Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134601 2025, 24(6) 1393-1415

Research Article

Electrospun chitosan-polycaprolactone fibers with thyme essential Oil/ZnO nanoemulsion: Fabrication and properties

Aghaei Marzango Y.[1], Nejatian M.[2,3*], Mousavi Nadushan R.[4], Ashrafi F.[5]

1Department of Food Science and Technology, NT.C., Islamic Azad University, Tehran, Iran
2Department of Nutrition Science and Food Hygiene, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran
3Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
4Department of Food Science and Technology, NT.C., Islamic Azad University, Tehran, Iran
5Department of Microbiology, NT.C., Islamic Azad University, Tehran, Iran
*Correspondence: mnejaatian@gmail.com

Keywords

Emulsion electrospinning,
Encapsulation,
Core-shell,
Active packaging,
Antimicrobial activity,
Hybrid structure

Article info

Received: March 2025
Accepted: July 2025
Published: November 2025

Abstract

This study developed and characterized electrospun chitosan/polycaprolactone (CS/PCL) nanofibers incorporating a thyme essential oil (TEO)/zinc oxide (ZnO) nanoemulsion for active food packaging applications. Nanofibers were fabricated via electrospinning, with TEO (3% and 6% w/w) and ZnO integrated through an emulsion method, and compared against a direct mixing approach. Scanning electron microscopy (SEM) revealed well-oriented, smooth, and bead-free nanofibers with average diameters ranging from 60 nm (with TEO) to 88 nm (without TEO). Antimicrobial efficacy, assessed over five days, indicated a time-dependent decrease for most nanofibers. Crucially, nanofibers prepared via the emulsion method demonstrated significantly sustained antimicrobial activity compared to those produced by direct mixing. Specifically, TEO-loaded nanofibers, encapsulated via emulsification, effectively maintained the total bacterial count of packaged meat within the standard range (<6 Log CFU/g) for up to 6 days. These findings highlight the significant potential of electrospun CS/PCL nanofibers incorporating emulsified TEO/ZnO for developing effective antimicrobial food packaging solutions, thereby enhancing food safety and extending shelf life.

Introduction

Food packaging is critical for preserving food quality and safety by safeguarding against environmental influences and microbial contamination, which helps maintain the quality and safety of commercial products (Gasti *et al.*, 2022). Traditionally, synthetic plastic-based materials have been widely employed for this purpose. However, growing concerns regarding these plastics stem from their degradation into micro- and nanoplastics through physical and chemical processes, which can contaminate the environment, enter the human food chain, and pose adverse health risks (Liu *et al.*, 2021). Consequently, there is a heightened interest in replacing conventional synthetic packaging with sustainable alternatives derived from natural polymers, such as polysaccharides and proteins (Liu *et al.*, 2022).

Chitosan stands out as a highly promising biomaterial for biodegradable film production, owing to its abundance, cost-effectiveness, excellent film-forming capabilities, low toxicity, and inherent preservative attributes (Cardoso *et al.*, 2019). Derived from the partial deacetylation of chitin, this polysaccharide uniquely possesses potent antimicrobial, antifungal, and antioxidant activities, rendering it particularly suitable for food packaging applications (Radulescu *et al.*, 2015). However, a significant limitation of pure chitosan films is their poor water resistance and low mechanical strength, which leads to softening and disintegration upon contact with water (Motelica *et al.*, 2020). These drawbacks severely restrict their utility in food packaging, especially in high-humidity environments. Consequently, research efforts are focused on strategies to enhance the physicochemical and functional properties of chitosan films, including blending with other polymers and incorporating nanoparticles (Cao and Song, 2019; Taherimehr *et al.*, 2021).

Polycaprolactone (PCL) is a widely studied biodegradable thermoplastic polymer, valued for its low melting point, excellent thermal processability, and low viscosity. Its widespread use in packaging applications is attributed not only to its biodegradability but also to its biocompatibility with other polymers and superior mechanical properties (Thakur *et al.*, 2021). To further enhance film performance, the incorporation of inorganic nanoparticles, such as silver or titanium dioxide, has been shown to improve mechanical attributes (Azari *et al.*, 2020). Similarly, zinc oxide (ZnO) nanoparticles (ZnO NPs) can be directly integrated into polymer matrices, including chitosan, to bolster mechanical and barrier properties. ZnO NPs also result in better packaging performance and longer food shelf-life. The primary antimicrobial mechanism is the production of reactive oxygen species, which can vary based on morphology and size. In general, ZnO NPs can halt the growth of fungi, Gram-positive, and Gram-negative bacteria, lowering the risk of cross-contamination and thereby increasing the longevity of products (Kim *et al.*, 2022; Nejatian *et al.*, 2023). For the fabrication of such advanced materials, electrospinning stands as the most common and effective method for producing nanofibers from biopolymers, yielding structures with high

surface-area-to-volume ratios and desirable mechanical characteristics (Phan *et al.*, 2021).

Chitosan electrospinning is enhanced by additives like chitin nanofibers (improved molecular orientation) or polyethylene oxide (increased chain entanglement via H-bonding) (Darbasizadeh *et al.*, 2018; Dobrovolskaya *et al.*, 2018). Combining chitosan with PCL in solvents like acetic/formic acid increases solution conductivity and viscosity, enabling electrospinning at lower concentrations (Van der Schueren *et al.*, 2012). Chitosan/PCL composite nanofibers effectively encapsulate bioactive compounds (*e.g.*, cinnamaldehyde (CIN), oregano oil (OEO), chrysanthemum oil (CHEO)), demonstrating sustained release (73-78% CIN retained after 96h) and potent antibacterial activity against food pathogens (Rieger and Schiffman, 2014; Lin *et al*., 2019; Ardekani-Zadeh and Hosseini, 2019; Wang *et al.*, 2019). Similar antimicrobial efficacy is shown by clove oil (CLV) in PCL/gelatin nanofibers, without cytotoxicity (Unalan *et al.*, 2019; Mohajeri *et al.*, 2023). Thyme essential oil (TEO) contains compounds such as thymol, carvacrol, and 1,8-cineole, such that the antimicrobial properties of the whole essential oil are greater than any of its components, indicating a synergistic effect of each of the compounds in the essential oil with each other. This plant is abundantly found and is also cultivated in plant breeding centers. Its essential oil is used in foods and, in addition to therapeutic and health applications, it is also consumed orally. Due to its excellent antimicrobial and antioxidant properties, thyme may be added to packaging materials to increase the shelf life of food products (Tajbakhsh and Moumeni, 2015; Nieto, 2020).

Most previous studies have focused on the direct addition of bioactive compounds to electrospinning solutions to fabricate nanofibers, which may result in a sudden and intense release of the bioactive compounds physically encapsulated on the fiber surface. For example, encapsulation of curcumin in gelatin nanofibers resulted in a release of more than 70% after only 4 hours (Deng *et al.*, 2017). In another study, where caffeine was directly mixed into a cellulose spinning solution, 60% of the caffeine was immediately released from the nanofibers into an aqueous solution (Furtado *et al.*, 2020). Additionally, storage time significantly impacts the antimicrobial and mechanical properties of bioactive-loaded nanofibers. Since essential oils are volatile and prone to oxidation, their gradual evaporation and degradation over time reduce their effective concentration in nanofibers, leading to diminished antimicrobial activity. Without controlled-release systems (*e.g.,* encapsulation in emulsion, cyclodextrins or liposomes), this decline becomes more pronounced (Aytac *et al.*, 2017). To overcome this challenge and improve the mechanical properties of the produced film, strategies such as encapsulation (*e.g.,* within nanoemulsions before electrospinning), polymer crosslinking, or combining EOs with stable antimicrobial agents (*e.g.,* silver or ZnO nanoparticles) are recommended to enhance the long-term stability and performance of nanofibers (Farahani *et al.*, 2020). This study innovates by incorporating TEO, either directly

encapsulated or hybrid nanoencapsulated, into chitosan/PCL nanofibers. It specifically investigates if TEO hybrid nanoencapsulation within the nanofibers provides more stable, sustained release than conventional TEO encapsulation, enhancing antimicrobial film longevity.

Materials and methods

Materials

PCL with a molecular weight of 80,000 Da and medium molecular weight chitosan (MMWC) with a deacetylation degree of 75-85% were purchased from Sigma-Aldrich Company. The required solvents were obtained from Dr. Mojallali Chemical Industries Complex (Tehran, Iran) and TEO was sourced from Barij Essential Pharmaceutical Company. Bacterial strains including *Listeria monocytogenes* ATCC 13932 and *Escherichia coli* ATCC 25922 were also prepared from the Industrial Research and Training Center of Iran.

Preparation of electrospinning solutions

Two solution strategies were adopted in polymer blending (Ardekani-Zadeh and Hosseini, 2019; Ma *et al.,* 2019). In the emulsion method, a given amount of chitosan (CS) was dissolved in acetic acid solution (2%, v/v) by stirring at 600 rpm for 3 h to obtain a clear 2% (w/w) solution (To remove any undissolved chitosan impurities, the chitosan solution was filtered through a 1 μm filter). The pH of the solutions was adjusted to 4 using a 5N of NaOH solution. Tween-80 (half the amount of essential oil) was added as a surfactant to the solution and stirred at 60 °C for 30 min to achieve a homogeneous mixture. After cooling, TEO was gradually added at different concentrations (3% and 6% w/w) into the aqueous phase under vigorous stirring for 4 minutes, followed by an additional 20 minutes of stirring. An 8% (w/w) PCL dispersion was prepared by dissolving certain amounts of PCL in dichloromethane. The chitosan (2%) and PCL (8%) dispersions were then mixed to form two distinct phases (aqueous and oil) in the emulsion at a volume ratio of 8:2. The prepared mixtures were stirred continuously for 8 hours at room temperature.

In the conventional mixing method, a 2% CS solution was first prepared by dissolving 2 g of CS powder in 100 ml of acetic acid (2% v/v). PCL was dissolved in acetic acid to prepare an 8% (w/v) solution. Then, these solutions were mixed for 72 hours at room temperature. Next, the essential oil was added to the above mixture at concentrations of 3 and 6% (w/w) and mixed for an additional hour.

Characterization of electrospinning solutions

The viscosity of the electrospun solutions was determined using a rotational viscometer (Brookfield, model DV III ULTRA, USA). For this purpose, concentric cylinder geometries including ULA, SC4-18, SC4-25, 31 and SC4-34 were used. During the test, the temperature of the sample inside the geometry was kept constant at 25°C by a circulator (Nejatian and Abbasi, 2019).

Electrospinning process

The electrospinning process performed in the present study was based on the slightly modified procedure described by Nejatian

et al. (2023). The upper surface of a clean polyethylene film was covered by the polymer solutions prepared in the previous step using an industrial electrospinning unit (INFL260B, Iran). This device is nozzleless and has 2 electrospinning units. The electrospinning process parameters such as spinneret and collector settings, electrospinning distance (12 cm), linear speed of 20 cm/s, temperature (30ºC), and humidity 40% were controlled and adjusted using an advanced integrated control system.

Characterization of electrospun fibers

Field emission scanning electron microscopy (FE-SEM) was used to characterize the morphology of the electrospun nanofibers, and the average fiber diameter was determined by Image J software by randomly measuring 50 fibers. Before imaging, the samples were coated with a thin layer of gold and imaging was performed at different magnifications. In addition, energy-dispersive X-ray spectroscopy (EDX, EDS) was used to study the chemical composition of the nanofibers formed on the film (Nejatian *et al.*, 2023).

The nanofibers were also characterized by Fourier transform infrared spectroscopy (FTIR) over the wavenumber range of 400-4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}. The crystal structure of the prepared nanofibers was investigated using an X-ray diffraction (XRD) device over the 2θ scanning range of 2-50º at a step size of 0.02°/min (Ardekani-Zadeh and Hosseini, 2019).

Water vapor permeability (WVP)

The WVP of the nanofibers was determined according to the American society for testing and materials E96 method (ASTM, 2002). For this purpose, 10 ml of distilled water was added to the permeability measurement cells. Then, the surface of the cells (with a diameter of 30 mm) was covered with nanofibers using silicone grease adhesive and the cells were placed in a desiccator including dried silica gel. The difference in humidity on both sides of the nanofibers at a temperature of 25°C creates a vapor pressure difference of 2.337×10^3 Pa. The weight changes of the cells were measured at 2-hour intervals over 12 hours using a digital precision scale with an accuracy of 0.0001 g. Then, by plotting the curve of cell weight changes versus time, a straight line was obtained. Next, WVP was measured according to the following equation (ASTM, 2002):

$$WVP = \frac{WVTR \times L}{\Delta P}$$

Where, WVP is the water vapor permeability in terms of g mm/kPa h m^2, WVTR is the water vapor transmission rate (obtained by dividing the slope of the drawn line by the film surface area), L is the average thickness of the sample (mm), and ΔP is the difference in vapor pressure inside and outside the cell, equivalent to 2.337×10^3 Pa.

Evaluation of antibacterial activity

The antibacterial activity of the sample was evaluated by the colony forming unit (CFU) method according to the reference. The samples were sterilized by 70% ethanol and physiological solution. Then the bacterial suspension was prepared according to the

McFarland standard and its OD was checked at λ 630 nm. 1000 µL of bacterial suspension was poured on the samples and incubated for 24 hours at 37±1°C. Next, dilution was done in a ratio of 10 to 90 and the diluted bacteria were placed on Nutrient Agar culture medium and incubated for 18 hours at a temperature of 37±1°C. After incubation, the average number of colonies was determined by the Image J software and the antibacterial activity of the samples was calculated using the following equation (Aytac *et al.*, 2017),

$$R(\%) = \frac{N_A - N_B}{N_A} \times 100$$

Where, R (%) denotes the antibacterial activity of the sample in percent. N_A is the number of colonies in the sample before contact with the antibacterial agent, and N_B is the number of colonies in the sample after contact with the antibacterial agent.

The use of electrospun nanofilm in the packaging of meat

ZnO/thyme electrospun nanofilm was used as the top layer of a cube-shaped polyethylene package (modified from Nejatian *et al.*, 2023). For this purpose, first, 250 g of meat was packed and sealed with a layer of the prepared nanofiber film. The control sample consisted of meat packaged in a completely polyethylene package. All samples were stored at refrigerated temperature and total bacterial counts of the samples were performed for 6 days.

Statistical analysis

Each experiment was carried out in triplicate. Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, version 15) and the Duncan's multiple range test was used to compare the mean values at a confidence level of $\alpha<0.05$.

Results

Fiber Morphology

Figure 1 illustrates the surface morphology of nanofibers produced from various formulations of chitosan, PCL, TEO and ZnO NPs. Pure chitosan/PCL nanofibers (containing no TEO) were successfully electrospun from a solution containing two polymers in a mass ratio of 2:8. As shown in this figure, these nanofibers exhibited a continuous cylindrical structure, were relatively well-oriented, bead-free, and featured a smooth surface.

Also, nanofibers were efficiently electrospun from solutions containing both essential oil concentrations (3 and 6%) prepared by two different loading types (emulsion and simple mixing) (images C, E, G, and I in Fig. 1). As shown in this figure, most of the nanofibers exhibited a linear morphology, a smooth surface, and a bead-free structure. However, when essential oil was added to the electrospun solutions prepared using the simple mixing method, the resulting fibers displayed a slight bead structure.

The viscosities of electrospinning solutions, along with the average diameter of the fibers prepared by them, are shown in Table 1. The control solution (containing a pure mixture of chitosan and PCL) has the highest viscosity (776.3±6.2 cP). After the control sample, the electrospinning solution prepared by the emulsion method showed a higher viscosity; as the viscosity of the emulsions containing 3 and 6% TEO was 517.9±4.1 and 258.8±8.3 cP, respectively.

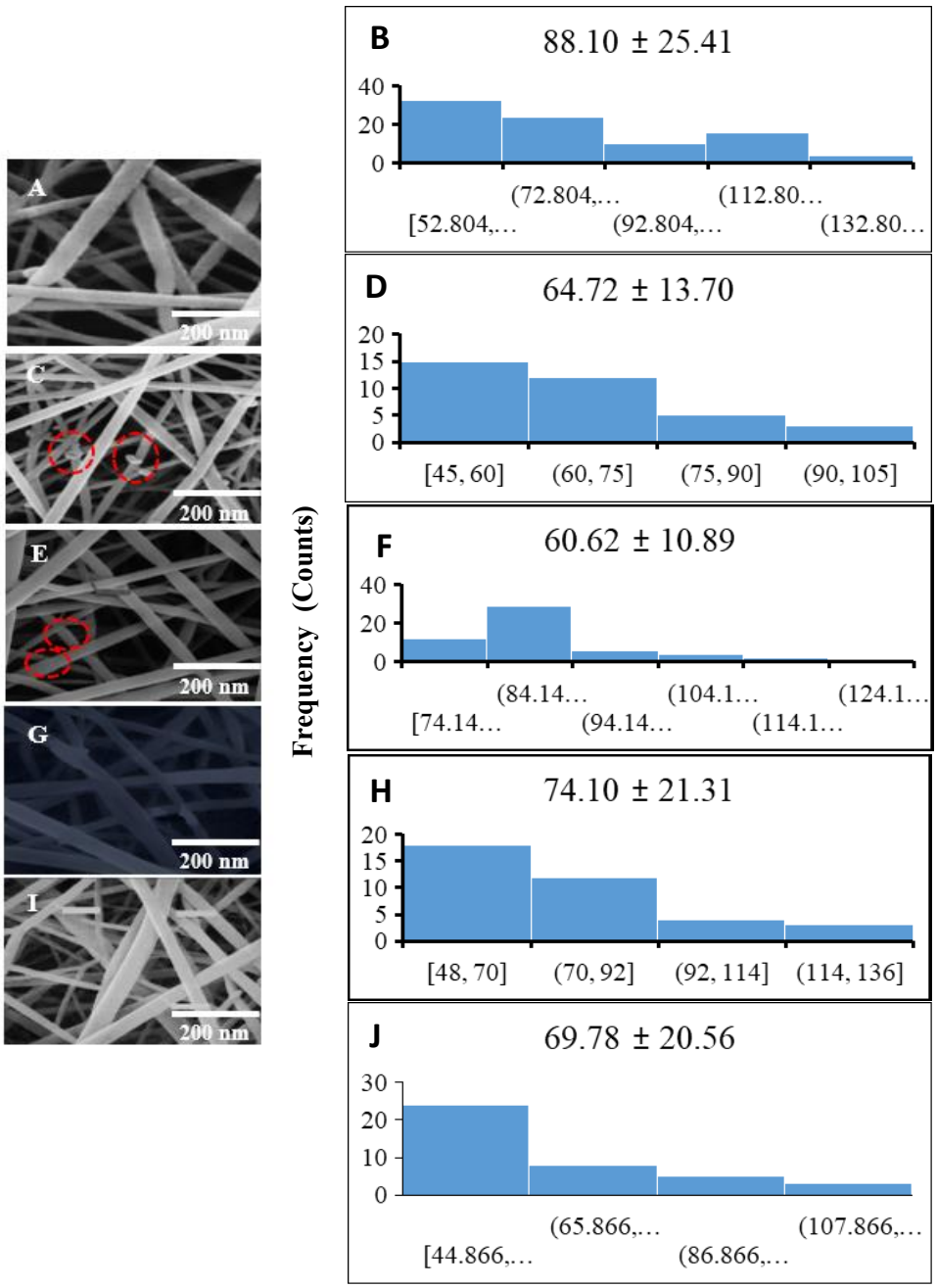

Figure 1: SEM images of pure CS/PCL (A) and thyme oil encapsulated nanofiber produced by conventional mixing (C: CM/3%EO; E: CM/6%EO) or emulsion (G: E/3%EO; E/6%EO) method. The equivalent fiber diameter distribution (B, D, F, H, J) along with the mean fiber diameter and standard deviation (n ≈ 50).

Table 1: Viscosity values of electrospinning solutions and size of the resulting nanofibers.

Fibers	Viscosity (cP or mPa.s)	Fiber diameter (nm)
CS/PCL (control)	776.3 ± 6.2	24.41 ±88.10
E/3%EO	517.9 ± 4.1	21.31 ±74.10
E/6%EO	258.8 ± 8.3	20.56 ±69.78
CM/3%EO	181.2 ± 2.7	13.70 ±64.72
CM/6%EO	155.3 ± 5.7	10.89 ±60.62

The lowest viscosity was related to the electrospinning solutions prepared by the simple mixing method, as the viscosity values of 181.2±2.7 and 155.3±5.7 were measured for the mixed solutions containing 3 and 6% TEO, respectively.

The elemental composition of the two nanofibers (E/3%EO and CM/3%EO) obtained from EDX analysis is shown in Figure 2. The peaks due to carbon, oxygen and zinc in the EDX spectra indicate the starting materials i.e. chitosan, PCL and ZnO NPs. The E/3%EO nanofiber showed 75.6 wt% carbon, 22.9 wt% oxygen, 0.8 wt% nitrogen and 0.7 wt% zinc. Carbon with 85.2 wt%, oxygen with 12.2 wt%, nitrogen with 2.4 wt% and zinc with 0.2 wt% were the constituent elements of the composition of the CM/3%EO fiber. Therefore, the peaks due to zinc element confirm the existence of ZnO NPs on the top surface of the fibers. In addition, almost no nitrogen peak was observed in the spectrum of fibers prepared by the emulsion method (in other words, the amount of nitrogen on the top surface of the fibers was very small), indicating that chitosan was not located in the surface structure of the fibers or was located in the central core of the fiber structure. On the other hand, the amount of nitrogen in the surface composition of fibers prepared by the conventional mixing method was significantly high. Another difference in the elemental composition analysis of the fibers prepared by the two different methods was the 3.5 times higher percentage of zinc element in E/3%EO fibers compared to nanofibers prepared by the conventional mixing method (CM/3%EO).

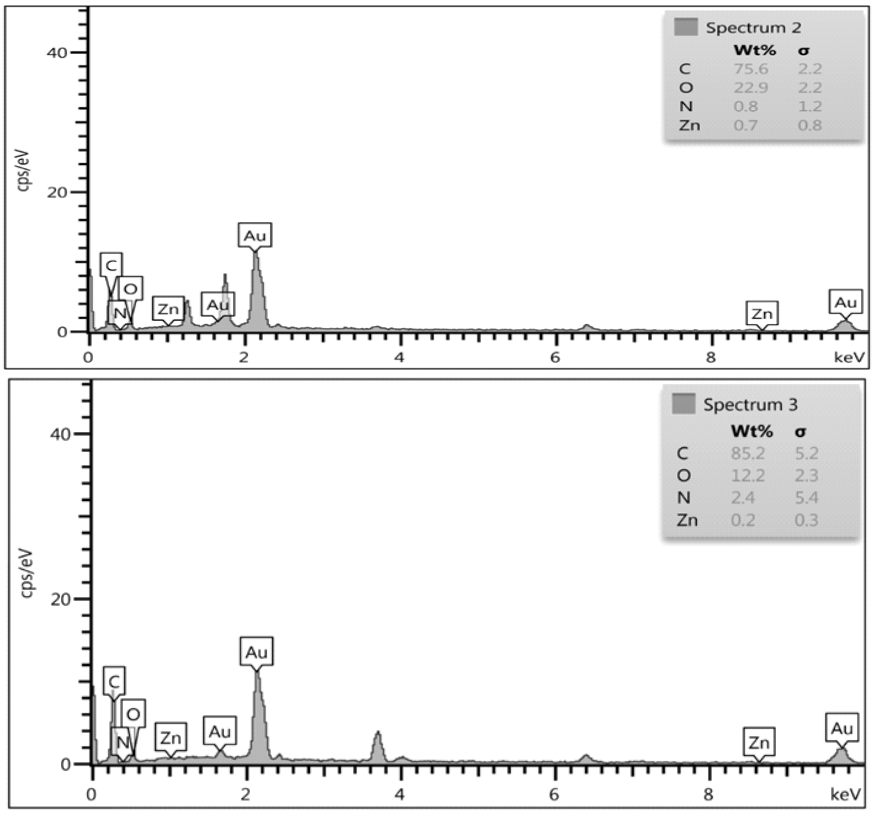

Figure 2: EDX elemental analysis of nanofibers prepared by emulsion method (top) and simple mixing (bottom).

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of electrospun nanofibers with/without TEO and prepared by different methods was evaluated to reveal any possible interactions between the CS/PCL biopolymer matrix. In the spectrum of the control nanofibers, peaks at 1370, 1464, 1746, 2849, 2932, and 3800-3500 cm^{-1} were identified. In general, the peaks remained unchanged after adding 6% TEO and ZnO NPs to the nanofibers prepared by both the emulsion (E/EO6%) and simple mixing (CM/EO6%) methods (Fig. 3).

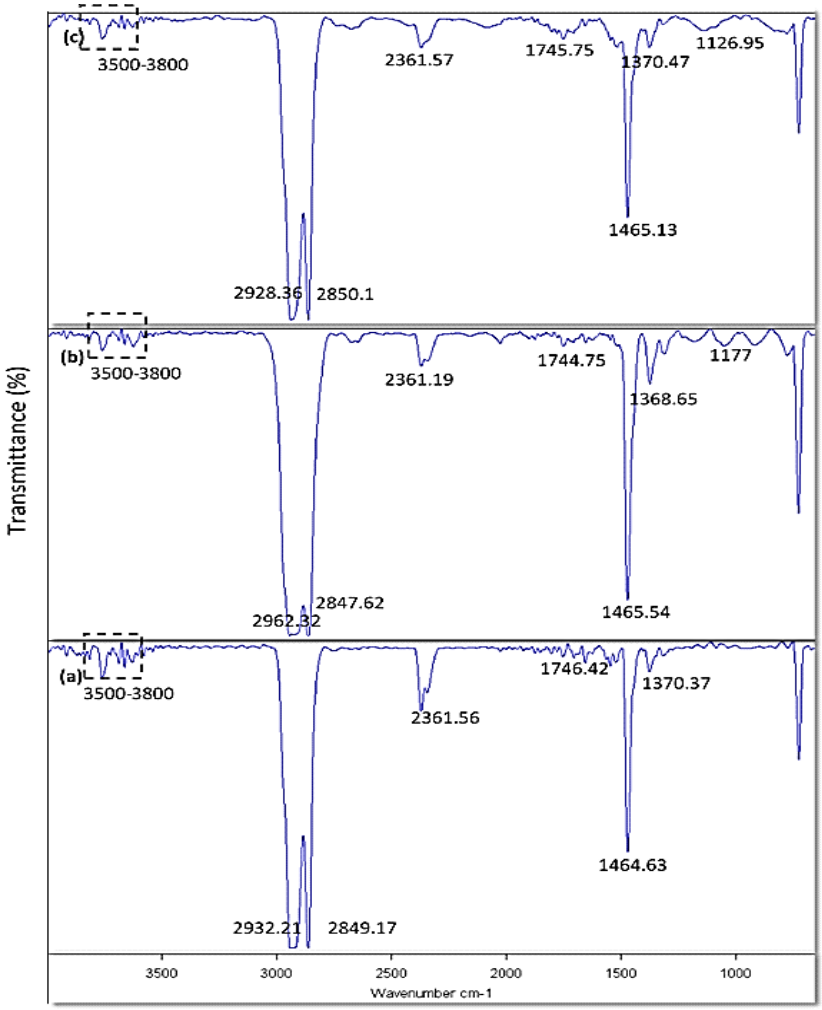

Figure 3: FTIR spectra of PCL/CS (a), E/6%EO (b) and CM/6%EO (c) nanofibers.

However, with the encapsulation of essential oil in the nanofibers, a new peak appeared at the beginning of the spectrum (1177 cm^{-1} for E/EO6% and 1127 cm^{-1} for CM/EO6%). In other words, the main peaks identified in the FTIR for E/EO6% nanofibers were 1177, 1368, 1465, 1744, 2847, 2932 and 3800-3500 cm^{-1}. Also, the FTIR of CM/EO6% nanofibers was primarily characterized by the peaks at 1127, 1370, 1465, 1746, 2850, 2928, and 3800-3500 cm^{-1}. In addition, three peaks at wavelengths of about 1464, 28450 and 2930 cm^{-1} were more intense in all 3

nanofibers. The characteristic peak at 3000-3800 cm^{-1} was related to the OH and NH stretching of the primary amino groups and the stretching vibration band of the CH_2 groups was located at 2868-2949 cm^{-1}. In more detail, the absorption peaks above 2925 cm^{-1} and around 2850 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the CH_2 groups, respectively. The absorption peak at 1746 cm^{-1} is attributed to the carbonyl (C═O) groups. In addition, the FTIR peaks at about 1460 cm^{-1} and 1370 cm-1 can be due to the bending vibration peaks of CH_2 and OH, respectively. The stretching vibration peaks of the C ─ O ─ C groups were located at around 1172-1240 cm^{-1}. It should be noted that the peak at 2361 cm^{-1} in FTIR is most likely related to the carbon dioxide (CO_2) functional group, which is usually observed due to the presence of carbon dioxide in the measuring device.

XRD analysis

X-ray diffraction test was performed to investigate the possible change in crystallinity of electrospun nanofibers. The results of this analysis for control nanofibers and nanofibers containing 6% essential oil and prepared by two different methods are shown in Figure 4. As is clear in Figure 4-a, control nanofibers exhibited two distinct peaks at 2θ=21.4 and 2θ=23.7 degrees, which indicates the semi-crystalline nature of the polymers, especially PCL. Nanofibers prepared by the emulsion method and containing 6% TEO showed the same distinct peaks of PCL in the X-ray diffraction test with only a slight shift (Fig. 4-b). In addition to a slight shift in the distinct peaks of PCL, CM/6%EO nanofibers showed significant differences in peak intensity (count) compared to the control sample (Fig. 4-c).

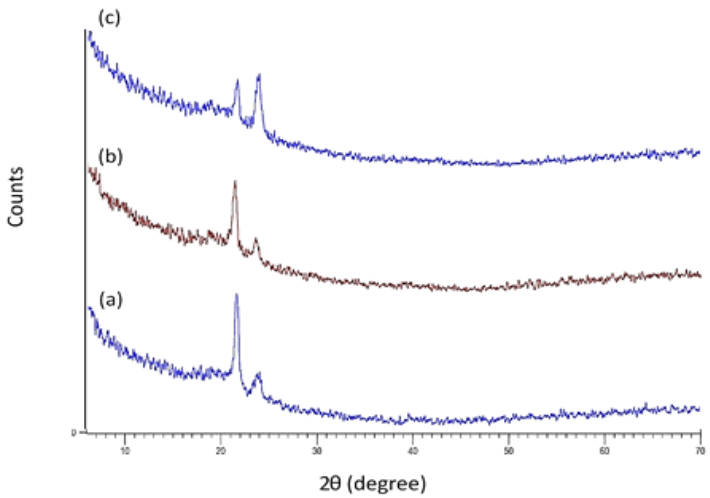

Figure 4: XRD spectra of control nanofibers (a), nanofibers prepared by the emulsion method (b), and nanofibers prepared by the conventional mixing method (c).

Water vapor permeability (WVP) of nanofibers

The WVP values of CS/PCL nanofibers containing different concentrations of TEO and prepared by different methods are shown in Table 2. The WVP values of electrospun nanofibers ranged from 0.075 to 0.276 g.mm/h.m^2.kPa. By adding TEO to

the composition of the produced films, the water vapor permeability increased. The lowest WVP value of nanofibers containing essential oil was related to E/3%EO (0.145±0.056 g.mm/h.m^2.kPa). With increasing the amount of essential oil (6%) in the samples prepared by the emulsion method, the WVP value increased significantly and reached about 0.231 g.mm/h.m^2.kPa. Nanofibers prepared by the conventional mixing method and containing both concentrations of TEO had the highest water vapor permeability (about 0.276 and 0.266 g.mm/h.m^2.kPa for films containing 3 and 6% essential oil, respectively). However, no significant difference was observed between the WVP values of these two films.

Table 2: WVP values of CS/PCL nanofibers containing different concentrations of thyme essential oil and prepared by different methods.

Fibers	Water Vapor Permeability (WVP g.mm/h.m^2.kPa)
CS/PCL (control)	0.075 ± 0.027^a
E/3%EO	0.145 ± 0.056^b
E/6%EO	0.231 ± 0.019^c
CM/3%EO	0.276 ± 0.102^c
CM/6%EO	0.266 ± 0.110^c

A different lowercase letter in a column indicates a significant difference.

Antibacterial activity of nanofibers

As seen in Figure 5, the antibacterial activity of the prepared nanofibers was examined against *Escherichia coli* (as a gram-negative bacterium) and *Listeria monocytogenes* (as a gram-positive bacterium). Also, the nanofibers exhibited antibacterial activity against both studied bacteria, with significantly higher effectiveness against *Escherichia coli* (68.73±5.50 %) than against *Listeria monocytogenes* (49.22±6.74 %). Encapsulating TEO and ZnO NPs within the fibers prepared by both methods notably enhanced their antibacterial activity. For example, nanofibers produced using the emulsion method and containing 3% and 6% TEO exhibited about 85% and 91% anti-growth activity of *Escherichia coli*, respectively. Also, the anti-growth activity of *Escherichia coli* in nanofibers fabricated using the conventional mixing method and containing 3% and 6% essential oil was estimated as about 96% and 98%, respectively. The antibacterial activity of various nanofibers against the gram-positive bacterium *Listeria monocytogenes* was significantly lower than that observed against *Escherichia coli*. The anti-*Listeria* activity of E/3%EO and E/6%EO nanofibers was about 68%, and 79%, respectively. The corresponding values for those prepared by the conventional mixing method and containing 3 and 6% thyme oil were about 80 and 86%, respectively (Fig. 5).

According to these results, the anti-*Escherichia* activity of different nanofibers was compared with each other over different times (1, 3, and 5 days). The antibacterial activity of nanofibers prepared by the emulsion method after 1, 3, and 5 days of storage was 85.37, 85.65, and 81.27% for samples containing 3% emulsion, respectively, and 90.69, 91.26 and 89.74% for samples containing 6% essential oil, respectively. Also, the antibacterial activity of nanofibers prepared by the conventional mixing method containing 3 and 6% essential oil increased from about 95.68% and 98.54% on the first day of storage to 94.87% and 97.49% on the third day. However, by the fifth day of

storage, the anti-*Escherichia* activity of CM/3%EO and CM/6%EO nanofibers decreased to about 45.50% and 44.46%, respectively. It is important to note that the sample without encapsulated essential oil (CS/PCL) displayed an anti-*Escherichia* activity of about 68% on the first day, which significantly decreased over time, dropping to about 57% and 33% on the third and fifth days, respectively (Fig. 6).

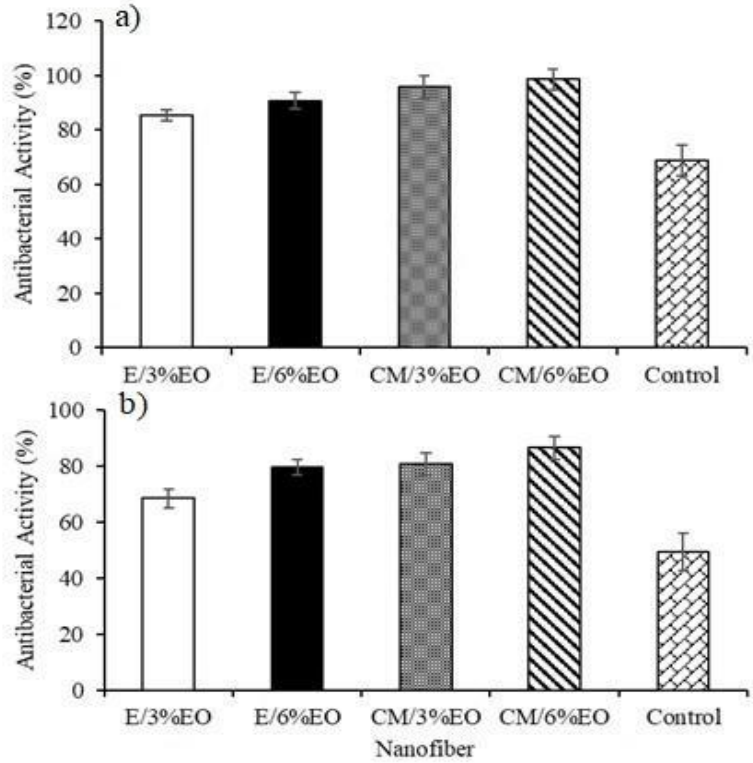

Figure 5: Antibacterial activity of nanofibers produced using various methods and different concentrations of thyme essential oil against *Escherichia coli* (a) and *Listeria monocytogenes* (b) on the first day of production.

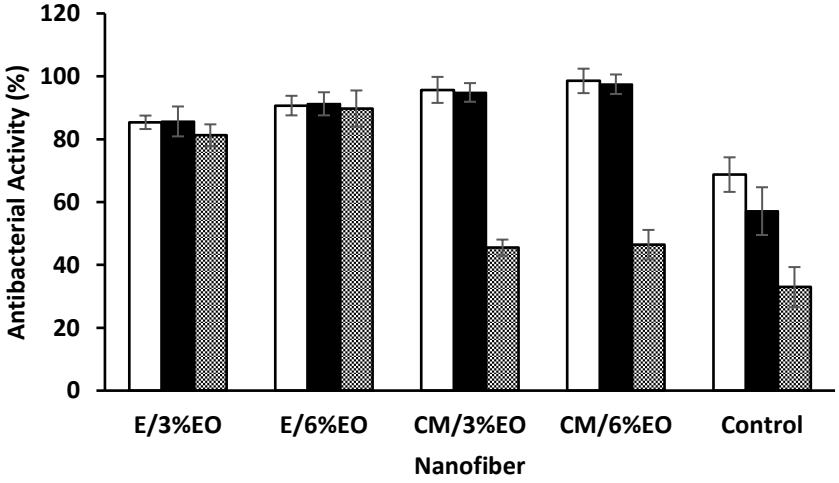

Figure 6: Antibacterial activity of nanofibers prepared by various methods and different concentrations of thyme essential oil against *Escherichia coli* at three different times (□ Day 1, ■ Day 3, and ▩ Day 5 of storage).

Total count of meat microorganisms during storage

The total bacterial count of meat during storage is an essential indicator of its quality and safety. This count reflects the changes in bacterial levels in the meat over time and can be used to monitor sanitation conditions and optimize shelf life. In this study, red meat samples were packaged using nanofibers produced through the emulsion method, incorporating 6% TEO and ZnO NPs (E/6%EO). These samples were stored at refrigerated temperatures for six days, with total bacterial counts measured every two days. The results showed a decrease in the number of bacteria in raw meat packaged with E/6%EO nanofibers and control fibers and stored at a refrigerated temperature from 5.6 Log CFU/g to 4.6 Log CFU/g and 4.5 Log CFU/g after two days of storage, respectively. Following this initial storage period, the bacterial count in meat samples packaged with nanofibers gradually increased, reaching 1.5 Log CFU/g and 7.5 Log CFU/g after 4 and 6 days, respectively. In contrast, the red meat samples packaged by control fibers exhibited total bacterial counts of 4.5 Log CFU/g and 1.6 Log CFU/g on the fourth and sixth days, respectively (Fig. 7).

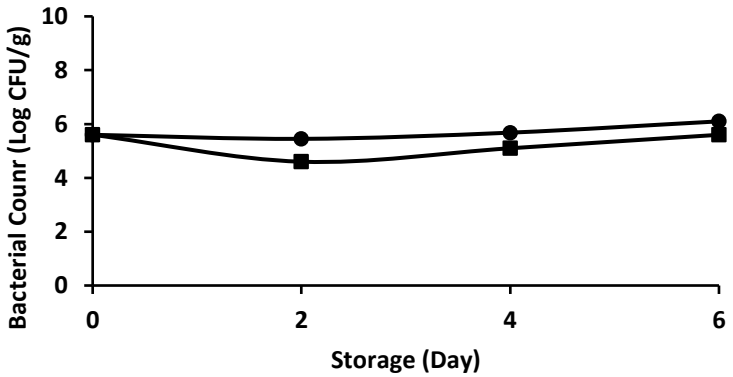

Figure 7: Results of total bacterial counts of red meat packaged in nanofibrous film (E/6%EO: ■) and conventional (control: ●) during a storage period of 6 days at refrigerated temperature.

Discussion

According to the results, it can be concluded that the electrospinning process of chitosan and PCL composite solutions with and without the presence of essential oil was successfully carried out through an industrial nanofiber production equipment, such that nanofibers with diameters ranging from 88 nm for the chitosan/PCL electrospinning solution to 60 nm for the chitosan/PCL electrospinning solution containing 6% essential oil and prepared by the conventional mixing method were obtained. Previously, fiber diameters of at least 200 nm for PCL containing carvacrol (Tampau *et al.*, 2018), about 50 nm for a chitosan/polyethylene oxide mixture containing cinnamaldehyde (Rieger and Schiffman, 2014), 63 nm for a PCL/chitosan mixture containing oregano essential oil (Ardekani-Zadeh and Hosseini, 2019), and 167 nm for a chitosan/PCL mixture containing cinnamaldehyde (Ghazaghi *et al.*, 2022), all prepared by the needle electrospinning method, were reported. This indicates that

the electrospinning device used in this study has a similar or even better ability than other types of nozzle electrospinning in the fiber formation.

The results showed that the addition of TEO to the electrospinning solution formulation significantly reduced their viscosity, with higher concentrations of the essential oil (6% vs. 3%) in solutions prepared by both emulsion and conventional mixing methods resulting in a greater reduction in solution viscosity. This observation can be attributed to the formation of hydrogen bonds between the essential oil and PCL molecules (Ramalingam *et al.*, 2019).

The same explanation can be applied to the difference in viscosity of electrospun solutions prepared by the two methods of emulsion and conventional mixing. As shown in Table 1, the viscosity of electrospun solutions prepared by the emulsion method (517.9±4.1 cP for 3% essential oil and 258.8±8.3 cP for 6% essential oil) was significantly higher than that of electrospun solutions prepared by the conventional method (181.2±2.7 cP for 3% essential oil and 155.3±5.7 cP for 6% essential oil). In other words, it is thought that in electrospun solutions prepared by the emulsion method, the essential oil is mainly incorporated in the chitosan core, and as a result, its interaction with PCL is limited. However, in electrospinning solutions prepared by conventional mixing, the essential oil has more opportunity to interact with the hydrogen bond formation sites of PCL.

Viscosity has been shown to play an important role in the electrospinning ability of mixed polymer solutions and subsequently the diameter of the resulting fibers. Lower viscosity results in more effective stretching of the polymer during the electrospinning process and consequently the formation of thinner fibers (Van der Schueren *et al.*, 2012). This was also clearly evident in the results obtained in this study, as (1) nanofibers containing encapsulated essential oils showed smaller fiber diameters compared to control nanofibers, (2) nanofibers prepared by conventional mixing method had lower viscosity compared to emulsion nanofibers and subsequently smaller fiber diameters were measured for them, (3) nanofibers containing higher concentrations of essential oils in both preparation methods had lower viscosity and consequently smaller fiber diameters. This is in agreement with the reports of Ghazaghi *et al.* (2022), who found that the addition of cinnamaldehyde (3–10%, w/w) to PCL/CS nanofibers decreased the fiber diameter (Ghazaghi *et al.*, 2022). Similarly, Ramalingam *et al.* (2019) showed that the addition of Gymnema sylvestre extract resulted in a significant reduction in fiber diameter compared to PCL fibers without the extract. These authors suggested that it is possible that the presence of phytochemicals such as gymnemic acid, gymnemagenin, lupeol, anthraquinones, and flavones in the extract of this plant increased the conductivity and decreased the viscosity of the resulting solution, which led to a reduction in the diameter of the composite fibers (Ramalingam *et al.*, 2019). Furthermore, in another study conducted by Aydogdu *et al.* (2019), it was reported that the diameter of composite nanofibers prepared by hydroxypropyl

methylcellulose (HPMC) and polyethylene oxide (PEO) decreased in the presence of gallic acid, which was attributed to the reduction in the viscosity of the electrospun solutions caused by the bioactive (Aydogdu *et al.*, 2019).

The absence of beads and the relatively smooth surface of the nanofibers indicate proper control of the electrospinning process parameters, including the applied voltage and the properties of the polymer solution used. These features, in turn, can affect the physical, mechanical, and biological properties of the aforementioned nanofibers (Ardekani-Zadeh and Hosseini, 2019; Zou *et al.*, 2020).

The absence of a significant nitrogen peak in the EDX spectrum of nanofibers prepared by the emulsion method (E/3%EO) compared to nanofibers prepared by the conventional mixing method (CM/3%EO) indicates that chitosan is not located in the surface structure of the fibers or, in other words, it is located in the central core of the fiber structure (Fig. 8). This evidence indicates the formation of a core-shell structure in nanofibers prepared by the emulsion method. Previously, Ma *et al.* (2019) had reported the formation of a core-shell structure in PCL/chitosan composite fibers by EDX analysis (Ma *et al.*, 2019).

According to FTIR analysis, the encapsulation of 6% TEO in nanofibers prepared by both emulsion (E/EO6%) and conventional mixing (CM/EO6%) methods created a new peak at the beginning of the spectrum (1177 cm^{-1} for E/EO6% and 1127 cm^{-1} for CM/EO6%). This effect may indicate possible physical interactions, such as electrostatic interactions and hydrogen bonding between the biopolymers and the essential oil (Ghorbani *et al.*, 2015). However, overall, the peaks associated with the mentioned functional groups in the control nanofibers remained almost unchanged after the addition of the essential oil, proving that the encapsulation of thyme in chitosan-PCL hybrid nanofibers does not disrupt their structural integrity. These results are in agreement with observations from previous studies (Ardekani-Zadeh and Hosseini, 2019; Tan *et al.*, 2021; Ghazaghi *et al.*, 2022) (Fig. 8).

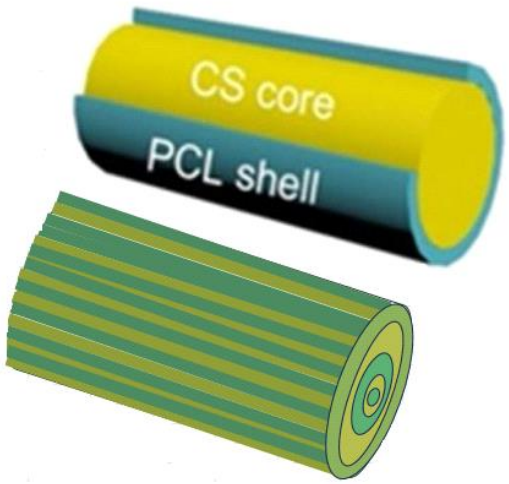

Figure 8: Schematic diagram for PCL/CS nanofibers with core-shell (top) and conventional (bottom) structures; it is obvious that the essential oil is encapsulated in the core in the core-shell structure, while in the conventional structure it is dispersed throughout the structure.

The X-ray diffraction peaks of CM/6%EO nanofibers showed a slight shift and significant differences in peak intensity (count) compared to the control sample. Such changes may reflect slight changes in the crystal structure of the fibers and the formation of weak intermolecular bonds between their constituents (chitosan, PCL, and TEO) (Fadaie *et al.*, 2018). However, overall, it can be concluded that the crystal

structure of PCL in the essential oil-containing composite nanofibers prepared by both methods was only slightly changed.

The results also showed that the incorporation of TEO into the nanofiber structure through both methods increased the WVP value. The amount and rate of water vapor transmission are directly proportional to the porosity of the nanofibers, which is a function of the fiber diameter. In fact, decreasing the fiber diameter leads to a decrease in fiber connections, which in turn increases the fiber porosity and, consequently, the WVP value (Liu *et al.*, 2009; Ardekani-Zadeh and Hosseini, 2019).

In addition, the method of encapsulation of essential oil had a significant effect on the water vapor permeability, as the nanofibers containing TEO prepared by the emulsion method had significantly lower WVP values compared to the nanofibers containing essential oil prepared by the conventional mixing method (Table 2). CM/6%EO and CM/3%EO nanofibers had the highest WVP values, equivalent to 0.266-0.276 g.mm/h.m^2.kPa. The reason for such a difference can also be explained by considering the relatively smaller diameter of the fibers in the samples encapsulated by the conventional mixing method (Table 1; Fig. 1). The measured WVP values of the nanofibers in this study are also in good agreement with previous studies. For example, WVP values ranging from 0.178 g.mm/h.m^2.kPa (control) to 0.367 g.mm/h.m^2.kPa (containing essential oil) were previously reported for chitosan-PCL-peel essential oil nanofibers (Ardekani-Zadeh and Hosseini, 2019).

The results of this study indicated that the control nanofibers exhibited antibacterial activity. They were approximately 1.4 times more effective against *Escherichia coli* compared to *Listeria monocytogenes*. Chitosan is known for its effective intrinsic antimicrobial properties, suggesting that chitosan-based films possess significant potential for food packaging applications (Mujtaba *et al.*, 2019; Song *et al.*, 2021). Several mechanisms have been proposed to explain the antimicrobial activity of chitosan films. It has been observed that these films can form a cellophane-like structure, creating a protective layer on food surfaces that helps prevent external microbial invasion. Additionally, chitosan films act as an effective oxygen barrier, limiting oxygen transfer to microbes and consequently inhibiting their growth on food (Nouri *et al.*, 2018). Protonated amino groups (NH_3^+ derived from NH_2 groups) in chitosan contribute critically to its antimicrobial action. These groups bind electrostatically to the negatively charged phosphoryl groups of phospholipids within the bacterial cell membrane. This interaction disrupts the lipopolysaccharide layer of the outer membrane, leading to substantial leakage of intracellular components and ultimately causing cell death (Elsabee and Abdou, 2013; Mohebi and Shahbazi, 2017). However, the scientific literature presents conflicting reports; while some previous studies found no antibacterial effect from pure chitosan films or chitosan composite films, others demonstrate efficacy. For instance, Zou *et al*. (2020) reported that PCL fibers alone showed no inhibitory effect, whereas PCL/chitosan composite

fibers exhibited clear inhibition zones with diameters of 13.17 mm against Escherichia coli and 11.40 mm against Staphylococcus aureus (Zou *et al.*, 2020).

Encapsulation of TEO at various concentrations, combined with ZnO NPs within nanofibers prepared by different methods significantly enhanced the antibacterial activity against *Escherichia coli* and *Listeria monocytogenes*. However, increasing concentration of TEO in both encapsulation methods did not result in a statistically significant change in antibacterial activity. This observation suggests that the antimicrobial effectiveness of TEO is influenced primarily by its intrinsic chemical composition, where synergistic interactions among its bioactive constituents contribute to specific antimicrobial mechanisms. Supporting this, Kang *et al.* (2018) reported that TEO, rich in p-cymene, thymol, and gamma-terpinene, inhibits Gram-positive bacterial growth by inducing membrane damage, altering cell morphology, and reducing intracellular ATP pool size (Kang *et al.*, 2018). The hyperpermeability of the bacterial cell membrane, which leads to the loss of membrane potential and the collapse of proton pumps and ATP depletion, and consequently the delay or inhibition of microbial growth, could be the main mechanism of action of TEO against gram-negative bacterial species (Guillín *et al.*, 2021).

Moreover, this antibacterial activity was greater on the gram-negative bacterium *Escherichia coli* compared to the gram-positive bacterium *Listeria monocytogenes*. In other words, *Listeria monocytogenes* showed greater resistance than *Escherichia coli* to the antimicrobial components encapsulated in the prepared fibers. Previous studies have repeatedly reported the greater sensitivity of gram-positive bacteria to antimicrobial compounds such as essential oils. This effect may be due to the absence of a lipopolysaccharide cell wall in these bacteria (Burt, 2004).

The antimicrobial efficacy of the prepared nanofibers decreased over time (from day 1 to day 5), such that the decreasing trend for nanofibers produced by the emulsion method was slower or did not change significantly compared to the conventional mixing method. For example, while the anti-*Escherichia* activity of E/6%EO nanofibers did not differ significantly from day 1 to day 5, CM/6%EO nanofibers showed a 50% reduction in anti-*Escherichia coli* activity. This level of antimicrobial activity retention of nanofibers prepared by the emulsion method in this study is a significant achievement compared to previous studies. For example, it has been previously reported that the antibacterial efficacy of chitosan-oregano essential oil-PCL nanofibers decreased significantly after only 6 hours of incubation (i.e., the exponential growth phase of bacteria). According to the explanation of these researchers, the possible reason is that with the rapid increase in the number of bacteria at this stage due to the high reproductive capacity of bacteria, nanofibers cannot prevent bacterial proliferation, which leads to a decrease in the inhibition efficiency (Ardekani-Zadeh and Hosseini, 2019). Therefore, it can be concluded that encapsulating essential oil within a hybrid structure of emulsion and fibers together

can control the release of the encapsulated bioactive compound and maintain the antibacterial activity of the fibers for a longer period of time. In the case of nanofibers prepared by the conventional method, the essential oil is encapsulated within a more open structure, and as a result, its release will be faster.

The total bacterial count for the raw meat used in this study was 5.6 Log CFU/g, indicating a relatively good microbial status. According to the Iranian National Standard No. 2349, a bacterial count of 6 logarithms of bacteria per gram is considered the critical bacterial count in meat (INSO, 2007). Meat packaging in a package with a nanofiber lid showed better results in terms of total bacterial count compared to conventional packaging (control). The total bacterial count of meat packaged in the nanofiber treatment initially decreased (a decreasing trend was observed until the second day) and then, despite a slight increase, remained within the standard range on the sixth day of refrigerated storage (5.7 Log CFU/g). It is likely that the migration of antimicrobial compounds of TEO from the packaging lid to the meat prevented bacterial growth. This was while in the control packaging, after 6 days of storage, the total bacterial count of the meat fell outside the standard range.

Conclusion

Novel antimicrobial electrospun nanofibers of chitosan/polycaprolactone/zinc oxide nanoparticles/thyme with diameters of 60–88 nm were fabricated by cartridge electrospinning and two preparation methods of electrospinning solution (conventional mixing and emulsion). The addition of the essential oil significantly reduced the viscosity of the electrospun solution and produced thinner fibers. Accordingly, the lowest viscosity was associated with electrospun solutions prepared by the conventional mixing method. Most of the nanofibers had a well-oriented, smooth surface and bead-free structure. However, the addition of TEO in electrospun solutions prepared by the conventional mixing method resulted in fibers with a slightly beaded structure. EDX results confirmed the formation of a core-shell structure in the nanofibers prepared by the emulsion method. The physical interactions and hydrogen bonding between chitosan/PCL and TEO were verified by FTIR, proving that the encapsulation of thyme in chitosan-poly-caprolactone hybrid nanofibers does not disrupt their structural integrity. Encapsulation of TEO in the nanofiber structure by both methods increased the WVP, such that nanofibers prepared by the emulsion method had significantly lower WVP values compared to nanofibers prepared by the conventional mixing method. Encapsulation of TEO at different concentrations and zinc oxide nanoparticles into nanofibers prepared by different methods significantly increased the antibacterial activity against *Escherichia coli* and *Listeria monocytogenes*. The antimicrobial efficacy of most of the produced nanofibers decreased over time (from day 1 to day 5), with the decrease trend for nanofibers produced by the emulsion method being slower or even not significantly different compared to the conventional mixing method. Finally, nanofibers containing

thyme essential oil encapsulated by the emulsion method were able to maintain the total bacterial count of meat within the standard range (Log CFU/g 6) for up to 6 days. In conclusion, nanofibers containing TEO encapsulated by the emulsion method can be used in the packaging of red meat products to improve shelf life as well as the safety of other potential foods.

Conflicts of interest

The authors declare that this research was conducted without any conflict of interest with any party.

References

Ardekani-Zadeh, A.H. and Hosseini, S.F., 2019. Electrospun essential oil-doped chitosan/poly (ε-caprolactone) hybrid nanofibrous mats for antimicrobial food biopackaging exploits. *Carbohydrate Polymers*, 223, 115108. DOI:10.1016/j.carbpol.2019.115108

ASTM, 2002. Standard test method for water vapor transmission of materials. E 96-00. Annual book of ASTM standards. ASTM Intl., Philadelphia. pp 1048-1053.

Aydogdu, A., Sumnu, G. and Sahin, S., 2019. Fabrication of gallic acid loaded Hydroxypropyl methylcellulose nanofibers by electrospinning technique as active packaging material. *Carbohydrate Polymers*, 208, 241-50. DOI:10.1016/j.carbpol.2018.12.065

Aytac, Z., Ipek, S., Durgun, E., Tekinay, T. and Uyar, T., 2017. Antibacterial electrospun zein nanofibrous web encapsulating thymol/cyclodextrin-inclusion complex for food packaging. *Food Chemistry*, 233, 117-124. DOI:10.1016/j.foodchem.2017.04.095

Azari, A., Anvar, S.A.A., Ahari, H., Sharifan, A. and Motallebi Moghanjoghi, A.A., 2020. Study on nanosilver-TiO2 photocatalytic nanocomposite coating with extrusion technique for increasing shelf life of Nile Tilapia (*Oreochromis niloticus*). *Iranian Journal of Fisheries Sciences*, 19(**5**) 2618-2633. DOI:10.22092/ijfs.2020.122566

Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(**3**), 223-253. DOI:10.1016/j.ijfoodmicro.2004.03.022

Cao, T.L. and Song, K.B., 2019. Active gum karaya/Cloisite Na+ nanocomposite films containing cinnamaldehyde. *Food Hydrocolloids*, 89, 453-460. DOI:10.1016/j.foodhyd.2018.11.004

Cardoso, R.V., Fernandes, Â., Gonzaléz-Paramás, A.M., Barros, L. and Ferreira, I.C., 2019. Flour fortification for nutritional and health improvement: A review. *Food Research International*, 125, 108576. DOI:10.1016/j.foodres.2019.108576

Darbasizadeh, B., Motasadizadeh, H., Foroughi-Nia, B. and Farhadnejad, H., 2018. Tripolyphosphate-crosslinked chitosan/poly (ethylene oxide) electrospun nanofibrous mats as a floating gastro-retentive delivery system for ranitidine hydrochloride. *Journal of Pharmaceutical and Biomedical Analysis*, 153, 63-75. DOI:10.1016/j.jpba.2018.02.023

Deng, L., Kang, X., Liu, Y., Feng, F. and Zhang, H., 2017. Effects of surfactants on the formation of gelatin nanofibres for controlled release of curcumin. *Food Chemistry*, 231, 70-77. DOI:10.1016/j.foodchem.2017.03.027

Dobrovolskaya, I.P., Yudin, V.E., Popryadukhin, P.V., Ivan'kova, E.M., Shabunin, A.S., Kasatkin, I.A. and Morgantie, P., 2018. Effect of chitin nanofibrils on electrospinning of chitosan-based composite nanofibers. *Carbohydrate Polymers*, 194, 260-6. DOI:10.1016/j.carbpol.2018.03.074

Elsabee, M.Z. and Abdou, E.S., 2013. Chitosan based edible films and coatings: A review. *Materials Science and Engineering: C*, 33(**4**), 1819-1841. DOI:10.1016/j.msec.2013.01.010

Fadaie, M., Mirzaei, E., Geramizadeh, B. and Asvar, Z., 2018. Incorporation of nanofibrillated chitosan into electrospun PCL nanofibers makes scaffolds with enhanced mechanical and biological properties. *Carbohydrate Polymers*, 199, 628-640. DOI:10.1016/j.carbpol.2018.07.061

Farahani, H., Barati, A., Arjomandzadegan, M. and Vatankhah, E., 2020. Nanofibrous cellulose acetate/gelatin wound dressing endowed with antibacterial and healing efficacy using nanoemulsion of Zataria multiflora. *International Journal of Biological Macromolecules*, 162, 762-773. DOI:10.1016/j.ijbiomac.2020.06.175

Furtado, L.M., Hilamatu, K.C., Balaji, K., Ando, R.A. and Petri, D.F., 2020. Miscibility and sustained release of drug from cellulose acetate butyrate/caffeine films. *Journal of Drug Delivery Science and Technology*, *55*, 101472. DOI:10.1016/j.jddst.2019.101472

Gasti, T., Dixit, S., Hiremani, V.D., Chougale, R.B., Masti, S.P., Vootla, S.K. and Mudigoudra, B.S., 2022. Chitosan/pullulan based films incorporated with clove essential oil loaded chitosan-ZnO hybrid nanoparticles for active food packaging. *Carbohydrate Polymers*, 277, 118866. DOI:10.1016/j.carbpol.2021.118866

Ghazaghi, A., Hosseini, S.F. and Rezaei, M., 2022. Electrospinning of cinnamaldehyde in chitosan/poly (ε-caprolactone) hybrid nanofibers: the investigation of physicomechanical, structural, and antimicrobial properties for food biopackaging exploits. *Innovative Food Technologies*, 9(**3**), 269-287. DOI:10.22104/ift.2022.5441.2087

Ghorbani, F.M., Kaffashi, B., Shokrollahi, P., Seyedjafari, E. and Ardeshirylajimi, A., 2015. PCL/chitosan/Zn-doped nHA electrospun nanocomposite scaffold promotes adipose derived stem cells adhesion and proliferation. *Carbohydrate Polymers*, 118, 133-142. DOI:10.1016/j.carbpol.2014.10.071

Guillín, Y., Cáceres, M., Torres, R., Stashenko, E. and Ortiz, C., 2021. Effect of essential oils on the inhibition of biofilm and quorum sensing in Salmonella enteritidis 13076 and Salmonella typhimurium 14028. *Antibiotics*, 10(**10**), 1191. DOI:10.3390/antibiotics10101191

INSO, 2007. Microbiology red meat - Carcasses, minced red meat -

Specifications and test methods. In (Vol. 2394). Iran National Standards Organisation, Iran. pp 1-14.

Kang, J., Liu, L., Wu, X., Sun, Y. and Liu, Z., 2018. Effect of thyme essential oil against Bacillus cereus planktonic growth and biofilm formation. *Applied Microbiology and Biotechnology*, 102, 10209-10218. DOI:10.1007/s00253-018-9401-y

Kim, I., Viswanathan, K., Kasi, G., Thanakkasaranee, S., Sadeghi, K. and Seo, J., 2022. ZnO nanostructures in active antibacterial food packaging: Preparation methods, antimicrobial mechanisms, safety issues, future prospects, and challenges. *Food Reviews International*, 38(**4**), 537-565. DOI:10.1080/87559129.2020.1737709

Lin, L., Mao, X., Sun, Y., Rajivgandhi, G. and Cui, H., 2019. Antibacterial properties of nanofibers containing chrysanthemum essential oil and their application as beef packaging. *International Journal of Food Microbiology*. 292:21-30. DOI:10.1016/j.ijfoodmicro.2018.12.007

Liu, F., Guo, R., Shen, M., Wang, S. and Shi, X., 2009. Effect of Processing Variables on the Morphology of Electrospun Poly[(lactic acid)-co-(glycolic acid)] Nanofibers. *Macromolecular Materials and Engineering*, 294(**10**), 666-672. DOI:10.1002/mame.200900110

Liu, Q., Chen, Z., Chen, Y., Yang, F., Yao, W. and Xie, Y., 2021. Microplastics and nanoplastics: emerging contaminants in food. *Journal of Agricultural and Food Chemistry*, 69(36), 10450-10468. DOI:10.1021/acs.jafc.1c04199

Liu, L., Swift, S., Tollemache, C., Perera, J. and Kilmartin, P.A., 2022. Antimicrobial and antioxidant AIE chitosan-based films incorporating a Pickering emulsion of lemon myrtle (Backhousia citriodora) essential oil. *Food Hydrocolloids*, 133, 107971. DOI:10.1016/j.foodhyd.2022.107971

Ma, L., Shi, X., Zhang, X. and Li, L., 2019. Electrospinning of polycaprolacton/chitosan core-shell nanofibers by a stable emulsion system. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 583, 123956. DOI: DOI:10.1016/j.colsurfa.2019.123956

Mohajeri, P., Hematian Sourki, A., Mehregan Nikoo, A. and Ertas, Y.N., 2023. Fabrication, characterisation and antimicrobial activity of electrospun Plantago psyllium L. seed gum/gelatine nanofibres incorporated with Cuminum cyminum essential oil nanoemulsion. *International Journal of Food Science & Technology*. 58(**4**), 1832-40. DOI:10.1111/ijfs.16324

Mohebi, E. and Shahbazi, Y., 2017. Application of chitosan and gelatin based active packaging films for peeled shrimp preservation: A novel functional wrapping design. *LWT-Food Science and Technology*, 76, 108-116. DOI:10.1016/j.lwt.2016.10.062

Motelica, L., Ficai, D., Ficai, A., Truşcă, R.D., Ilie, C.I., Oprea, O.C. and Andronescu, E., 2020. Innovative antimicrobial chitosan/ZnO/Ag NPs/citronella essential oil nanocomposite—Potential coating for

grapes. *Foods*, 9(**12**), 1801. DOI:10.3390/foods9121801

Mujtaba, M., Morsi, R.E., Kerch, G., Elsabee, M.Z., Kaya, M., Labidi, J. and Khawar, K.M., 2019. Current advancements in chitosan-based film production for food technology; A review. *International Journal of Biological Macromolecules*, 121, 889-904. DOI:10.1016/j.ijbiomac.2018.10.109

Nejatian, M. and Abbasi, S., 2019. Formation of concentrated triglyceride nanoemulsions and nanogels: natural emulsifiers and high power ultrasound. *RSC Advances*, 9, 28330. DOI:10.1039/C9RA04761A

Nejatian, M., Yazdi Ghandehari, A.P., Khorasani, S. and Simal-Gandara, J., 2023. Increasing the shelf life of fresh in-hull pistachio using nanocomposite packaging of zinc nanoparticles and pistachio green hull essential oil. *Scientia Horticulturae*, 313, 111888. DOI:10.1016/j.scienta.2023.111888

Nieto, G., 2020. A review on applications and uses of thymus in the food industry. *Plants*, 9(**8**), 961. DOI:10.3390/plants9080961

Nouri, A., Yaraki, M.T., Ghorbanpour, M., Agarwal, S. and Gupta, V.K., 2018. Enhanced Antibacterial effect of chitosan film using Montmorillonite/CuO nanocomposite. *International Journal of Biological Macromolecules*, 109, 1219-1231. DOI:10.1016/j.ijbiomac.2017.11.119

Phan, D.N., Khan, M.Q., Nguyen, V.C., Vu-Manh, H., Dao, A.T., Thanh Thao, P., Nguyen, N.M., Le, V.T., Ullah, A. and Khatri, M., 2021. Investigation of mechanical, chemical, and antibacterial properties of electrospun cellulose-based scaffolds containing orange essential oil and silver nanoparticles. *Polymers*, 14(**1**), 85. DOI:10.3390/polym14010085

Radulescu, M., Ficai, D., Oprea, O., Ficai, A., Andronescu, E. and M Holban, A., 2015. Antimicrobial chitosan based formulations with impact on different biomedical applications. *Current Pharmaceutical Biotechnology*, 16(**2**), 128-136. DOI:10.2174/1389201016021501121 51157

Ramalingam, R., Dhand, C., Leung, C.M., Ong, S.T., Annamalai, S.K., Kamruddin, M., Verma, N.K., Ramakrishna, S., Lakshminarayanan, R. and Arunachalam, K.D., 2019. Antimicrobial properties and biocompatibility of electrospun poly-ε-caprolactone fibrous mats containing Gymnema sylvestre leaf extract. *Materials Science and Engineering: C*, 98, 503-514. DOI:10.1016/j.msec.2018.12.135

Rieger, K.A. and Schiffman, J.D., 2014. Electrospinning an essential oil: Cinnamaldehyde enhances the antimicrobial efficacy of chitosan/poly (ethylene oxide) nanofibers. *Carbohydrate Polymers*, 113, 561-8. DOI:10.1016/j.carbpol.2014.06.075

Song, X., Liu, L., Wu, X., Liu, Y. and Yuan, J., 2021. Chitosan-based functional films integrated with magnolol: Characterization, antioxidant and antimicrobial activity and pork preservation. *International Journal of*

Molecular Sciences, 22(**15**), 7769. DOI:10.3390/ijms22157769

Taherimehr, M., YousefniaPasha, H., Tabatabaeekoloor, R. and Pesaranhajiabbas, E., 2021. Trends and challenges of biopolymer-based nanocomposites in food packaging. *Comprehensive Reviews in Food Science and Food Safety*, 20(**6**), 5321-5344. DOI:10.1111/1541-4337.12832

Tajbakhsh, E. and Moumeni, M., 2015. Detection of staphylococcus areus and salmonella typimurium in traditional and industrial olivie salads in shahrekord city. *Journal of Food Microbiology*, 2(**1**), 39-48.

Tampau, A., González-Martínez, C. and Chiralt, A., 2018. Release kinetics and antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocolloids*, 79, 158-69. DOI:10.1016/j.foodhyd.2017.12.021

Tan, L.F., Elaine, E., Pui, L.P., Nyam, K.L. and Aniza, Y., 2021. Development of chitosan edible film incorporated with Chrysanthemum morifolium essential oil. *Acta Scientiarum Polonorum Technologia Alimentaria*, 20(**1**), 55-66. DOI:10.17306/J.AFS.0771

Thakur, M., Majid, I., Hussain, S. and Nanda, V., 2021. Poly (ε-caprolactone), A potential polymer for biodegradable food packaging applications. *Packaging Technology and Science*, 34(**8**), 449-461. DOI:10.1002/pts.2572

Unalan, I., Endlein, S.J., Slavik, B., Buettner, A., Goldmann, W.H., Detsch, R. and Boccaccini, A.R., 2019. Evaluation of electrospun poly (ε-caprolactone)/gelatin nanofiber mats containing clove essential oil for antibacterial wound dressing. *Pharmaceutics*, 11(**11**), 570. DOI:10.3390/pharmaceutics11110570

Van der Schueren, L., Steyaert, I., De Schoenmaker, B. and De Clerck, K., 2012. Polycaprolactone/chitosan blend nanofibres electrospun from an acetic acid/formic acid solvent system. *Carbohydrate Polymers*, 88(**4**), 1221-1226. DOI:10.1016/j.carbpol.2012.01.085

Wang, K., Lim, P.N., Tong, S.Y. and Thian, E.S., 2019. Development of grapefruit seed extract-loaded poly(ε-caprolactone)/chitosan films for antimicrobial food packaging. *Food Packaging and Shelf Life*, 22:100396. DOI:10.1016/j.fpsl.2019.100396

Zou, Y., Zhang, C., Wang, P., Zhang, Y. and Zhang, H., 2020. Electrospun chitosan/polycaprolactone nanofibers containing chlorogenic acid-loaded halloysite nanotube for active food packaging. *Carbohydrate Polymers*, 247, 116711. DOI:10.1016/j.carbpol.2020.116711