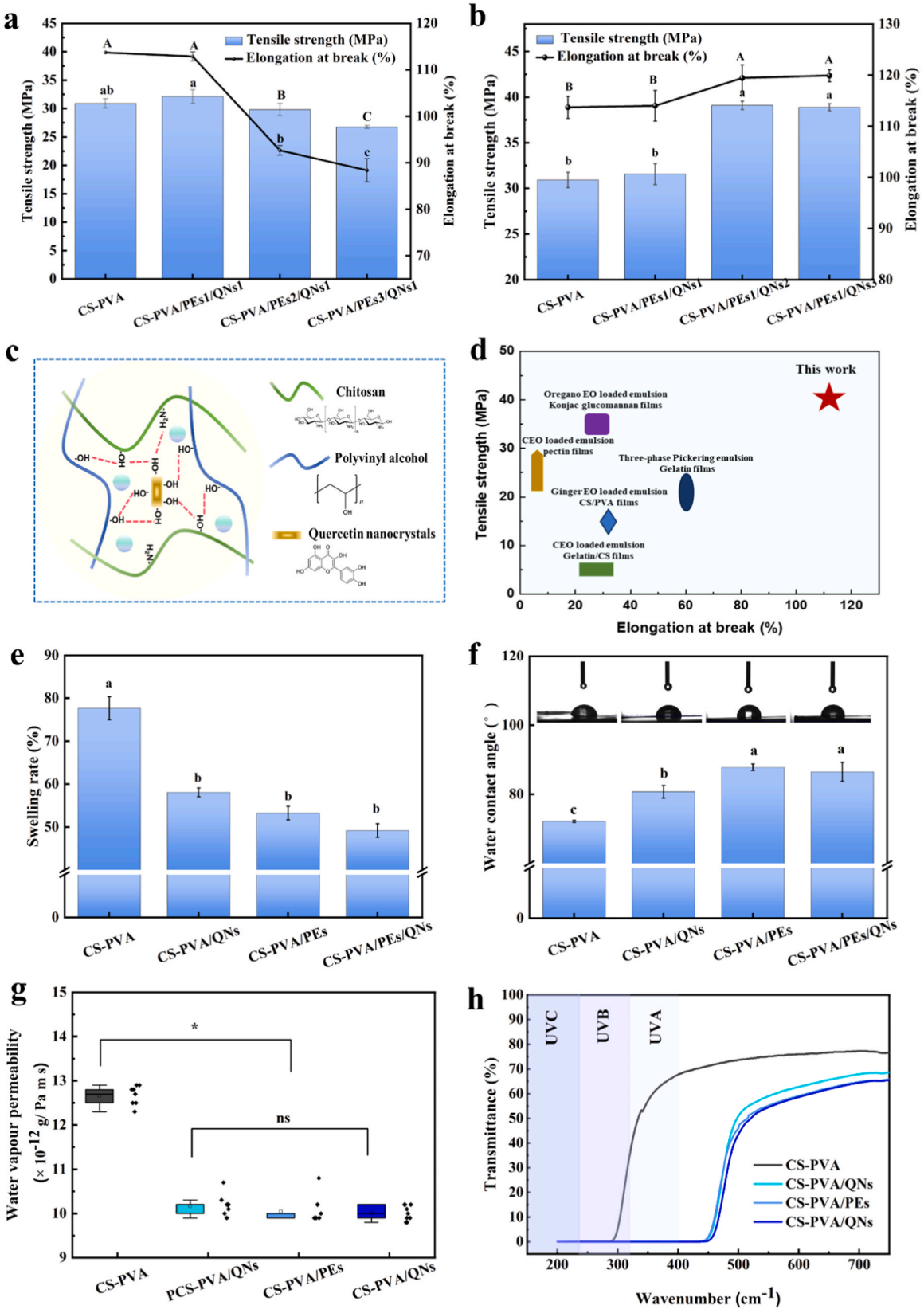


Fig. 2. Mechanical properties of the quercetin nanocrystals enhanced chitosan/polyvinyl alcohol/CEO loaded-Pickering emulsion composite films (CS-PVA/PEs/QNs), tensile strength and elongation at break of films with different CEO-loaded PE (a) and QNs concentrations (b). The 5 %, 10 %, and 15 % (v/v) addition level of QNs were labeled as QNs_1, QNs_2, and QNs_3; the 5 %, 10 %, and 15 % (v/v) addition level of CEO-loaded PE were labeled as PEs_1, PEs_2, and PEs_3. Schematic diagram of the internal cross-linking structure of CS-PVA/PEs/QNs (c). Comparison of the mechanical properties for CS-PVA/PEs/QNs compared with other essential oils-loaded PE films (d). Water resistance of CS-PVA/PEs/QNs, swelling rate (e) and water contact angle (f). The barrier properties of CS-PVA/PEs/QNs against water vapor (g) and ultraviolet light (h).

Table 1
Water content (WC), water solubility (WS) and transparency of QNs enhanced chitosan/polyvinyl alcohol/CEO loaded Pickering emulsion composite films (CS-PVA/PEs/QNs).

	WC (%)	WS (%)	Transparency
CS-PVA	28.46 ± 0.55a	24.65 ± 0.93a	1.98 ± 0.01c
CS-PVA/QNs	22.43 ± 0.39b	19.99 ± 0.66b	2.90 ± 0.02b
CS-PVA/PEs	23.81 ± 0.96b	19.67 ± 0.77b	3.22 ± 0.06a
CS-PVA/PEs/QNs	21.88 ± 1.05b	18.47 ± 1.00b	3.30 ± 0.03a

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

QNs, both of which introduced structural heterogeneity and enhanced light scattering within the film matrix. Fig. 2h depicted the UV-shielding properties of films intuitively. UVA (320–400 nm) and UVB (280–320 nm) irradiation had negative effects on food, particularly those with high fat content (Y. Zhang & Jiang, 2023). The barrier of the CS-PVA/PEs/QNs to ultraviolet light (280–400 nm) was almost 100 %, indicating a strong UV-shielding capacity of CS-PVA/PEs/QNs. This can be mainly attributed to the UV absorption ability of quercetin and reductive aldehyde groups in CEO. The results of optical properties showed that the CS-PVA/PEs/QNs had significant protective effects on food when used as UV protection packaging film.

3.3. Antimicrobial and antioxidant activity of the CS-PVA/PEs/QNs

The rapid proliferation of spoilage microorganisms is a primary factor contributing to the reduced shelf life of food. The antibacterial efficacy of the prepared films was evaluated against representative spoilage bacteria: *A. pullicarnis* MN21 (Gram-negative), *P. paralactis* MN10 (Gram-negative), and *L. sakei* VMR17 (Gram-positive). As shown in Fig. 3a, no colony-forming units of MN10, MN21, or VMR17 were

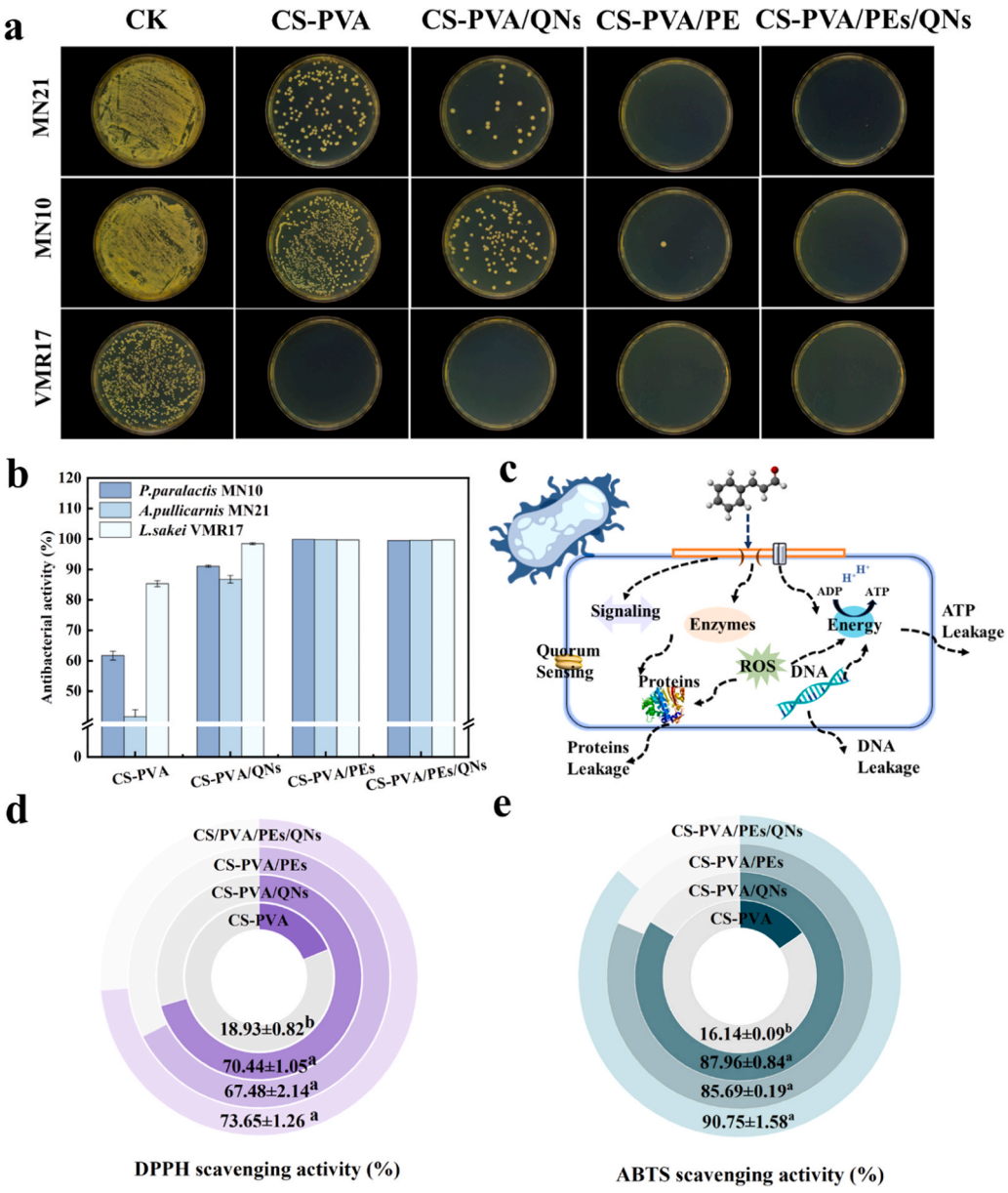


Fig. 3. Antimicrobial properties of quercetin nanocrystals enhanced chitosan/polyvinyl alcohol/CEO-loaded Pickering emulsion composite films (CS-PVA/PEs/QNs) against spoilage bacteria *P. paralactis* MN10, A. *pullicarnis* MN21, and *L. sakei* VMR17. Photographs of colony growth in LB agar plates (a) and ratio of antimicrobial activity (b) after treatment with different films. CS-PVA/PEs/QNs exhibits antimicrobial mechanism through multiple pathways (c). DPPH scavenging activity (d) and ABTS scavenging activity (e) of CS-PVA/PEs/QNs.

observed on the agar plates after treatment with the CS-PVA/PEs/QNs film, demonstrating its excellent antibacterial efficacy. The corresponding inhibition rates were then quantified and are presented in Fig. 3b. Both CS-PVA/PEs and CS-PVA/PEs/QNs films exhibited near-complete (approaching 100 %) antimicrobial efficacy against all three bacterial strains. This strong antibacterial effect is primarily attributed to the presence of cinnamaldehyde and other bioactive components in CEO. When the hydrophobic CEO diffused through the PEs barrier and was released from the film matrix, active molecules such as cinnamaldehyde initially disrupted the integrity of bacterial cell walls and membranes (Fig. 3c). This was followed by the inhibition of nucleic acid and protein synthesis, along with interference in other essential metabolic processes, ultimately leading to bacterial cell death. Previous studies have reported that Gram-positive bacteria are generally more susceptible to cinnamaldehyde than Gram-negative bacteria, which can be explained by structural differences in their cell envelopes (Lucas-González et al., 2023). Moreover, the superior performance of the CS-PVA/PEs/QNs film can be ascribed to the synergistic contribution of QNs. In addition to reinforcing the film's structural integrity, QNs may also exert direct antimicrobial effects, further enhancing the overall antibacterial activity of the composite film.

Oxidative degradation is a major contributing factor to the shortened shelf life of food products. As illustrated in Figs. 3d and 3e, the CS-PVA/PEs/QNs composite film exhibited strong radical scavenging activity, with DPPH and ABTS inhibition rates of 73.65 ± 1.26 % and 90.75 ± 1.58 %, respectively. These values significantly exceeded those of the pure CS-PVA film, showing an approximately fivefold enhancement, which aligns with our initial postulation. The markedly improved antioxidant performance can be ascribed to the synergistic activity between QNs and CEO, both of which are rich in phenolic compounds capable of donating hydrogen atoms to neutralize free radicals. The compelling combined antimicrobial and antioxidant properties underscore the potential of the CS-PVA/PEs/QNs film as a promising material for multifunctional active packaging applications.

3.4. Release behavior and mechanism analysis

The release profile of the active compounds was governed by the film's composition and microstructure, as well as the type of food simulant used (Zhu et al., 2025). The films containing only CEO-loaded PEs (CS-PVA/PEs) and those reinforced with QNs (CS-PVA/PEs/QNs) were select to monitor the release behavior of CEO in different food simulants over a 120-h period. As shown in Fig. 4a-b, the cumulative CEO release from the films was evaluated in two food simulants. Notably, the composite film did not exhibit an ideal sustained-release profile in the 50 % ethanol environment (Fig. 4a). This can be attributed to the relatively hydrophilic nature of 50 % ethanol, which induced moderate swelling of the CS-PVA matrix. The consequent opening of the polymer network pores allowed for relatively unrestricted diffusion of CEO, whose release was ultimately governed by its limited solubility in this medium. As a result, a rapid initial release followed by an early plateau was observed. Conversely, in a 95 % ethanol environment (Fig. 4b), all films exhibited similar release profiles characterized by a sustained and gradual release of CEO, which is indicative of an effective diffusion barrier within the polymer matrix in high-fat food simulants. This finding aligns with our previous report on chitosan/gelatin films containing CEO-loaded PEs (Fan et al., 2023). To quantitatively compare the release profiles, we applied a mixed-effects model to the cumulative release rate data in 95 % ethanol. The model identified a significant overall difference ($p < 0.05$), with the CS-PVA/PEs/QNs film exhibiting a notably slower release rate and an extended T50 during the early phase (1–24 h). This statistically grounded evidence confirms that QNs act as an effective release-modifying agent, resulting in slower release kinetics and validating our core assertion of their role in enhancing the sustained-release performance. The incorporated QNs not only increased the tortuosity of the diffusion path for CEO molecules but also densified the polymer matrix *via* strong hydrogen bonding. This created a more robust and intricate network that effectively retarded the diffusion of CEO, highlighting the critical role of QNs in achieving sustained release in aqueous food simulants.

The CEO release data were fitted to several mathematical models, including Zero-order, First-order, Higuchi, and Korsmeyer-Peppas. As summarized in Table 2, the Korsmeyer-Peppas model provided the best fit, with a high regression coefficient ($R^2 > 0.98$) for the CS-PVA/PEs/QNs film, indicating a diffusion-controlled release mechanism. The release exponent (n) values for this film were determined to be 0.002 and 0.15 in 50 % and 95 % ethanol, respectively. These values are significantly less than 0.45, which is characteristic of a Fickian diffusion process. This confirms that the release of CEO is primarily governed by molecular diffusion through the polymer matrix, driven by the concentration gradient of CEO.

3.5. Application of meat preservation

To evaluate the practical efficacy of the developed films, fresh meat samples were cut into appropriate portions, placed in polypropylene containers, and covered with polyethylene (as the control, CK group), CS-PVA, CS-PVA/QNs, or CS-PVA/PEs/QNs films. All packaged samples were stored at 4 °C for 11 days.

3.5.1. Sensory evaluation and physiochemical properties

Sensory evaluation provides a direct and intuitive assessment of meat freshness and quality from the consumer's perspective. Initially, all meat samples exhibited a bright red color, distinct muscle fibers, and

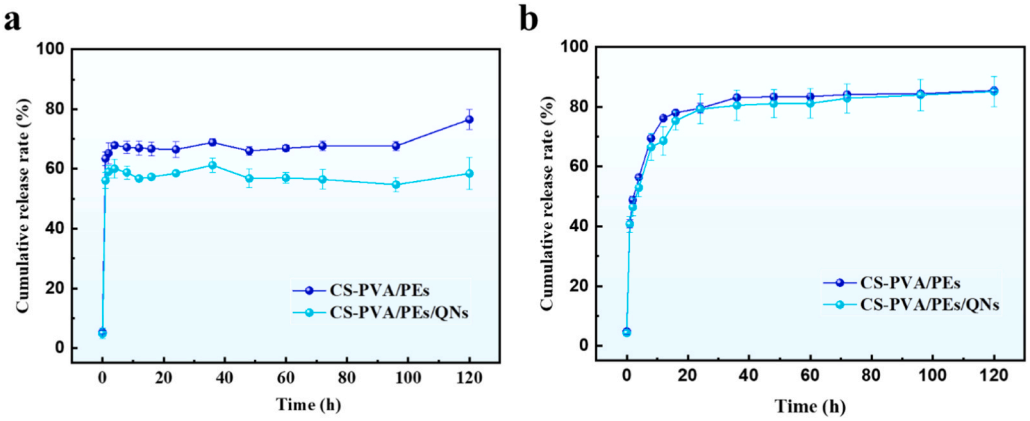


Fig. 4. The release kinetics profiles of CEO in 50 % ethanol (f) and 95 % ethanol (g) for films containing only CEO-loaded Pickering emulsion (CS-PVA/PEs) and films reinforced with quercetin nanocrystal-enhanced (CS-PVA/PEs/QNs).

Table 2
Kinetic release model fitting for CEO release at different food simulants.

Fitting Model	Formula	Sample	Ethanol (%)	Fit value		R^2
				k	n	
Zero order	$M_t/M_\infty=kt$	CS-PVA/PEs	50	0.60	-	0.31
			95	0.23	-	0.55
		CS-PVA/PEs/QNs	50	0.14	-	0.06
			95	0.95	-	0.47
First order	$M_t/M_\infty=1-e^{-kt}$	CS-PVA/PEs	50	2.74	-	0.99
			95	0.23	-	0.94
		CS-PVA/PEs/QNs	50	3.68	-	0.98
			95	0.49	-	0.96
Higuchi	$M_t/M_\infty=kt^{1/2}$	CS-PVA/PEs	50		-	
			95	0.45	-	0.55
		CS-PVA/PEs/QNs	50		-	
			95	1.89	-	0.47
Korsmeyer Peppas	$M_t/M_\infty=kt^n$	CS-PVA/PEs	50	66.58	0.005	0.99
			95	57.49	0.10	0.98
		CS-PVA/PEs/QNs	50	58.07	0.002	0.98
			95	44.27	0.15	0.98

Note: M_t represents the cumulative release amount at time *t*, M_∞ represents the cumulative release amount at infinite time, Mt/M∞ represents the cumulative release percentage at time *t*. The best fitting result is determined based on the highest correlation coefficient (R).

firm texture, with no noticeable differences among groups. However, as storage progressed, meat packaged in the CS-PVA/PEs/QNs maintained a stable red color and firm structure, whereas the control (CK) group showed obvious discoloration and softening (Fig. 5a). A sensory panel evaluated appearance, texture, odor, and overall acceptability (Fig. S5). By day 7, CK samples showed discoloration, stickiness, and strong odor, with scores below 4. In contrast, CS-PVA/PEs/QNs samples retained good sensory quality, indicating better preservation and extended shelf life.

TVC value also reflects the freshness of meat. As shown in Fig. 5b, the starting value of TVC of meat was 2.80 log CFU/g, during storage, the TVC value of the CK group increased rapidly to 7.72 log CFU/g, exceeding the acceptable limit of 6 log CFU/g on day 7, suggesting significant microbial spoilage. In contrast, meat samples packaged with CS-PVA/PEs/QNs film showed significantly slower microbial growth, with TVC remaining below the threshold even after 7 days. This significant inhibition of bacterial growth indicates the enhanced antimicrobial activity of the CS-PVA/PEs/QNs film.

As microbial proliferation intensifies and proteolytic activity increases, leading to the release of nitrogenous compounds. Consequently, TVB-N serves as an important spoilage marker that reflects the extent of protein decomposition in meat. The TVB-N value at 0 d was 6.24 ± 0.42 mg/100 g and exhibited a rising trend during the storage period in all groups (Fig. 5c). The TVB-N value of CK group was 15.61 ± 0.26 mg/100 g by 7 days of storage, which exceeded the acceptability maximum limit by Chinese Standard GB 2707–2016 (15 mg/100 g). In contrast, the CS-PVA/PEs/QNs group maintained a significantly lower TVB-N level of 13.82 ± 0.30 mg/100 g even on day 11, indicating its superior ability to retard protein degradation. Throughout the storage period, the CS-PVA/PEs/QNs films consistently suppressed TVB-N accumulation compared to other groups.

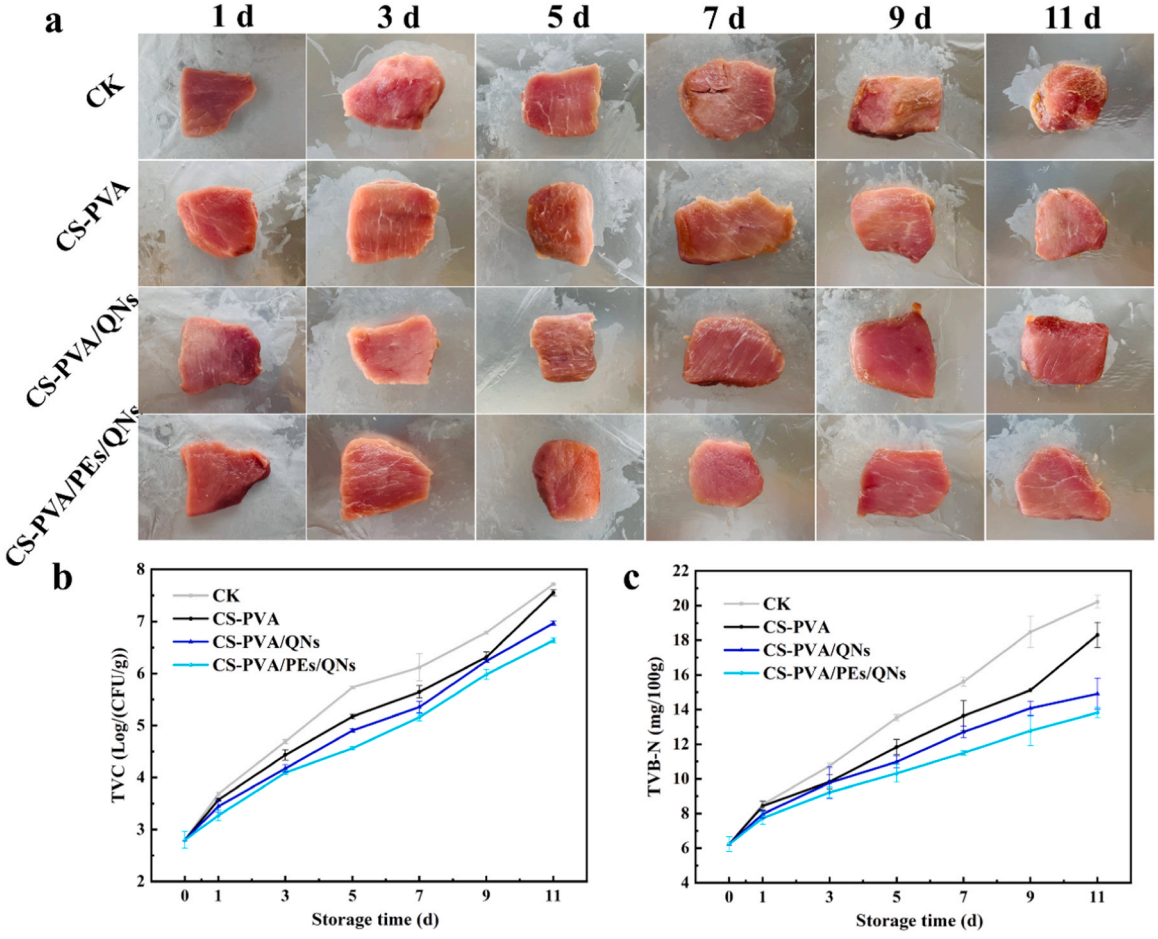


Fig. 5. Preservation performances of the quercetin nanocrystals enhanced chitosan/polyvinyl alcohol/CEO-loaded Pickering emulsions composite films (CS-PVA/PEs/QNs) on meat. Digital pictures (a), TVC (b) and TNB-N (c) of meat samples during storage at 4 °C.

3.5.2. Microbial diversity changes

The diversity and richness of microbial communities are related to the spoilage process of meat, which could be investigated by 16S rRNA high-throughput gene amplicon sequencing method (X. Wen et al., 2022). Based on the physiochemical results, key time points were designated as representative, including 0d, 1d, 3d, 9d and 11d. As shown in Fig. 6a, the OTUs of samples of CS-PVA/PEs/QNs group was 1334, which was lower than that of other film treated group, indicating fewer microorganism species in samples of CS-PVA/PEs/QNs group. Alpha diversity analysis confirmed that the richness and diversity of the microbiota were markedly reduced by CS-PVA/PEs/QNs treatment, as evidenced by a decreased Shannon index and an increased Simpson index (Table S1). The bacterial community structure of meat samples at the phylum level (Fig. 6b), further confirmed the reduced microbial diversity in the CS-PVA/PEs/QNs group. This result is highly consistent with Wang et al. (2022), who treated meat with thyme essential oil nanoemulsions, indicating that CS-PVA/PEs/QNs exhibit a strong antibacterial effect (Y. Zhao et al., 2022). Bacterial genus heatmap (Fig. 6c) and PCoA (Fig. 6d) portrayed the dynamic bacterial shifts. Clustering analysis of the heatmap showed that the microflora composition of CS-PVA/PEs/QNs group on the first storage day was basically similar to that of fresh samples (0 d), emphasizing the effective of CS-PVA/PEs/QNs in early storage. According to the PCoA analysis findings, the CS-PVA/PEs/QNs group samples clustered tightly together and were distinctly separated from the CK group samples, indicating that the CS-PVA/PEs/QNs film significantly altered the microbial community structure. The long inhibition effect on the microbial growth of meat was attribute to the slow-release of antibacterial substances in CS-PVA/PEs/QNs. These outcomes underscore that meat samples packaged by CS-PVA/PEs/QNs profoundly influence the microbial community complexity.

3.5.3. Preservation mechanism

During meat storage, the CEO encapsulated within the PEs migrates from the CS-PVA/PEs/QNs film to the meat surface. The incorporation of QNs enhances the compactness of the film matrix, forming a robust barrier that facilitates the slow and sustained release of CEO. This controlled release effectively inhibits the growth of spoilage microorganisms on the meat, delays microbial decay, and extends the product's shelf life. Meanwhile, the strong antioxidant capacity of the CS-PVA/PEs/QNs film-attributable to the synergistic action of QNs and CEO-helps suppress lipid oxidation, thereby maintaining the meat's color and overall quality. Furthermore, the multi-scale barrier structure of the film, reinforced by QNs as nanofillers and the inherent hydrophobicity of the CEO-loaded PEs, effectively reduces quality deterioration caused by moisture transfer and ultraviolet radiation during storage.

4. Conclusions

In this study, a controlled-release active packaging film was developed by incorporating QNs and CEO-loaded PEs into a CS-PVA composite, and its efficacy in meat preservation was demonstrated. FTIR analysis confirmed the formation of hydrogen bonds among QNs, CEO-loaded PEs, and the CS-PVA matrix, which contributed to the structural

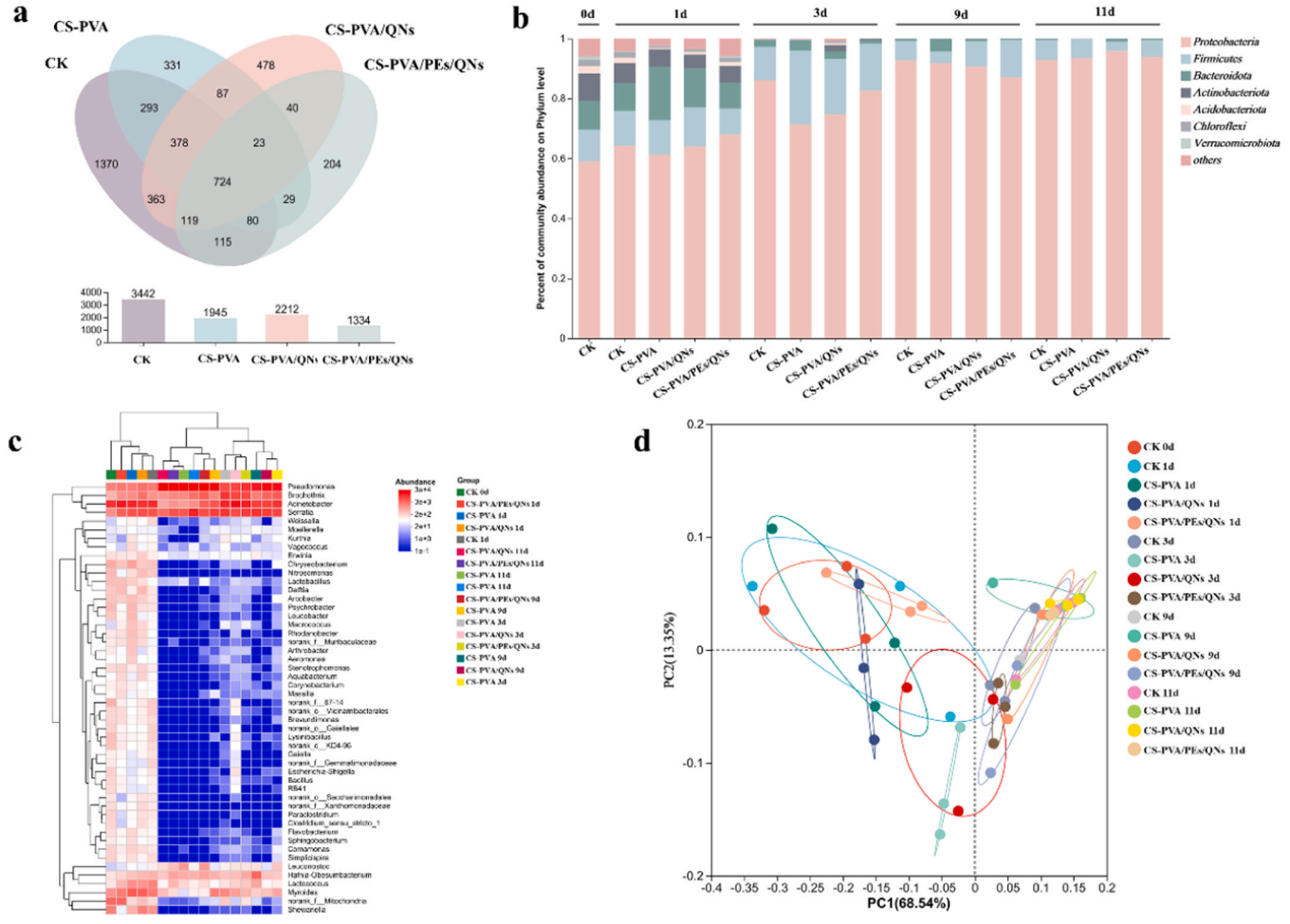


Fig. 6. Microbial communities' diversity of packaged meat during storage. The Venn diagrams of OTU richness distribution (a), bar charts (b), heatmap (c) and principal coordinate analysis (d) of the top 50 abundant genera displaying relative abundance of microbes on phylum level.

integrity of the composite. XRD and TGA further indicated that the inclusion of QNs improved the crystallinity and thermal stability of the film. Mechanical characterization revealed that QNs significantly enhanced both the TS and EAB of the CS-PVA/PEs/QNs film, outperforming other essential oil-loaded PE-based films reported in the literature. The CS-PVA/PEs/QNs also exhibited excellent barrier properties against water vapor and UV light. Moreover, owing to the synergistic effect between QNs and CEO-loaded PEs, the CS-PVA/PEs/QNs film showed notable antimicrobial and antioxidant activities. The release of CEO followed a sustained profile over 120 h, governed primarily by Fickian diffusion. The film was also verified to be biodegradable and environmentally friendly. In practical application, the sustained release of CEO from CS-PVA/PEs/QNs effectively reduced microbial diversity and extended the shelf life of fresh pork to 11 days at 4 °C. Overall, this work presents a novel and sustainable strategy for designing high-performance antimicrobial packaging with potential for broad use in food preservation.

CRediT authorship contribution statement

Aurore Richel: Writing – review & editing, Supervision. **Chaoqiao Zhu:** Validation, Formal analysis. **Cheng Li:** Writing – review & editing, Formal analysis. **Marie-Laure Fauconnier:** Writing – review & editing, Supervision. **Xin Li:** Software, Investigation. **Ming Tian:** Methodology, Data curation. **Chengli Hou:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Simin Fan:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Qingfeng Yang:** Validation, Methodology, Formal analysis. **Dequan Zhang:** Supervision, Resources, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2025.101678.

Data Availability

Data will be made available on request.

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