Preparation of Humidity-Responsive Cinnamon Essential Oil Nanomicelles and its Effect on Postharvest Quality of Strawberries

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Abstract
Biodegradable materials have attracted considerable attention to be applied in maintaining food quality and safety due to their ability of a sustained release of antimicrobial agents. In this study, cinnamon essential oil (CEO) loaded poly (ethylene glycol)-poly (ε-caprolactone) (PEG-PCL) micelles (CEO-micelles) were prepared for humidity-controlled CEO release and preservation of strawberry quality. The CEO-micelles with spherical shape and uniform size were obtained, and a high encapsulation rate (92.00 ± 1.77%) of CEO was achieved. X-ray diffraction demonstrated that the CEO was successfully encapsulated in PEG-PCL micelles. The release rate could be controlled by adjusting the relative humidity (RH) and 75% RH was favorable for CEO release from micelles (with 72% total amount release in 7 days). The encapsulation of CEO with high concentrations in PEG-PCL micelles reduced the cytotoxicity. Additionally, CEO-micelles exhibited high antifungal activity against Botrytis cinerea, the main pathogenic fungus of strawberry. Finally, the application of CEO-micelles to the preservation of strawberries had a positive effect on changes in decay rate, weight loss, firmness, color, and total soluble solids. These findings suggested that the CEO-micelles could be fabricated to humidity responsible nano-vesicles for preservation of fruit or vegetable with controllable release profile.

Keywords Cinnamon essential oil · Amphiphilic micelle · Humidity-responsive · Controlled release · Strawberry

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| CEO-micelles | Cinnamon essential oil loaded poly (ethylene glycol)-poly (ε-caprolactone) micelles |
| EOs          | Essential oils |
| CEO          | Cinnamon essential oil |
| PEG-PCL      | Poly (ethylene glycol)-poly (ε-caprolactone) |
| PEG          | Polyethylene glycol |
| PCL          | Poly (ε-caprolactone) |
| CCK-8        | Cell Counting Kit-8 |
| blank-micelles | The micelles without CEO |
| PDI          | Polydispersity index |
| DLS          | Dynamic laser light scattering |
| TEM          | Transmission electron microscopy |
| XRD          | X-ray diffractometer |
| EE%          | The encapsulation efficiency percentage |
| HPLC         | High-performance liquid chromatography |
| PDA          | Potato dextrose agar |
| ITS          | Internal transcribed spacer |
| N-J          | Neighbor-joining methods |
| PET          | Polyethylene terephthalate |
| CK           | Control |
| CEO-2        | The ratio of CEO content to the volume of air in the clamshells (0.6 L) was 2 μL/L |
| CEO-5        | The ratio of CEO content to the volume of air in the clamshells (0.6 L) was 5 μL/L |

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The ratio of CEO content to the volume of air in the clamshells (0.6 L) was 10 μL/L.

CEO-micelles-2: CEO-micelles with the same amount of CEO as the CEO-2 treatment group.

CEO-micelles-5: CEO-micelles with the same amount of CEO as the CEO-5 treatment group.

CEO-micelles-10: CEO-micelles with the same amount of CEO as the CEO-10 treatment group.

TSS: Total soluble solids.

**Introduction**

Strawberry (*Fragaria ananassa*) is known as the “Queen of Fruits” because of its unique color and flavor, as well as its high nutritional value (Nowicka et al., 2019). However, strawberries have a short shelf life of 1–2 days at room temperature, which is mainly related to the postharvest mechanical damage and fungal infection. Currently, the control of postharvest pathogenic microorganisms in fruits mainly relies on the use of chemical synthetic fungicides (Romanazzi et al., 2016). However, the potential toxicity of fungicides as well as the residues of synthetic fungicides can have a negative impact on the environment and human health. Also, the pathogens can develop resistance to synthetic fungicides. All of these may retard the further application of these chemicals in preservation of fruits (Bahrami et al., 2020; Romanazzi et al., 2016).

As natural preservatives, plant essential oils (EOs) are attracting attention for their non-toxic and antibacterial properties, and are gradually being used in food preservation, especially in highly perishable fruits (Mutlu-Ingok et al., 2020). Cinnamon essential oil (CEO) is extracted from the bark or leaves of the cinnamon tree and is a widely used as flavoring substance and food additive with good broad-spectrum antimicrobial properties (Clemente et al., 2016; Sharma et al., 2021). But in fact, like other EOs, CEO has poor bioavailability, low solubility, and easy to volatilize, thus limiting its application (Prakash et al., 2018).

The essential oil could be encapsulated to nano/micro-vesicles, such as nanomatrix, microcapsule, liposomes, for the purpose of reducing the evaporation and decreasing the rate of diffusion of EOs with controlled release profile to the external environment (Das et al., 2021; Göktepe et al., 2021; Hemmatkhah et al., 2020; Radi et al., 2022). It is reported that the cinnamaldehyde (CA) loaded nanostructure lipid carriers could be utilized as edible coating to extend the shelf life of Mazafati date fruit (Akhanv et al., 2021). Also, the jasmine essential oil loaded chitosan nanomatrix could exhibit excellent effect to prohibit *Aspergillus flavus* with good biocompatibility (Kujur et al., 2021). It is reported that the quality of fresh-cut carrot slices could be maintained by the thyme essential oil by the microencapsulation method in chitosan edible coating, which reduce the microbial counts from day 6 till the end of storage comparing with free thyme essential oil group (Viacava et al., 2022). These works revealed that the nanoencapsulation technique could be used to enhance the efficacy of the essential oil in the food system.

Micelles are generally composed of amphiphilic blocks that self-assemble into a shell-core structure in an aqueous medium, consisting of hydrophilic blocks that form a shell and hydrophobic block that form a core (Behl et al., 2020). The hydrophobic drug can be physically entrapped in the core of the block copolymer micelles. Biodegradable polymer micelles have been extensively studied as nano-carriers of hydrophobic drugs or bioactive compounds (Tyrrell et al., 2011; Xiao et al., 2020). Among them, poly (ethylene glycol)-poly (ε-caprolactone) (PEG-PCL) copolymers are widely used in the preparation of polymeric micelles due to their good biocompatibility and biodegradability (Danafar et al., 2014). Polyethylene glycol (PEG) is the most used hydrophilic shell-forming segment with good water solubility, non-toxic or low toxicity, biocompatibility, etc. It could form a hydrophilic shell around the micelle core to raise solubility of hydrophobic substances in a hydrophilic environment (Yang et al., 2009). Poly (ε-caprolactone) (PCL) is most used as the hydrophobic segment for encapsulating hydrophobic drugs due to the toxicity-free, biodegradable, as well as the excellent biocompatibility. Granata et al. (2018) reported that PCL nanocapsules could be used to load *Thymus capitatus* and *Origanum vulgare* EOs by a nano-precipitation method. The nanocapsules illustrated high efficiency of encapsulation (96 ± 4% and 91 ± 1%), which demonstrated that the good compatibility could be achieved between EOs and PCL polymer. Micelles formed by self-assembly of diblock copolymers are good candidates for encapsulating CEO as it can improve the stability and dispersion of hydrophobic CEO in a hydrophilic environment. Additionally, hydrophilic polymer PCL molecules can expand or degrade when exposed to moisture environment, which can be utilized to adjust the release of the encapsulated bioactives (Lee et al., 2021; Min et al., 2021).

Based on the research works of the EOs, the lipid was commonly used to fabricate the nano-vesicles (Akhanv et al., 2021; Radi et al., 2022). However, the mechanical stability of the lipid is limited, and the neutral lipid formed liposomes were easy to aggregation in water (Pinilla et al., 2020). Other micro-sized vesicles have some difficulty to disperse in solution for long time, or with relatively low encapsulate rate (Plati & Paraskevopoulou, 2022). The humidity plays a key role in the growth of the microorganism (Del Toro-Sánchez et al., 2010). High RH is a typical environment for some food packaging and is prone to microbial growth (Balaguer et al., 2013; Paris...
et al., 2020). It is proposed that the environmental humidity responsible system could be desired for controlled release of EOs. To address these issues, the di-block copolymer PEG-PCL was used for the formulation of the nanomicelles in the purpose of controlling the release of the CEO, especially response to the high humidity environment. The CEO-micelles were characterized by their physicochemical and evaluated humidity-controlled release properties. In addition, its efficacy as an antifungal agent for food was explored by applying it to the preservation of strawberries.

**Materials and Methods**

**Materials**

Cinnamon essential oil (CEO) was obtained from Yilong Chemical Co., Ltd. (Guangdong, China). The PEG_{2k}-PCL_{5k} and PEG_{2k}-PCL_{8k} diblock copolymers were purchased from Daigang biological engineering Co., Ltd. (Shandong, China). Dichloromethane (analytical grade) was purchased from Nanjing Chemical Reagent Co., Ltd. (Jiangsu, China). Methanol and acetonitrile (HPLC grade) were purchased from TEDIA High Purity Solvent Co., Ltd. (Jiangsu, China). Murine fibroblast (L929) cell line was purchased from cell bank of ATCC. Cell Counting Kit-8 (CCK-8) was purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Jiangsu, China).

**Preparation of CEO-Loaded Micelles**

The CEO loaded PEG-PCL micelles (CEO-micelles) were prepared as follows. Briefly, 100 mg PEG-PCL was dissolved in 3 mL dichloromethane and constant stirring (500 rpm) at room temperature for 3.5 h, then 100 mg of CEO was added into the solution and continued stirring to remove the solvent. The film was formed by blowing with nitrogen for the evaporation of the residue organic solvent. After that, 2 mL ultrapure water was added drop-wise by syringe pumps (Shenzhen Hoke Medical Equipment Co., Ltd., Shenzhen, China) at 1 mL/h into the solution with continuous stirring. Finally, CEO-micelles were obtained by adding 3 mL of ultrapure water to the solution and filtering by using 0.45-μm filter membranes. The micelles without CEO (blank-micelles) were also prepared as describing above.

**Analysis of the CEO-Micelles**

**Particle Size, Polydispersity Index, and Zeta Potential Measurements**

The particle size, polydispersity index (PDI), and zeta potential were determined by dynamic laser light scattering (DLS) using Malvern Nano Zetasizer ZS (Malvern Instruments, Ltd., Worcestershire, UK) at room temperature. All samples were dispersed in Milli-Q water with the concentration of 1 mg mL\(^{-1}\). The scattering angle was 90° and the refractive index was 1.330. All measurements were repeated three times.

**Morphological Characterization**

A drop of polymer micelles solution was placed on the carbon-coated copper grid and allowed completely dry at room temperature. The morphology of CEO-micelles was observed by transmission electron microscopy (TEM) (JEM-1400, Japan) at an accelerating voltage of 80 kV.

**X-ray Diffractometer (XRD) Analysis**

The Ultima IV combined multifunctional horizontal X-ray diffractometer (XRD Ultima IV, Rigaku, Japan) was used to test the crystal structure of the characterized samples with tube voltage of 40 kV, tube current of 40 mA, scan speed of 0.02° s\(^{-1}\), and scan range from 5 to 40°.

**Encapsulation Efficiency of the CEO-Micelles**

The encapsulation efficiency percentage (EE%) was determined by high-performance liquid chromatography (HPLC) equipped with a UV detector at 290 nm and a Welchrom® C18 (5 μm, 4.6 × 150 mm) maintained at 30 °C. The mobile phase was acetonitrile–water (50:50) at a flow rate of 0.8 mL/min. The injection volume was 10 μL and the samples were previously filtered through 0.22-μm pore size nylon membranes. The precisely weighed standard cinnamaldehyde (the major component of CEO) was dissolved with methanol and diluted to 1, 5, 10, 50, 100, and 500 mg/L. The linear regression of the peak area of the UV detector on concentration was performed, and the regression equation was calculated.

The total CEO content in the CEO-micelles suspension was determined by dissolving the CEO-micelles suspension in dichloromethane, sonicating it for 20 min, and measuring by HPLC method. The amount of free-CEO was determined using the filtration–centrifugation technique (Granata et al., 2018), with some modifications. To separate free-CEO from micelles, the CEO-micelles suspension was placed in the Amicon Ultra-filtration centrifuge tubes (30 kDa MWCO, Millipore, Germany) and was centrifuged at 16,000 rpm for 10 min in 4 °C. The amount of free-CEO was determined by measuring the absorbance of CEO in the sample collected from the lower chamber of the filter. Each experiment was repeated for three times to obtain the average values. EE% was calculated using the following Eq. (1).
EE% = \left( W_r - W_f \right) / W_i \times 100 \quad (1)

where the $W_i$ is the total amount of CEO and the $W_f$ is the free content of essential oil in CEO-micelles suspensions, respectively.

**Release Characteristic of CEO from the Micelles**

The amount of CEO released from micelles at different relative humidity was determined. Concisely, CEO-micelles solutions were sprayed on food-grade paper cards (1.5 cm × 1.5 cm × 0.1 cm) and placed in a fume hood to remove moisture for subsequent experiments. All samples were placed in a constant temperature and humidity chamber incubated at 25 °C (15% RH; 35% RH; 75% RH). At specific time intervals (0, 3, 6, 12, 24, 36, 48, 72, 120, and 168 h), the remaining CEO was determined by adding 0.5 mL dichloromethane to each sample to dissolve the encapsulated CEO. The release studies of free-CEO were also tested as above, and performed in a constant temperature and humidity chamber incubated at 25 °C (75% RH).

Each sample was analyzed by HPLC according to the method reported in the “Encapsulation Efficiency of the CEO-Micelles” section. Each experiment was repeated for three times to obtain the average values. The cumulative release of CEO from micelles was calculated according to the following Eq. (2):

\[
\text{CEO cumulative release (\%)} = \frac{M_f - M_i}{M_i} \quad (2)
\]

where $M_i$ is the initial amount of CEO in the sample and $M_f$ is the remaining amount of CEO in the sample at each specific time.

**Cytotoxicity Studies**

The in vitro cytotoxicity of free-CEO, blank-micelles, and CEO-micelles were assessed on murine fibroblasts L929 using a CCK-8 assay. The samples were diluted to concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL. Murine fibroblasts L929 cells were cultured in DMEM medium containing 10% fetal bovine serum. After digestion with 0.25% trypsin, L929 cells were cultured in DMEM medium containing trypsin concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL. Murine fibroblasts using a CCK-8 assay. The samples were diluted to concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL. Murine fibroblasts

**Testing of Antifungal Activity**

**Isolation and Molecular Identification of Fungi**

In order to isolate the main pathogenic fungi of strawberries postharvest, isolation and identification were performed with reference to the method described by Kamaruzzaman et al. (2018). The strawberry fruits were stored at room temperature (25 °C), and after the characteristic colonies grown, the fungal mycelium was picked out from the infected strawberry with sterile forceps and placed on potato dextrose agar (PDA) medium. The strains were isolated and purified by repeated scratch isolation, and the isolated strain was named CM-H1.

Then the strains were sent to Sangon Biotech (Shanghai) Co. Ltd for internal transcribed spacer (ITS) sequence determination. The resulting ITS sequences were subjected to BLAST homology search in the NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) database, and the homologous sequences were compared with the target sequences using MEGA 6.0 software (version 6.0, Mega Limited, Auckland, New Zealand) to construct a phylogenetic tree by using the neighbor-joining methods (N-J) and analyze the affinities.

**Effects of CEO and CEO-Micelles on Inhibiting Fungal Mycelial Growth**

The antifungal activity of CEO and CEO-micelles against the main postharvest pathogenic fungi of strawberries was assessed on fungal-inoculated PDA medium using a paper disk diffusion method (Lin et al., 2021). After the PDA medium had solidified, holes were punched through the middle of the fresh medium using a sterile 0.7-cm diameter punch. Mycelial disks (Φ = 0.7 cm) of the Botrytis cinerea were cut from the 7-day cultures and placed in the center of a PDA dish (Φ = 9 cm). Paper loaded with different concentrations of CEO and CEO-micelles solutions were placed in the center of the petri dish lids, with final concentrations of 10, 20, 40, and 80 μL/L air (the ratio of CEO content to the volume of air in the petri dish (0.06 L)). Finally, all samples were sealed with parafilm and
incubated at 26 °C for 7 days. Each treatment was tested in triplicate. The radial growth (mm) of the fungus was measured by a digital Vernier caliper (mm) (SJ-455615, Haining Shangjiang Tools Co. Ltd., China). The MIC (minimum inhibitory concentration) was determined as the lowest concentration of antimicrobial substance that could inhibit the growth of pathogenic fungus in the culture medium.

**Effect of CEO-Micelles on the Postharvest Physicochemical Quality of Strawberry Fruits**

**Fruit Material Preparation**

To evaluate the application of CEO-micelles as antifungal agents in postharvest fruits and vegetables, we selected a representative strawberry fruit in our experiment. In this study, fresh strawberries (*Fragaria ananassa* Duch. “Hongyan”) were harvested from an experimental orchard in Nanjing (Jiangsu, China). Strawberries were transferred to the agricultural products storage and preservation laboratory of Nanjing Forestry University immediately after picking. Berries with uniform size, uniform color, no mechanical damage, and no fungal infection were selected for the following experiment.

**Treatments and Experimental Design**

CEO and CEO-micelles solutions were sprayed on food-grade paper cards and dried for subsequent experiments. The strawberry fruits were randomly divided into seven groups and each group contains 72 strawberries. Six strawberries with the total weight of 90 ± 10 g for each group were placed randomly in polyethylene terephthalate (PET) clamshells (0.6 L). The food-grade paper card was attached to the top center of the clamshell. Seven different treatments group were as follows: T1 (CK), control, without any treatment; T2 (CEO-2), the ratio of CEO content to the volume of air in the clamshells (0.6 L) was 2 μL/L; T3 (CEO-5), the ratio of CEO content to the volume of air in the clamshells (0.6 L) was 5 μL/L; T4 (CEO-10), the ratio of CEO content to the volume of air in the clamshells (0.6 L) was 10 μL/L; T5 (CEO-micelles-2), CEO-micelles with the same amount of CEO as the CEO-2 treatment group; T6 (CEO-micelles-5), CEO-micelles with the same amount of CEO as the CEO-5 treatment group; T7 (CEO-micelles-10), CEO-micelles with the same amount of CEO as the CEO-10 treatment group. All clamshells were packaged in PE film and stored at 25 ± 1 °C for 4 days. The physicochemical properties of the fruits were evaluated on 0, 1, 2, 3, and 4 days and each sampling point contained 18 strawberries and was considered as three replications (6 strawberries in each replication).

**Determination of Decay Rate of Strawberries**

Fruits were considered infected when soft rot, disease spots, or fungal infections were observed. The results were expressed as fruit decay rate. The decay was counted daily and calculated according to the following formula (4).

\[
\text{Decay rate} (%) = \frac{\text{number of decay fruits}}{\text{number of total fruits}} \times 100
\]

**Assessment of Strawberries Physicochemical Quality**

Quality parameters of the strawberries were measured daily during the 4-day storage period, including weight-loss rate, firmness, skin color (L* (lightness) and a* (greenness/redness) colorimetric values), and total soluble solids (TSS). Measurements were taken at 1, 2, 3, and 4 days.

To determine the weight loss rate of the strawberries, the strawberries were weighed accurately every day during storage period. The weight-loss rate was calculated as a percentage of the initial weight and all measurements were taken by recording the weight of three clamshells.

Strawberry firmness was determined with a texturometer (model TA XT Plus, TA Instruments, Surrey, UK) by measuring the force to penetrate 6 mm (2-mm probe) into strawberry flesh at rate of 2 mm/s. Berries were cut into two pieces and measured on both sides at the highest elevation closer to the bottom. Hardness units are expressed in newtons (N) and six strawberries from each clamshell was measured.

L* (lightness) and a* (greenness/redness) colorimetric values were measured by a colorimeter (Chroma Meter CR – 400, Konica Minolta, Japan), which indicates the intensity of color saturation. Three measurements were taken on the surface of each six strawberries from the clamshell.

TSS content was analyzed from strawberry juice using a digital saccharimeter(WYT, Chendu United Optical Instruments Co. Ltd) Juice was obtained by homogenizing the berries in a juice extractor; the homogenates were then filtered with triple gauze. Six strawberries from each clamshell were tested.

**Statistical analyses**

All the results are expressed as mean ± standard deviation of three replications. The data were analyzed using GraphPad Prism 9 (GraphPad Software Inc., USA) and SPSS 23.0 (IBM SPSS Statistics, v. 23.0, USA). The difference significance between treatments was assessed using Duncan’s multiple range test at \( p < 0.05 \).
Results and Discussion

Analysis of CEO-Micelles

Particle Size, Polydispersity Index, and Zeta Potential

Size distribution of blank-micelles and CEO-micelles were measured by DLS. It indicates that the PDI of the samples ranged from 0.259 to 0.276 (Table 1) and exhibits a single-peaked distribution (Fig. 1A and Supporting information Fig. S1A). The result indicates that the particles are uniformly distributed in the suspension. Unloaded micelles (blank-micelles) presented a mean size diameter of 109.7 ± 0.23 nm (Table 1). The micelles average diameter was enlarged by incorporation of CEO (CEO-micelles), which was 218.3 ± 2.55 nm (Table 1). This result is expected because the incorporation of CEO into the hydrophobic core could increase the average size of the micelles (Xiao et al., 2021).

The zeta potential is an important factor to stability of colloidal dispersion system (Azouz et al., 2016). As shown in Table 1, the zeta potential values of blank-micelles and CEO-micelles were −14.9 ± 0.76 and −10.4 ± 0.06, respectively. The PEG chains on the surface of the micelles could improve the steric stability of micelles in aqueous solution (Ocal et al., 2014).

Morphological Characterization

The morphology of CEO-micelles was observed by TEM. It revealed that the micelles were uniformly distributed (Fig. 1B), with a nano-spherical shape. The result substantiated the formation of micellar nanostructures. The average sizes observed by TEM were slightly smaller than those measured by DLS (Table 1). The reasons for this result are the micelles average particle sizes were determined by DLS indicates the hydrodynamic diameter, while that observed by TEM means the collapsed micelles diameter after water evaporation (Venkatraman et al., 2005; Xiao et al., 2021). This seems to be consistent with the results of Xiao et al. (2021) who found that the particle size results from TEM analysis are smaller than the sizes obtained from the DLS for

Table 1 Mean particle size, PDI, zeta potential, and EE% of micelles

| Micelle              | Particle size (nm) | Polydispersity (PDI) | Zeta potential (mV) | EE%     |
|----------------------|--------------------|----------------------|---------------------|---------|
| PEG_{2k}-PCL_{5k}    | 109.7 ± 0.23       | 0.259                | −14.9 ± 0.76        | –       |
| PEG_{2k}-PCL_{5k}-CEO| 218.3 ± 2.55       | 0.276                | −10.4 ± 0.06        | 92.00 ± 1.77 |
| PEG_{2k}-PCL_{3k}    | 142.1 ± 0.44       | 0.381                | −3.21 ± 0.18        | –       |
| PEG_{2k}-PCL_{3k}-CEO| 238.5 ± 4.53       | 0.435                | −1.14 ± 0.09        | 75.77 ± 3.69 |

The values are expressed as the mean ± standard deviations of three experiments.

Fig. 1 A Size distribution curves of particle sizes of CEO-micelles (PEG_{2k}-PCL_{5k}-CEO); B transmission electron micrographs of CEO-micelles (PEG_{2k}-PCL_{5k}-CEO); C X-ray diffraction spectra of lyophilized blank-micelles and lyophilized CEO-micelles (PEG_{2k}-PCL_{5k}-CEO); D in vitro release of free-CEO at 75% RH, CEO-micelles (PEG_{2k}-PCL_{5k}-CEO) at 15% RH, 35% RH and 75% RH, values are the means ± SD.
agarwood essential oil loaded nanocellulose crystal-grafted polylactic acid copolymer micelles.

**X-ray Diffractometer (XRD) Analysis**

The XRD technique is used to identify the crystalline phase of a material. Verification of the formation of inclusion complexes can be based on analysis of crystallinity (Wang et al., 2013). Figure 1C illustrates the XRD diffractograms of blank-micelles and CEO-micelles. Blank-micelles appears strong diffraction signals of PEG-PCL (Liu et al., 2010). Similar peaks appeared for CEO-micelles, the diffraction peaks at 22.94° with a lower intensity than unloaded micelles (Fig. 1C). These results suggest that there may be an interaction between PEG-PCL and CEO, as the crystallization pattern of CEO-micelles changes when the host molecule was present in its inner core (Wang et al., 2013; Xiao et al., 2021).

**Encapsulation Efficiency Percentage (EE%)**

The calibration curve for the calculation of cinnamaldehyde concentration was linear in the range of 1–500 mg/L, and the equation was $y = 126,302x + 95,339$ ($y$: the area of peak; $x$: the concentration of cinnamaldehyde) with a related coefficient of $R^2 = 1$ (Supporting information, Fig. S2). The EE% of CEO-micelles could reach 92.00 ± 1.77% in PEG2k-PCL5k group, which is much higher than that of the PEG2k-PCL9k (75.77 ± 3.69%) (Table 1). It suggested that PEG-PCL nanomicelles have good encapsulation efficiency for CEO. The encapsulation efficiency of PEG-PCL nanomicelles for cinnamon essential oil is much higher than those of chitosan-based and β-cyclodextrin nanomicelles, which are widely used in the food industry (Das et al., 2021; Kujur et al., 2021; Yin et al., 2021). This is because the PCL can provide sufficient hydrophilic interaction with the CEO to allow its well-preserved encapsulation in the micelles core (Granata et al., 2018; Thonggroom et al., 2016).

**Release Characteristic of CEO from the Micelles**

Relative humidity is one of the most important factors in the storage of fruits and vegetables (Mascheroni et al., 2011). The sensitivity of PEG-PCL polymers to water molecules probably affected the release flux of CEO from the micelles (Min et al., 2021). Figure 1D illustrates the release characteristics of the CEO subject to humidity variations at a constant temperature. Free-CEO undergoes a sudden release in a short period of time, reaching 88% of the release in 12 h (Fig. 1D). On the contrary, encapsulated CEO (CEO-micelles) released only 6%, 10%, and 16% (Fig. 1D) under different RH conditions (15%, 35%, and 75% RH) in 12 h, respectively. This result demonstrates that encapsulation of essential oils can retard the rapid release of essential oils. Furthermore, CEO-micelles could respond to the different humidity to achieve the controlled release profile. The release rate of encapsulated CEO from micelles was achieved after 7 days at 75% RH, with 72% total amount release, while the release rate was lower at 15% RH (less than 30%) and 35% RH (less than 35%) (Fig. 1D). The effect of RH on the release of active compounds from amphiphilic polymer micelles perhaps attributed to the interaction of water molecules with PEG, which drives a degradation of the PCL block copolymer, resulting in the releasing of the CEO (Min et al., 2021). Low relative humidity conditions may be suitable for storage of micelles to avoid significant loss of essential oils prior to use. High RH probably conducive to achieving long-term and effective release of active compounds from amphiphilic polymer micelles for the purpose of control the growth of pathogenic microorganisms during the storage (Mascheroni et al., 2011).

**Cytotoxicity Studies**

Essential oils have many beneficial effects such as antifungal, antioxidant, and anti-inflammatory properties, but apart from these properties, EOs may also have potentially toxic effects (Llana-Ruiz-Cabello et al., 2015; Prakash et al., 2018). In addition, the incorporation of essential oils in active food packaging may lead to human exposure to these compounds, requiring in vitro toxicity assessment prior to use in food contact materials (Llana-Ruiz-Cabello et al., 2015). Figure 2 exhibits the results of cytotoxicity tests of free-CEO, blank-micelles, and CEO-micelles on L929 cell lines at different concentrations ranging from 0.25 to 4 mg/mL. The results indicated that blank-micelles did not induce toxic effects on cells after 24-h treatment at all experimental concentrations, and exhibits well biocompatibility. Free-CEO and CEO-micelles illustrated a concentration-dependent toxicity effect of L929 cell (Fig. 2B). Low concentrations (below 0.5 mg/mL) of CEO have a proliferative effect on cells, while at high concentrations it has a significant toxic effect on the cells, as this also can be seen from the microscopic images in Fig. 2A. The CEO encapsulated by micelles exhibits lower toxic effects than free-CEO at the same concentration; no significant toxic effects on cells were observed until concentrations higher than 2 mg/mL (Fig. 2B). This is due to the encapsulation and controlled release of essential oils of the non-toxic nanomicellar material reducing the toxicity to cells. Similar results were obtained by Suksiriworapong et al. (2012). In general, the result suggests that nanoencapsulation of essential oils is a feasible and effective way to reduce the toxic effects associated with high dose use of essential oils.
Testing of Antifungal Activity

Isolation and Identification of Fungi

The colony morphology of strain CM-H1 which isolated from infected strawberry was shown in supporting information Fig. S3. The fungal colonies were gray-white fluffy, later became dark gray, spore production after the color deepened, later light brown or black, the back of the colony initially light gray, later brown. These morphological features were similar with the *Botrytis cinerea* isolated by Kamaruzzaman et al. (2018).

ITS sequences are highly conserved sequences that have been shown to be useful for phylogenetic analysis of identified microorganisms. The ITS sequences of CM-H1 were analyzed by BLAST alignment and selected homologous sequences for phylogenetic analysis (Fig. 3A), CM-H1 was grouped with *Botrytis cinerea* (GenBank: KT006154) as a species with the similarity was 85%, therefore, combining the morphological identification and molecular biology identification results, the pathogenic bacterium CM-H1 screened in this study was identified as *Botrytis cinerea*.

Antifungal Activity

To assess the antifungal activity of the pure compounds, the minimum inhibitory concentration (MIC) was estimated. Figure 3B, C indicates that the MIC value of CEO and CEO-micelles were 80 μL/L of air. The morphological images and diameters of colonies of *Botrytis cinerea* during 7 days of culturing on PDA medium were showed in Fig. 3B, C. Colonies in the blank-micelles and control groups manifested no difference in diameter from each other (*p* > 0.05). The free and encapsulated forms of CEO presented antifungal properties against *Botrytis cinerea* and the antifungal activity improved significantly (*p* < 0.05) with the increased concentration (Fig. 3C). Regarding the antifungal mechanism of CEO, it has been reported in the literature that the volatile substances released by the CEO interact with fungal cell membranes, thus affecting their mobility and permeability, leading to morphological deformation, collapse, and deterioration of conidia or mycelium (Chaudhari et al., 2020; Clemente et al., 2016; Manso et al., 2013). Both free and encapsulated forms
of CEO at the same concentration presented the activity on control the mycelial growth of *Botrytis cinerea* ($p > 0.05$) (Fig. 3C). However, the mycelium of *Botrytis cinerea* treated with the CEO-micelles presented slightly smaller colony diameters than the free-CEO treated group, indicating that the CEO-micelles has a better in vitro antifungal activity than the free-CEO against *Botrytis cinerea* (Fig. 3C). This result could be attributed to the fact that the encapsulated CEO was slowly released from its carrier to the target microorganism at a relatively low rate, which can maintain an effective inhibitory concentration over a long period of time (Granata et al., 2018; Viacava et al., 2022).

**Effect of CEO-Micelles on the Postharvest Physicochemical Quality of Strawberry Fruits**

**Decay Rate of Strawberry**

The rate of fruit decay is the main apparent index to evaluate the storage effect. Most of the strawberries in the untreated group showed spots and moldy tips in 2 days (Fig. 4). After 4 days, there was a large area of mold infestation and a rotten odor, almost all of the strawberries rotted (83% decay rate) (Fig. 5A). The different concentrations of CEO and CEO-micelles have a significant positive effect on delaying
strawberry fruit decay during postharvest storage, with a concentration-dependent effect on slowing down strawberry decay. There was no significant ($p < 0.05$) difference in the inhibition effect between the same concentration of CEO-2 and CEO-micelles-2 treatment groups (Fig. 5A). Furthermore, strawberries of CEO-micelles-10 treatment group still retaining high commercial value in terms of apparent morphology at the end of the storage period (only 24% decay rate), reducing the decay rate by 59% compared to the control group (Fig. 5A).

### The Weight Loss of Strawberry

The weight loss of the fruits is mainly caused by respiration and transpiration. The changes of weight loss rate in different packing groups are shown in Fig. 5B. The increasing trend of weight loss rate in the control group indicates that the storage time will accelerate the decay and deterioration of strawberry. In contrast, CEO released from CEO and CEO-micelles could effectively delay the weight loss rate, indicating that the respiratory metabolism of strawberries could be inhibited (Alikhani & Daraei Garmakhany, 2012; Peretto et al., 2014).

### The Hardness of Strawberry

Hardness is an important parameter that reflects the degree of ripening and softening of the fruits. The tendency of strawberries to soften during storage is mainly related to the action of enzymes (pectinase, amylase, polyphenol oxidase, etc.), which greatly affects their postharvest life and susceptibility to fungal contamination (Peretto et al., 2014). As shown in Fig. 5C, the hardness of strawberries showed a gradual decrease during storage. The hardness of strawberries in the control group tended to decrease significantly during storage, reaching 6.8 N after 2 days, which was 35% of the initial value, and 2.6 N after 4 days, which was only 13% of the initial value (Fig. 5C). In contrast, the decreasing trend of strawberry hardness in the CEO and CEO-micelles treatment groups was slower. The fruit hardness of strawberries in the CEO and CEO-micelles groups with CEO addition of 10 μL/L was retained to 23% of the initial value after 4 days (Fig. 5C). This is because the released CEO probably slowed down the decomposition of enzymes inside the strawberry fruits, thus causing a slow decrease in hardness (Chu et al., 2020; Peretto et al., 2014).

### The Soluble Solid of Strawberry

TSS content is one of the important indicators of fruit ripeness and quality. The effects of different treatment conditions on the TSS content of strawberries during storage are shown in Fig. 5D. The TSS content of all treatment groups illustrated an increasing trend at the beginning of storage, and then decreased continuously with the extension of storage time (Fig. 5D). The TSS content of strawberry fruits in both CEO and CEO-micelles treatment groups was significantly ($p < 0.05$) higher than that of the control group at the end of the storage period (Fig. 5D). This may be due to the increased metabolic activity of the fruits and the rapid growth of microorganisms, resulting in extensive strawberry spoilage and loss of juice (Alikhani & Daraei Garmakhany, 2012).

### The Color of Strawberry

Color is a very important factor that affects consumers’ judgment of fruits and vegetable quality. The effect of CEO and CEO-micelles on skin color of strawberries during storage is measured by L* and a* values. The a* (greenness/redness) values of CEO and CEO-micelles treated strawberry fruits were not significantly ($p > 0.05$) different compared to the control group throughout the storage period (Fig. 5F). The L* (brightness) of strawberries in the CK group and all treatment groups
(Fig. 5E) was significantly ($p < 0.05$) affected by storage time, reflecting that the values in the time period from 0 to 4 days decreased. Changes in strawberry brightness were mainly associated with increased ripeness and oxidation via oxidative enzymes after picking, with later darkening associated with microbial infection (Alikhani & Daraei Garmakhany, 2012; Mitcham, 2007). On day 4, strawberries treated with the CEO and CEO-micelles groups were significantly brighter than the control samples, which may be related to storage conditions as well as fungal infections (Fig. 5E).
Conclusion

CEO loaded PEG-PCL micelles with a high encapsulation rate were successfully prepared in this study. XRD demonstrated that the CEO was successfully encapsulated in PEG-PCL micelles. The release rate could be controlled by adjusting the RH which could provide the feasibility of dynamic controlled releasing of CEO. The formed CEO-micelles had high antifungal activity against the main pathogenic fungus (Botrytis cinerea) of strawberries. The application of CEO-micelles to preserve strawberries also had a positive effect on changes in postharvest quality of strawberries. These findings imply that encapsulation of CEO in PEG-PCL micelles could be provided a new method for antifungals utilizations in active packaging products.

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Data Availability All data generated or analyzed during this study are included in this published article and supplementary information files.

Declarations

Competing Interests The authors declare no competing interests.

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