Modification of Poly Lactid Acid (PLA)/Chitosan with cinnamon essential oil for antibacterial applications

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Abstract. The active packaging material used for the manufacture of food packaging that has antimicrobial properties. The purpose of this research is to inhibit the growth of microorganisms on food surfaces, to improve nutritional quality and to extend the shelf life of food and reduce the environmental impact of packaging. The preparation is done Cinnamon essential oil (CEO) is a natural antimicrobial being investigated for food packaging as a substitute for synthetic chemicals due to consumer concerns over food safety. In this study, lactic acid poly (PLA) and chitosan were successfully modified with essential oil (CEO) at concentrations (1.5%, 2%, 2.5%, and 3% v/v) that formed composite fibres with using a simple electrospinning method. The morphology of the composite fibres can be seen from PLA / CS-CEO-2.5 showing good stability of the CEO so that the antimicrobial activity is increased compared to other blends. PLA / CS-CEO fibre shows a high level of long-term inactivation against Escherichia coli and Staphylococcus aureus due to the CEO's ongoing release, indicating that advanced PLA / CS-CEO fibres have great potential for active food packaging applications.

1. Introduction
At present, many new active packaging materials are receiving increasing attention in the food packaging industry. Active packaging can inhibit the growth of microorganisms on the surface of food, improve the quality of nutrients and sensory foods, extend shelf-life of certain foods, and reduce the environmental impact of packaging.

Cinnamon essential oil (CEO) is a natural antimicrobial being investigated for food packaging as a substitute for synthetic chemicals due to consumer concerns over food safety. Specifically, CEOs have low toxicity, low environmental impact, and high antibacterial and antioxidant activity [1].

However, the application of CEOs in active packaging is challenging because of its hydrophobicity, high volatility, and susceptibility to degradation from exposure to oxygen, heat, and light [2]. To overcome this challenge, many studies have shown that the most effective method is to encapsulate essential oils in a carrier to extend its application. Many polymers, such as liposomes, sodium alginate, and chitosan, have also been widely used to encapsulate and enhance the stability and bioactivity of essential oils [3].

Chitosan is a natural analogue of chitin formed by deacetylation of chitin. This has great potential for use as an active package because of its antibacterial and antifungal properties [3]. Chitosan has been loaded with cinnamon essential oil, Eucalyptus staigeriana essential oil, oregano essential oil, and limonene essential oil with the aim of increasing their stability under given environmental or
processing conditions and maintaining their antimicrobial activity [4,5]. Research shows that the incorporation of this essential oil increases thermal stability and water-holding capacity of CS and shows high bioactive properties for future active packaging applications [5,6]. Sotelo-Boyas et al. comparing the antibacterial activity of CS nanoparticles and nanocapsules combined with essential lime oil prepared by the nanoprecipitation and nanoencapsulation methods. They observed the highest antibacterial activity against S. dysenteriae for composite particles synthesized by nanoprecipitation [7,8].

CEO / CS nanoparticles obtained using these methods showed some antibacterial activity but did not meet all the requirements for good packaging materials. Among the encapsulation methods, electrospinning is a maturity technique that facilitates the production of polymer fibres combined with CEO-loaded particles.

Therefore, CS is often mixed with synthetic polymers such as PVA, poly (caprolactone), and poly (lactic acid) (PLA) to increase the spinnability of the CS polymer and improve the mechanical properties of the fibers produced [9,10]. Wen et al. reported polyvinyl alcohol / CEO / b-cyclodextrin (PVA / CEO / b-CD) electrospun, which showed excellent antimicrobial activity against Escherichia Coli (E. Coli) and Staphylococcus Aureus (S. Aureus) showing effective extension of shelf-life packaged strawberries [10,11]. In this study, we examine the effect of different CEOS (1.5%, 2%, 2.5%, and 3% v/v) on the encapsulation of efficiency, particle size, and antibacterial properties of CS nanoparticles combined with this essential oil. In addition, the structural and morphological properties of PLA / CS fibres with different CEO loads were evaluated. This biodegradable PLA / CS / CEO fibre is proposed to have excellent antimicrobial activity, making it a promising active food packaging material.

2. Method

2.1. Material and research tools
This research was synthesized with PLA (Mw = 15,000) by lactide and opening ring polymerization as previously reported [11]. CS (Mw 8000-1, 2000; 85% deacetylation) was given by Sinopharm Chemical Reagent Co., Ltd. (Chengdu, China). Acetic acid (>99.7% purity) and pentasodium tripolyphosphate (TPP) purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetic acid, glycerol, and Tween 80 were purchased from Meck (Darmstadt, Germany). Cinnamon essential oil is purchased from Hengcheng Natural Flavors Co. (Jiangxi, China). Difco Luria-Bertani (LB) broth was purchased from BD Life Sciences (Franklin Lakes, NJ, USA). All other chemicals and solvents which are of higher reagent grade or purity and are purchased from Chengdu Kelong Reagents Co. (Chengdu, China) unless otherwise stated.

2.2. CS-CEO nanoparticle preparation
CS-CEO nanoparticles were prepared following the published literature with some modifications [6]. Briefly, CS (0.5% w / v) was dissolved in 40 mL aqueous acetic acid solution (1% (v / v)) while continuing to be stirred under ultra sonication for 1 hour. Then surfactant 80 (1: 1, v / v) was added to CS solution with constant stirring at 50°C for 1 hour for the solution to dissolve completely. Then the CEO ethanol solution (20 mg / mL) is slowly added to the CS solution at a rate of 5 mL / h under strong stirring continuously for 50 minutes to get a CS: CEO volume ratio of 1.5%, 2%, 2.5 % and 3%. After the formation of a new homogenization, 15 mL TPP solution is slowly dropped into the solution continuously stirring for 50 minutes. CS nanoparticles are also synthesized using the same solvent.

2.3. PLA preparation and mixed with CEO-CS
The PLA is dried at 60 C in a vacuum oven overnight, and then dissolved at 25% by weight in 80:20 (v/v) trio, chloroacetic acid: dichloromethane, stirred for 12 hours at room temperature, and then mixed with CEO / CS targeted at a ratio of 1.5%, 2%, 2.5% and 3% (v/v), where the blended
The electrospun blender is denoted as PLA / CS, PLA / CS-CEO-1.5, PLA / CS-CEO-2, PLA / CS-CEO-2.5, and PLA / CS-CEO-3, respectively. The solution is then loaded at 1.0 mL/h and 40-50% humidity into a round capillary metal with an inner diameter of 0.7 mm, using a syringe pump (Zhejiang Medical University Instrument Company, Zhejiang, China) with a 5 mL syringe. The high voltage station (Tianjin High Voltage Power Supply Co., Tianjin, China) is then used to apply the voltage difference between the 20 kV syringe nozzle and the grounded collector placed 15 cm apart. Plate-type collector covered in aluminium foil is used to collect mats. The collectors are vacuum-dried at room temperature for two days to completely remove residual solvents.

2.4. Characteristics of Fourier Transform Infrared (FTIR)
Infrared spectroscopy of polyurethane obtained by KBr pellets using the Shimadzu FTIR spectrophotometer. The spectra were obtained in the mid-infrared region (4000-400 cm⁻¹) at room temperature.

2.5. Characteristics of Scanning Electron Microscope (SEM)
An electron gun produces electron beams and is obtained by the anode. Magnetic lenses focus electrons toward the sample. The focused electron beam scans the entire sample directed by the scanning coil. When electrons hit the sample, the sample will issue new electrons which will be received by the detector and sent to the monitor. SEM analysis to observe the surface of objects directly.

2.6. Characteristics of antibacterial properties
The antimicrobial effectiveness of PLA / CS-CEO fibres against E. coli and S. aureus can be determined [9]. An electrospun mat (10 mm × 20 mm) dipped in a test tube containing 4 mL of isotonic solution where 0.5 mL of E. coli and S. aureus inoculum, adjusted for cell concentration of 10⁷ CFU/mL, were given. The test tube is stirred at 200 rpm at 37 °C and the suspension solution is sampled from the test tube at each time the analysis and is serially diluted with peptone buffer water. A sample of 100 µL suspension is then spread to LB plates using the spread plate method. This plate was incubated for 24 hours at 37 °C and the colony-forming unit (CFU) was counted. Per cent reduction in bacteria calculated according to:

Reduction of bacteria (%) = (B - A) / B × 100  (1)

Where a log is 10 bacteria density for the treated substrate and B is the untreated substrate. There are no mat fibres and PLA fibres used as control samples.

3. Result and discussion

3.1. Morphological analysis using SEM
The surface of the resulting fibre can be seen in Figure 1. PLA / CS-CEO fibre folds are successfully produced. PLA composites show a smooth surface structure. PLA and Chitosan composites show that the surface is not good and visible particles that are not fused. PLA and CS composites modified with different CEO concentrations show a smooth surface structure, and fiber also with a smooth surface (without beads) and no visible separation of particles from the fibre matrix. Such morphology confirms that CS-CEO particles were successfully encapsulated into PLA fibres. It is interesting to note that when the CS-CEO concentration increases to ≥ 3%, the fiber has a smaller diameter and the simultaneous formation of the beads is observed [12].
3.2. Morphological analysis using FTIR

The FTIR test can be seen in Figure 2 showing the FTIR spectrum of a typical PLA, CEO, CS, and PLA / CS-CEO-2.5 sample. The CEO Spectrum shows characteristic bands at 1720 and 1706 cm\(^{-1}\) according to the vibrational framework associated with stretching C - C on the benzene ring and the carboxyl group (C = O). In addition, peaks in the region from 2000-1650 cm\(^{-1}\) in the spectrum of pure CEOs are caused by bending aromatic C-H ring bonds. In the CS spectrum, the characteristics of the absorption band were observed at 2800 cm (N-H bending), 3400 cm (amide I stretching), 3310 cm (amide II bending), 2500 cm (C-H stretching), along with peak widths between 3400 and 3700 cm that corresponds to stretch O-H. In the PLA spectrum, characteristic peaks were observed at 2992 cm (–CH asymmetric stretching), 1225 cm (–C- O stretching), 1454cm (–CH bending –CH3), and 870 cm (–C -C stretching), as reported in the literature. In the case of PLA / CEO-CS fibers, the peak characteristics of PLA and CS are observed. In general, the intensity of most CEO peaks depends on the summarized CEO concentration. This shows effective CEO encapsulation in CS and their good interactions. Its peak at 1020 cm\(^{-1}\) indicates the presence of an amino group in CS. In addition, the CEO characteristic peak at 1625 cm\(^{-1}\) and 1678 cm\(^{-1}\) shifted to 1628 cm and 1680 cm\(^{-1}\), respectively, in the PLA / CS-CEO-2.5 spectrum.

3.3. Antibacterial test

As shown in Figure 3, pure PLA does not show antibacterial activity, whereas PLA / CEO-CS fibre shows antimicrobial activity against E. coli and S. aureus for a period of 120 hours (5 days), where antibacterial activity increases with increasing concentration CEO. Although PLA / CS-CEO-1.5 shows some inhibition of E. coli and S. aureus, antimicrobial activity depends on CS, CEO concentration, and time. However, in this study, there were also obstacles from PLA / CS-CEO-2.5
and PLA / CS-CEO-3 fibres gradually decreasing after 70 hours of treatment time, which is similar to the study release. After 70 hours, PLA / CS-CEO-2.5 and PLA / CS-CEO-3 fibre have released almost all CEOs into the medium. Therefore, the final number of CEOs released to the medium slows down over time. Then, this indicates that CEO release is controlled from composite films is very important to ensure antibacterial activity against this type of test [13]. The maximum concentration was achieved in the PLA / CS-CEO-2 sample, which showed complete inhibition of E. coli and S. aureus during the incubation period, and the highest antibacterial efficiency was 99.3% and 98.4%, respectively. This may be because the strong interaction between CS and CEO and high CEO crystallinity results in lower PLA solubility, allowing CEOs to do the same with antimicrobial action even when released slower [15]. It is interesting that the PLA / CS-CEO is more effective against Gram-negative E. coli than that of Gram-positive S. aureus.

Figure 3. Graph of antimicrobial activity against E. coli and S. aureus.

4. Conclusion
CS - CEO nanoparticles are used as carriers for the addition of CEOs to active packaging materials. PLA / CS-CEO fiber is obtained by electrospinning to achieve release from the CEO concentration. The influence of the number of CEOs is loaded on the structure and morphology of the fibers viewed using FTIR, SEM, and antibacterial analysis. The results show that combining CEOs can enhance the antibacterial properties of PLA / CS-CEO fibers. The optimal composition was found PLA / CS-CEO-2, which showed the highest antibacterial efficiency for a long time. Therefore, such as PLA / CS-CEO fibers exhibited significant potential for active food packaging and other applications where antibacterial activity is required.

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