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Peppermint essential oil-loaded solid lipid nanoparticle in gelatin coating: Characterization and antibacterial activity against foodborne pathogen inoculated on rainbow trout (*Oncorhynchus mykiss*) fillet during refrigerated storage

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Abstract

The present study was conducted to determine the characterization and antibacterial activity of peppermint essential oil-loaded solid lipid nanoparticle (PEO–SLN) and its impact on the quality of trout fillet stored at $4 \pm 1°C$ for 12 days. The SLNs were prepared through a bath sonication technique. PEO–SLNs contained 0.2% (w/v) PEO in 2% of lipid phase glycerol monostearate (GMS) and tween 80 (1% w/v) used as a surfactant in the aqueous phase. The characterization parameter of PEO–SLN was evaluated, and the antibacterial activity of PEO–SLNs was conducted under in vitro conditions. Trout samples were analyzed for inoculated *Pseudomonas aeruginosa, Listeria monocytogenes*, and *Escherichia coli* O157:H7 during refrigerated storage. The mean particle size of PEO–SLNs was 154.83 ± 1.21 nm with a polydispersity index (PDI) of 0.35 ± 0.01 and zeta potential was about -24.16 ± 0.51 mV. The results indicated that PEO–SLN had higher antibacterial activity than the free form of PEO and also when used in combination with gelatin coating (gel + PEO–SLN) had a significant effect on preventing microbial growth in trout fillets ($p < 0.05$). The most decreasing rate of *P. aeruginosa* (1.92 log CFU/g), *E. coli* O157:H7 (0.71 log CFU/g), and *L. monocytogenes* count (1.69 log CFU/g) was seen in gel + PEO–SLN. These findings illustrated that PEO–SLNs could potentially be utilized in the food industry to increase the shelf life of fish fillets.

KEYWORDS

antimicrobial properties, gelatin coating, peppermint, rainbow trout, SLN

1 | INTRODUCTION

Seafood plays an essential role in human health due to its high nutritional value. They are precious because of their significant sources of protein, fat, minerals, and vitamins (Kumari et al., 2017). However, the composition of fish meat creates a favorable situation for the growth and spread of spoilage microorganisms and common pathogens in food; therefore, spoilage of fish that occurs during storage is mainly due to microbial activity (Hosseini et al., 2016; Jasour et al., 2015; Nair et al., 2020). Thus, it is necessary to take some measures to delay the

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deterioration of fish quality and prolong the shelf life of fish by inhibiting or slowing the growth of microorganisms. In recent years, edible coatings containing antimicrobial agents have been used to prevent food spoilage (Keykhosravy et al., 2020; Mohajer et al., 2021; Nair et al., 2020). Many studies have been performed on polysaccharide coatings such as alginate (Hu et al., 2019; Khanzadi et al., 2020) and chitosan (Hassanzadeh et al., 2018; M. Raeisi et al., 2020). Gelatin is a protein derived from the hydrolysis of collagen that has multiple nutritional properties. The main benefit of gelatin coatings is preventing the penetration of oxygen, which delays microbial spoilage and prevents fat oxidation in meat (Sarika & James, 2016). The most remarkable feature of fish gelatin is that it is widely accepted in all cultures and is not associated with the risk of developing bovine spongiform encephalopathy (BSE) (Karim & Bhat, 2009; Wasswa et al., 2007). In addition, fish skins are significant byproducts of the fishery industry that causes environmental pollution and could be kept clean by using these pollutants (Djagny et al., 2001). The development of antioxidant and, or antibacterial, gelatin coatings has received much attention from researchers, primarily through incorporating natural ingredients into the polymer matrix (Maryam Adilah & Nur Hanani, 2016). Fish gelatin coating could be fortified by adding of EOs, such as fennel, peppermint, thyme, rosemary, and so forth (Aitboulahsen et al., 2018; Jridi et al., 2020). Peppermint is an aromatic herb in the mint family with the scientific name *Mentha piperita*. Peppermint essential oil (PEO) has antimicrobial properties containing: menthol, limonene, and flavonoid (Camele et al., 2021). Various kinds of research evaluated EO effectiveness against different types of bacteria (Ehsani & Mahmoudi, 2013; Marwa et al., 2017) and fungi (Desam et al., 2019; Nasseri et al., 2016).

Among the various methods of encapsulating plant essential oil compounds, solid lipid nanoparticles (SLNs) are the newest colloidal system used in the food industry (Katouzian et al., 2017). SLNs are lipid nanocarriers similar to other emulsions that differ significantly in lipid type. These carriers are made by substituting solid lipids or a mixture of solid lipids instead of liquid oil in oil-in-water nanoemulsion. Lipid particles in this system are solid at room temperature and body temperature, and their size is usually in the range of 40 to 1000 nm (Shtay et al., 2019). The most crucial advantage of SLNs is controlling active substance release over other nanocarriers. Another profit is the high encapsulation efficiency, protecting the essential oil from oxygen, light, and moisture. Due to the small size of these nanocarriers, it reduces transmission resistance and enhancement antimicrobial activity (Salminen et al., 2019). There are several methods for producing SLNs, including high-pressure homogenization, microemulsification, solvent evaporation, ultrasonication, and double-emulsion. In general, all methods are effective and are selected according to the compound encapsulated and the final use or route of administration (Garces et al., 2018).

Numerous studies have evaluated the EO-loaded SLN in in vitro conditions (Bazzaz et al., 2018; Ghodrati et al., 2019; Nasseri et al., 2016). Their results indicated that the antimicrobial activity of EO notably improved when encapsulated in SLN. Likewise, researchers assessed SLN loading with beeswax (B-SLN) on strawberry preservation (Zambrano-Zaragoza et al., 2020) and curcumin (CU-SLN) against pathogens in food models (Alanchari et al., 2021). Their result represented that curcumin and beeswax, when used in the SLN system, had higher antibacterial activity than their free form. To the best of our knowledge, no study has been done to assay the effect of PEO–SLN containing edible coating in food models, yet. Therefore, this research aims to (1) prepare, characterize, and evaluate the in vitro antibacterial activity of SLNs and (2) use this nanocarrier in gelatin coating to examine the antimicrobial activity of PEO–SLN against foodborne pathogen inoculated on rainbow trout (*Oncorhynchus mykiss*) fillet during refrigerated storage.

2 | MATERIALS AND METHODS

2.1 | Materials

Glycerol monostearate (GMS; Alfa Aesar, Haverhill, MA, USA), tween 80 (Merck, Germany), gelatin (cold water fish skin; Sigma-Aldrich, Germany), and PEO (Nader agro-industry, Mashhad, Iran) were purchased. Lyophilized strains of *P. aeruginosa* (ATCC: 1430, 27853, 1707, 1074), *L. monocytogenes* (ATCC: 7644, 82119, CIP: 7834, and NCTC: 10671), and *E. coli* O157:H7 (NCTC: 12900) were received from the microbial laboratory of food hygiene (Mashhad, Iran). Moreover, all cultural media were purchased from Merck (Darmstadt, Germany).

2.2 | Preparation and analysis of PEO

Peppermint essential oil was purchased from Nader agro-industry, Mashhad, Iran. The analysis of PEO was performed using gas chromatography (Agilent 7890A/5975C) equipped with a capillary column (50 m × 250 μm × 0.12 μm) according to the method described by Mahmoudi (2014).

2.3 | Preparation of SLN

GMS (2% w/v) was melted at 75°C and then PEO (0.5% w/v) was added (lipid phase). The aqueous phase was prepared through dissolving tween 80 (1% w/v) in double-distilled water and was added to the lipid phase at the end of the melting process to prevent evaporation of PEO. The emulsion was prepared using a bath sonication (Power sonic 505; Hwashin Technology, Gyeonggi-do, South Korea) at three cycles with 15 min and intervals of 15 s. The PEO–SLN was obtained by cooling it at room temperature (Fathi et al., 2013).

2.4 | Evaluation of SLN characteristics

2.4.1 | Particle size and zeta potential

Average particle size (Z diameter), PDI, and zeta potential of PEO–SLN were evaluated by dynamic light scattering (DLS) (ZetaSizer NanoZS; Malvern Instruments Ltd, UK) (Mäder & Mehnert, 2005).

2.4.2 | Calibration curve of PEO

The calibration curve of PEO was performed by using a UV/visible spectrophotometer (CE 9500, Buck Scientific, Inc., East Norwalk, CT, USA). Seven concentrations of PEO insolvent (methanol) were prepared. Then, the spectrophotometer was set on the maximum wavelength of the PEO, and their absorption was read. Maximum absorbance was examined by scanning PEO between 200 nm and 800 nm. The highest absorbance value was then used to calibrate the curve of PEO (235.1 nm). The calibration curve was obtained over the concentration range of 14.4–924.9 µl/ml (Rodrigues et al., 2011).

2.4.3 | Encapsulation efficacy

The dialysis bag method was used to measure the encapsulation efficacy (EE) of SLN (Li et al., 2016). This study used a dialysis tubing cellulose membrane with a pore size of 12 kDa (Sigma-Aldrich Co., Madrid, Spain). The dialysis bags were filled with a known amount of PEO–SLN. Then, it was put on a magnetic stirrer at 75 rpm for 24 h to purify completely. Afterward, 50 µl of the sample with 1950 µl methanol was mixed, and then its absorbance was measured at 235.1 nm in a UV spectrophotometer. The amount of encapsulation was calculated utilizing a calibration curve and using the following Equation:

$$\mathrm{EE}\,(\%) = \mathrm{W}\,(\mathrm{encapsulate})/\mathrm{W}\,(\mathrm{initial}) \times 100 \qquad (1)$$

where $W_{encapsulates}$ is the total amount of loaded PEO, and $W_{initial}$ is the amount of initial PEO used for loading in the SLN system.

2.5 | Antibacterial activity of PEO–SLN

2.5.1 | Disc diffusion method

Three pathogenic bacteria (four strains of *P. aeruginosa* and *L. monocytogenes*, and one strain of *E. coli* O157:H7) were provided by culturing in BHI broth. 0.5 McFarland standard turbidity was prepared and diluted to gain 1.5×10^7 CFU/ml bacterial suspensions. Next, Mueller-Hinton agar was inoculated with 0.1 ml of the bacterial suspension via surface culture, and then sterile discs were put on the surface of the plates. Twenty microliters of each sample (PEO and PEO–SLN) were added onto the disc and were incubated at 37°C for 24 h. Also, a blank disc (without sample) served as a negative control, while chloramphenicol was used as a positive control. At the end of incubation, the inhibition zones are measured by a caliper (Khanzadi et al., 2020).

2.5.2 | Minimum inhibitory concentration and minimum bactericidal concentration

The microdilution method was performed to evaluate the antibacterial activity of PEO–SLN (Sharifi et al., 2017). Bacterial cultures were adjusted to standard turbidity (0.5 McFarland) and diluted to reach 1.5×10^7 CFU/ml. PEO–SLN (1000 µl, which contains 5.5 mg PEO) was serially diluted (1:2) to obtain in the range of 5.5—0.17 mg/ml by dissolving in BHI, and then 160 µl BHI broth, 20 µl bacterial suspensions, and 20 µl PEO–SLN were added into each well. Positive and negative control were 180 µl BHI broth, 20 µl bacteria, and 180 µl BHI broth, 20 µl PEO–SLN, respectively. Next, the microplate was inoculated at 37°C/24 h. The minimum inhibitory concentration (MIC) with no visible bacterial growth is represented as the MIC value for SLN containing PEO for the tested bacteria. For minimum bactericidal concentration (MBC) determination, 10 µl from wells with nonturbidity was spread on agar and incubated at 37°C for 24 h, the minimum concentration without any bacterial growth represented as MBC.

TABLE 1 Experimental treatments of this research

	Treatments	Explanation
1	Con	Without any coating
2	Gel	Gelatin 5% (w/v)
3	Gel + PEO	Gelatin coating with PEO 0.2% (w/v)
4	Gel + PEO–SLN	Gelatin coating with PEO–SLN 0.2% (W_{EO}/V)

Abbreviations: PEO, peppermint essential oil; SLN, solid lipid nanoparticle.

2.6 | Preparation of trout sampling

Fresh trout fillets (600–700 g) were purchased from a seafood market in Mashhad, Iran. Fish were skinned, beheaded, gutted, washed carefully, and then filleted aseptically. Trout fillets cutting into 2 × 4 × 1 cm and weighed to be 10 g. To sterilize the trout fillet, ethanol (96% v/v) was sprayed. Then four strains of *L. monocytogenes* and *P. aueroginosa* as a cocktail along with one strain of *E. coli* O157:H7 (100 μl of each culture at a concentration of 10^8 CFU/ml) were inoculated (using micropipettes) on each side of trout fillet pieces to gain a final concentration of 10^6 CFU/g. Next, samples were immersed in various coating solutions (Table 1) for 2 min, and after draining, they were packed in sterile bags and kept in refrigerated storage (4 ± 1°C) for the microbial analysis on days 0, 1, 2, 4, 6, 8, and 12 (S. Raeisi et al., 2016).

2.7 | Preparation of coating solutions

To prepare gelatin coating, 5 g cold fish gelatin (5% w/v) was dissolved in sterilized distilled water and placed on a hot plate magnetic stirrer at 75 rpm and 70°C until completely solved (group 2). Then, PEO 0.2% (w/v) was added to gelatin as the oily phase, and with 0.2% Tween 80 (w/v) thoroughly mixed until a homogenous mixture was obtained (group 3). Group 4 was prepared by mixing gelatin and PEO–SLN 0.2% (W_{PEO}/V).

2.8 | Microbial analysis

To evaluate the count of the inoculated bacteria, trout fillets (10 g) were blended with 90 ml of 0.1% sterile peptone water using a stomacher (Seward Medical, London, UK) for 2 min at 240 rpm. Next, 100 μl of serially diluted (1:10) homogenates was cultured via a surface method on plates containing Palcam agar, Sorbitol MacConkey, and Pseudomonas agar for enumeration of *L. monocytogenes*, *E. coli* O157:H7, and *P. aueroginosa*, respectively, and incubated at 37°C/24 h.

2.9 | Statistical analysis

Data attained in triplicate were statistically analyzed by SPSS software (version 21) using the repeated measures ANOVA, Bonferroni post hoc test, and Dunnett's T3 test. $p < 0.05$ was considered statistically significant.

3 | RESULTS AND DISCUSSION

3.1 | GC-MS analysis of PEO

As shown in Table 1, GC-MS analysis of peppermint essential oil identified the 26 volatile components representing 92.8% of the total oil. Based on obtained findings, the main constituents of the PEO were iso-menthol (24.69%), carvone (19.89%), menthone (12.35%), and limonene (10.88%). The following major components were trans-dihydrochalcone (4.06%), 1, 8-cineole (3.81%), neo-menthyl acetate (2.88%), iso-menthone (2.59), menthol (2.33%), and piperitenone (2.11%). Similarly, Badea et al. (2019) found that menthol and menthone are the main constituents of PEO examined in their study. The obtained result demonstrated that the main component of *Mentha piperita L.* using GC-MS methods is menthol, while 1,8-cineole is the main compound of *Mentha piperita L.* using the hydrodistillation system (Moradi & Najafian, 2015). The obtained data through the GC/MS analysis emphasized that the most abundant chemical composition of PEO examined in our study are monoterpenes. Noori et al. (2018) identified more compounds in the essential oil of *Mentha piperita* L. using hydrodistillation methods. This difference could be due to different extraction methods, plant organs, and distillation conditions.

3.2 | Characterization of SLN

The droplet size of SLN is associated with several parameters such as types of components, manufacturing process, and environmental conditions (Koshani & Jafari, 2019). As shown in Table 2, the mean droplet size and zeta potential of PEO–SLN were 154.83 ± 1.21 nm and −24.16 ± 0.51 Mv, respectively. Moreover, a PDI value of 0.35 ± 0.01 and size dispersion by the intensity of PEO–SLN showed a stable dispersion (Figure 1). PDI between 0.1 and 0.25 indicates low particle dispersion and a narrow particle dispersion diagram, whereas PDI greater than 0.5 indicates high particle dispersion. These data were in line with the findings of Fathi et al. (2013). Nasseri et al. (2016) reported the average particle size (255.5 ± 3 nm) and PDI (0.369 ± 0.05) for *Zataria multiflora*-loaded SLNs. Their

TABLE 2 Z-average, zeta Potential, PDI, and encapsulation efficiency of PEO–SLN

Formulation	Size (nm)	Zeta potential (Mv)	PDI	Encapsulation efficiency (%)
GMS: 2% (w/v) Tween 80: 1% (w/v) PEO: 0.5% (w/v)	154.83 ± 1.21	−24.16 ± 0.51	0.35 ± 0.01	55.5 ± 1.81

Abbreviations: PDI, polydispersity index; PEO, peppermint essential oil.

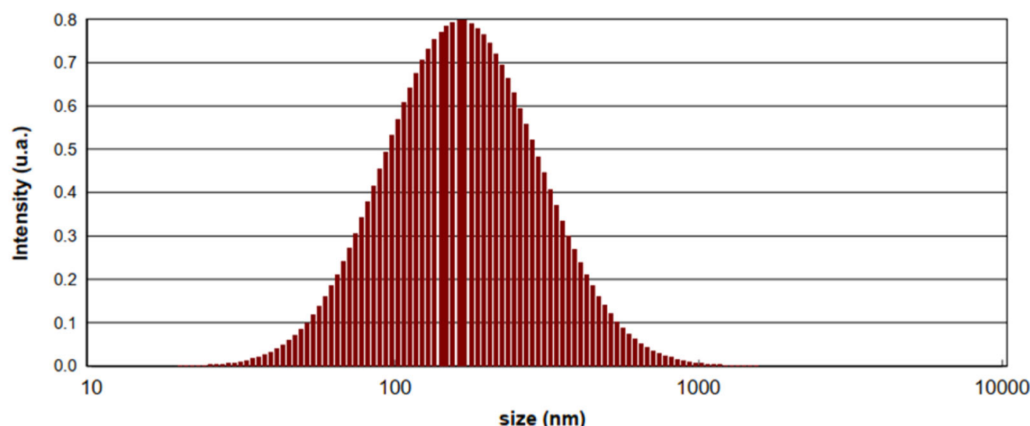

FIGURE 1 Size dispersion by intensity of PEO–SLN

result showed that after 4 weeks of storage, the particle size of SLN was more than 300 nm. Some researchers presented that a higher amount of surfactant can cause smaller particle sizes. Additionally, an increase in the amount of lipid component of the formulation leads to a larger size and broader distribution (Bazzaz et al., 2016; Lim et al., 2020).

The maximum absorption wavelength of PEO was observed at 235.1 nm. Figure 2 shows the pattern of light absorbance for PEO scanned between 200 nm and 800 nm. One peak could be observed, with the highest absorbance of 235.1 nm, indicating the wavelength of maximum sensitivity to PEO samples. Therefore, all further analyzed samples read in this wavelength. To determine EE, the calibration curve of PEO in the concentration range of 14.4–924.9 µl/ml in methanol. EE of PEO–SLN was about 55.5% which was in agreement with the result of Fathi et al. (2013), who showed that hesperetin-loaded SLN with different amounts of GMS and stearic acid had an EE range of about 39–63%. Bazzaz et al. (2018) obtained that the EE of SLN–EO of *Eugenia caryophyllata* was approximately 70%. They also observed that increasing the amount of EO could cause saturation of the lipid matrix and reduce EE. Prombutara et al. (2012) reported that using more than 2% (w/w) nisin loading decreased EE from 73.6% to 69.2%. They found that because of the hydrophobic nature of the lipid ingredients of the carrier system, peptides have the desire to adsorb onto the surface. Such adsorption can result in a significant loss of the amount of peptide available for delivery. Azizi et al. (2018) found that quercetin-loaded SLN improved encapsulated echium oil by about 78%. SLN formulations containing 1-laurin-3-palmitin (LP) for retaining thymol represented superior efficiency compared with the commonly used SLN matrix (GMS and tripalmitin glyceryl). 1.3 LP SLN shows the highest thymol trap efficiency and improved stability during long-term storage (Shi et al., 2019). Also, He et al. (2019) reported that the EE of carvacrol-loaded SLN was more than 98% in all formulations. Their result showed that SLNs formulations with equal masses of propylene glycol monopalmitate and GMS had better consistency of carvacrol during storage.

3.3 | In vitro antibacterial assay of PEO–SLN

The antimicrobial activity of PEO and PEO–SLN was determined against three foodborne pathogens shown as the inhibition zone in Table 3. The results demonstrated that the inhibition zone diameter of the PEO–SLN was greater than pure PEO for all tested bacteria. The most potent antimicrobial activity of PEO–SLN against *L. monocytogenes* with the approximately inhibition zones of 21 mm for all strains. *E. coli* O157:H7 and *P. aeruginosa* had an almost similar inhibition zone (∼ 19 nm), while the

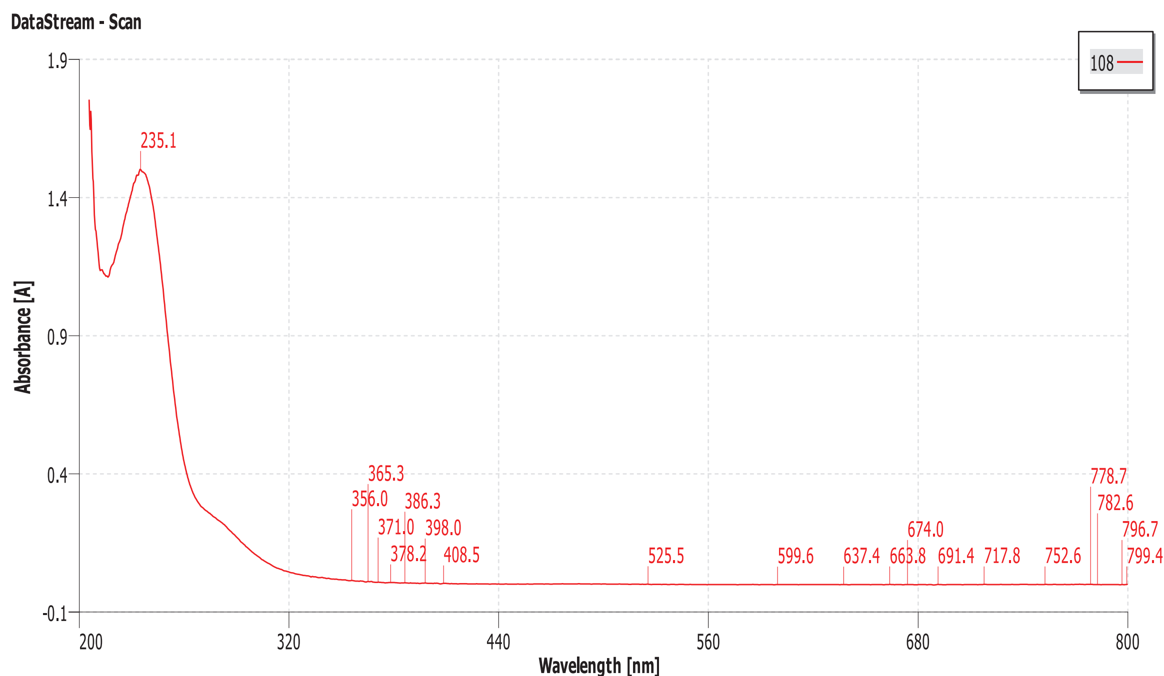

FIGURE 2 Pattern of light absorbance for PEO scanned between 200 nm and 800 nm

TABLE 3 Inhibition zone of PEO–SLN and PEO against *L. monocytogenes*, *P. aeruginosa*, and *E. coli* O157:H7 determined by agar disc diffusion method

Strains	inhibition zone (mm)	
	PEO–SLN	Peppermint
Listeria monocytogenes (ATCC 7644)	21.33 ± 1.1	19.11 ± 1.5
Listeria monocytogenes (CIP 7834)	21.21 ± 1.1	19.33 ± 0.5
Listeria monocytogenes (NCTC 10671)	21.66 ± 0.5	19.33 ± 0.5
Listeria monocytogenes (ATCC 82119)	21.10 ± 1.7	19.33 ± 0.5
Pseudomonas aeruginosa (ATCC 1430)	19.66 ± 0.7	17.33 ± 1.5
Pseudomonas aeruginosa (ATCC 27853)	20.66 ± 1.5	18.33 ± 1.5
Pseudomonas aeruginosa (ATCC 1707)	19.66 ± 1.0	18.66 ± 0.5
Pseudomonas aeruginosa (ATCC 1074)	19.33 ± 1.5	17.33 ± 0.5
E.coli O157:H7 (NCTC: 12900)	19.33 ± 1.5	17.66 ± 0.5
Chloramphenicol	32.00 ± 00	29.00 ± 00

antibacterial activity of PEO was ~ 17 nm against *E. coli* O157:H7 and *P. aeruginosa*. These data confirm the previous studies, indicating that the nanocarrier system enhances the antibacterial effect. In another study, Alanchari et al. (2021) examined the antibacterial activity of curcumin-loaded-SLN (CU-SLN) and free form of curcumin against *S. aureus* and *E.coli* by agar well diffusion. They reported that the encapsulation of curcumin improves the antibacterial activity of curcumin. Furthermore, they represented that the zone inhibition of curcumin was 7 mm for *E.coli*, while it was 12 mm for CU–SLN. The enhancement of the antibacterial activity of encapsulated EO over the free form can be because of two primary reasons. First, the SLNs' capability in improving interaction with microbial cell membranes. Therefore, they can be used as an alternative to treat microbial infections. Second, enhancing the stability and solubility of EO in aqueous media develops the maintenance or improvement of its biological activity.

As shown in Table 4, MIC values of *L. monocytogenes* were lower than *E. coli* O157:H7 and *P. aeruginosa*. The obtained results represented that the MIC and MBC values of PEO for *L. monocytogenes* and *P. aeruginosa* were similar between all strains examined. PEO represented an antibacterial activity against *L. monocytogenes* with MIC values of 0.13 mg/ml and 0.27 mg/ml for *E. coli* O157:H7 and *P. aeruginosa*. According to the findings, gram-positive bacteria were more sensitive to antibacterial impacts of PEO than gram negative, which was consistent with prior literature (Hashemi et al., 2013; Ozogul et al., 2017; Sharifi et al., 2017). Gram-positive bacteria have a thick peptidoglycan layer and no outer lipid membrane, while

TABLE 4 MIC and MBC activity of PEO–SLN against *L. monocytogenes*, *P. aeruginosa*, and *E. coli* O157:H7 by microdilution method

Strains	MIC (mg/ml)	MBC (mg/ml)
Listeria monocytogenes (ATCC 7644)	0.13	0.27
Listeria monocytogenes (CIP 7834)	0.13	0.27
Listeria monocytogenes (NCTC 10671)	0.13	0.27
Listeria monocytogenes (ATCC 82119)	0.13	0.27
Pseudomonas aeruginosa (ATCC 1430)	0.27	0.55
Pseudomonas aeruginosa (ATCC 27853)	0.27	0.55
Pseudomonas aeruginosa (ATCC 1707)	0.27	0.55
Pseudomonas aeruginosa (ATCC 1074)	0.27	0.55
E.coli O157:H7 *(NCTC: 12900)*	0.27	0.55

Abbreviations: MBC, minimum bactericidal concentration; MIC minimum inhibitory concentration.

gram-negative bacteria have a thin peptidoglycan layer with an outer lipid membrane, which creates a barrier against essential oils (Nazzaro et al., 2013; Trombetta et al., 2005). Ghodrati et al. (2019) obtained the same result. They encapsulated PEO with an NLC system, and their antibacterial results showed that the lowest MIC value was found in *P. aeruginosa*. In other research work, Keykhosravy et al. (2020) evaluated the antibacterial effect of *Zataria multiflora* and *Bunium persicum* nanoemulsion. Their result represented that both gram-negative and positive bacteria were sensitive to EO nanoemulsion. Shetta et al. (2019) determined the in vitro properties of PEO and green tea oil (GTO) in chitosan nanoparticles (CN) and represented that CN/GTO was more powerful than CS/PEO. MBC values of CN/GTO and CS/PEO were 1.15 mg/ml and > 2.72 mg/ml against *E. coli*, respectively.

3.4 | Enumeration of inoculated bacteria

3.4.1 | *E. coli* O157:H7

Figure 3 shows the growth of *E. coli* O157:H7 inoculated on trout fillets during refrigerated storage. The initial count of *E. coli* O157:H7 was 5.63 ± 0.11 log CFU/g in Con, which was notably reduced and reached 4.05 ± 0.01 log CFU/g on day 12. The growth rate of mentioned bacteria was notably higher in Gel and Con groups than those in other treated samples ($p < 0.05$). Obtained data are in line with the research performed by Sharifi et al. (2017), who showed that alginate coating enriched with *Zataria multiflora* significantly reduced the growth of *E. coli* O157:H7 in trout fillet during storage. In the current study, the lowest bacterial count was observed in Gel + PEO–SLN (2.43 ± 0.18). As shown in Table 5, the maximum reduction rate of *E. coli* O157:H7 (∼ 1 log CFU/g) was obtained in Gel + PEO–SLN samples compared with the Con group. The obtained results indicated that gelatin coating enriched with PEO and PEO–SLN could reduce the growth of mentioned bacteria in trout fillets during 12 days of storage. Hence, previous studies demonstrated that the encapsulated PEO enhances the antibacterial activity (Rajkumar et al., 2020). Similarly, remarkable improvements in antibacterial activity were seen for the encapsulated green tee oil against *E.coli*. This finding was in agreement with the result of Esmaeili and Asgari (2017), who found that encapsulated *Carum copticum* essential oils (CEOs) boosted their antibacterial activity of them over pure CEO.

3.4.2 | *P. aeruginosa*

As depicted in Figure 4, the number of the *P. aeruginosa* in the Con and Gel group increased and reached 6.81 ± 0.03 and 6.57 ± 0.04 log CFU/g at the end of storage, respectively. Psychotropic bacteria such as *P. aeruginosa* can grow in refrigerated storage and spoiled fish fillet. Moreover, the results showed that Gel coating alone could not be effective to prevent psychotropic growth. Similarly, Andevari and Rezaei (2011) showed that the quality of trout fillet was not affected by Gel coating alone during cold storage. In addition, Ou et al. (2002) reported that the tilapia fillet stored in the refrigerator showed no significant difference in psychotropic content in samples coated with Gel compared to control. However, samples treated with Gel incorporated with PEO and PEO–SLN significantly prevent *P. aeruginosa* growth during the cold storage period compared to Con and Gel ($p < 0.05$). The sample coated with Gel + PEO–SLN exhibited the maximum antibacterial effect against the mentioned bacteria ($p < 0.05$). Following the obtained results, the number of *P. aeruginosa* bacteria decreased in groups 3 and 4 until day 6 and then increased until the end of the period storage. These results revealed that gelatin-containing PEO–SLN could preserve trout fillet for 6 days, and after that, the mean bacterial count would be raised. PEO-loaded SLN containing gelatin as an edible coating extends the shelf life of trout fillet during refrigerated storage for approximately 6 days. After 12 days, *P. aeruginosa* counts were reduced to 0.21, 1.66, 1.92 log in Gel, Gel + PEO, and Gel + PEO–SLN

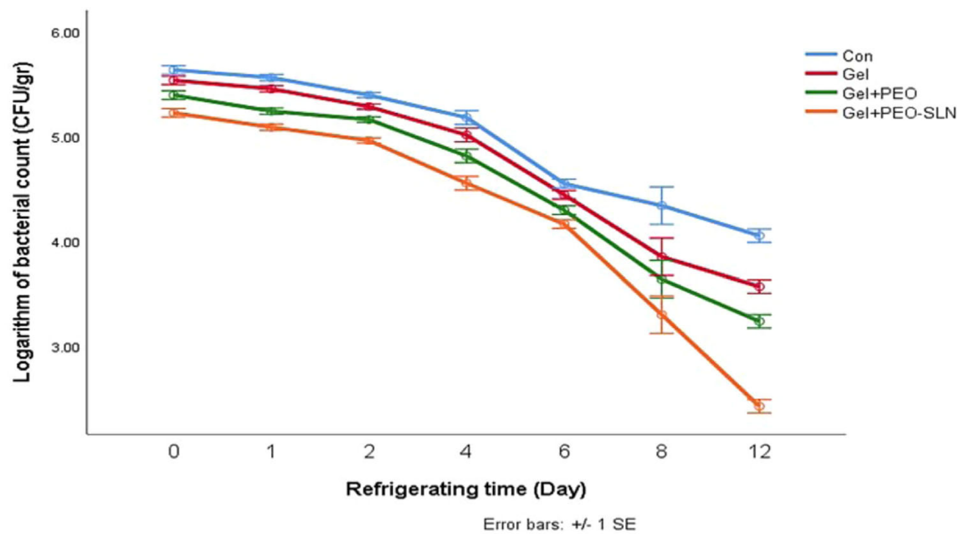

FIGURE 3 Changes in *E. coli* O157:H7 count (log CFU/g) of rainbow trout samples in different groups during 12 days of storage at 4°C. Data are expressed as mean ± SD ($n = 3$)

TABLE 5 Average reduction rate of *E. coli* O157:H7, *P. aeruginosa*, and *L. monocytogenes* count (log CFU/g) among treatments when compared together during 12 days of storage

		Mean difference (I–J)			
Attributes	**Group (I)**	**Group (J)**	**Gel**	**Gel + PEO**	**Gel + PEO–SLN**
E. coli O157:H7*P. aeruginosa*	Con		0.22[a]	0.41[a]	0.71[a]
	Gel			0.19[a]	0.49[a]
	Gel + PEO				0.29[a]
	Con		0.21[a]	1.66[a]	1.92[a]
	Gel			1.45[a]	1.70[a]
	Gel + PEO				0.25[a]
L. monocytogenes	Con		0.29[a]	1.39[a]	1.69[a]
	Gel			1.10[a]	1.40[a]
	Gel + PEO				0.29[a]

Note: Data are expressed as mean difference.
Abbreviations: PEO, peppermint essential oil; SLN, solid lipid nanoparticle.
[a]Indicate a statistically significant difference ($p < 0.05$).

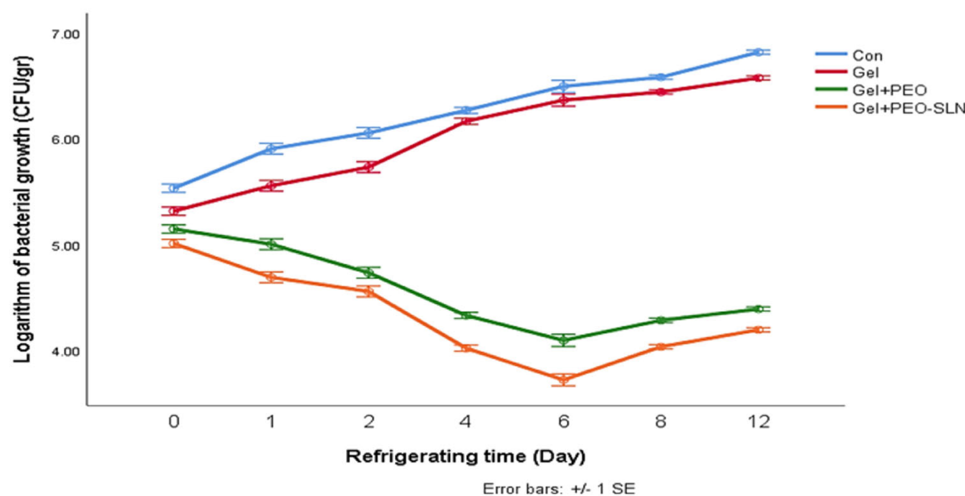

FIGURE 4 Changes in *Pseudomonas aeruginosa* count (log CFU/g) of rainbow trout samples in different treatments during 12 days of storage at 4°C. Data are expressed as mean ± SD ($n = 3$)

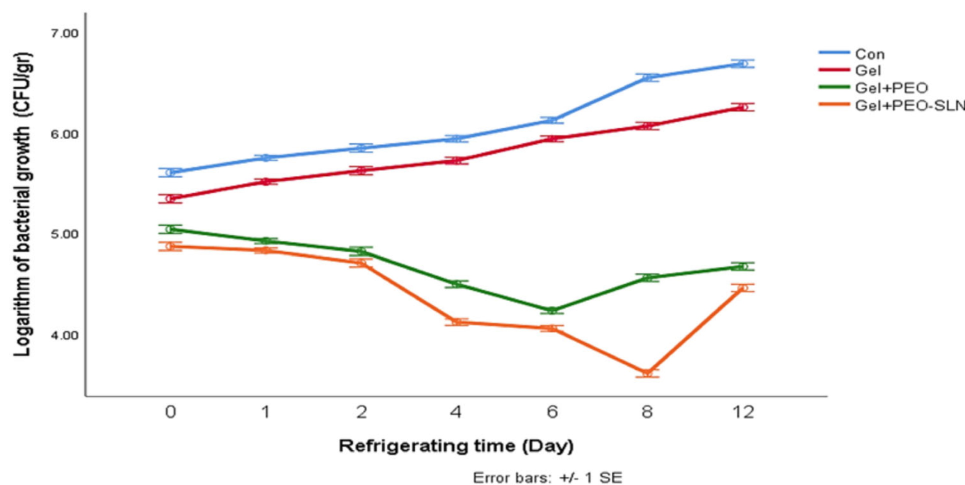

FIGURE 5 Changes in *Listeria monocytogenes* count (log CFU/g) of rainbow trout samples in different treatments during 12 days of storage at 4°C. Data are expressed as mean ± SD ($n = 3$)

treatments, respectively, compared with the Con (Table 5). As reported in previous studies, treatment with edible coating enriched with EOs has effectively slowed psychotropic growth (Ceylan et al., 2020; Meral et al., 2019; Moghimi et al., 2016). Durmus et al. (2019) announced that citrus EO (orange, lemon and tangerine)-based nanoemulsions effectively slowed the growth of psychrophilic bacteria on rainbow trout fillets. They confirmed that using these systems could extend 4 days for orange and lemon and 6 days for mandarin.

3.4.3 | *L. monocytogenes*

Figure 5 illustrates the growth of *L. monocytogenes* inoculated on trout fillets during refrigerated storage. The Con and Gel samples had the highest number of *L. monocytogenes* (6.68 and 6.25 log CFU/g, respectively) on day 12, which was in line with the result of Sharifi et al. (2017) and S. Raeisi et al. (2016). Psychotropic bacteria such as *L. monocytogenes* could grow on fish fillets stored in cold conditions. As displayed in Figure 5, Gel coating could not inhibit the growth of *L. monocytogenes*. These findings are in agreement with the results obtained by Ye et al. (2011). They reported that edible coating without an antibacterial agent could not delay the growth of *L. monocytogenes*. Similarly, Andevari and Rezaei (2011) found that Gel coating alone exhibited no substantial antimicrobial effect on rainbow trout fillet. According to the findings of the present study, in group 4 (Gel + PEO–SLN), the final number of *L. monocytogenes* reached blow 4 log CFU/g on day 8 of storage, while the final count of samples coated with Gel (group 2) was found as 6.06 log CFU/g at the same day. Similarly, Shahbazi and Shavisi (2018) found the chitosan film incorporated with essential oil was more effective than chitosan alone to reduce the growth of bacteria in trout fillets. Moreover, previous studies demonstrated that edible coatings like chitosan, alginate, and gelatin represented the antimicrobial effect against foodborne bacteria. However, the inhibition of their growth was remarkably lower than designated coats enriched with EOs. As tabulated in Table 5, the highest reduction rate of *L. monocytogenes* (1.69 and 1.40 log CFU/g) was obtained in Gel + PEO–SLN samples compared with Con and Gel samples, respectively. The results showed that group 3 (Gel + PEO) and group 4 (Gel + PEO–SLN) had a notable difference in mean log bacterial compared with group 2 (coating with Gel, $p < 0.05$). However, Gel treatment had a substantial difference compared with the Con, too. Samples coated with chitosan-loaded nanoemulsion containing EO showed decreasing ∼ 3 log CFU/g of *L. monocytogenes* during 18 days of storage (keykhosravy et al., 2020). PEO showed significant antibacterial activity because some compounds such as menthol and menthone act on cell membranes, causing significant morphological damage and destabilizing microbial membranes. According to the findings of this study, the encapsulated PEO showed a significant improvement in antibacterial activity. The enhancement of the antibacterial activity of PEO after encapsulation could be attributed to the protective effect of the encapsulation, which reduces the evaporation rate of EOs during the assay (Zhang et al., 2019).

4 | CONCLUSION

This study was created to assess the antibacterial activity of PEO in both (free and encapsulated) forms in the edible coating against foodborne pathogens under

real food processing conditions. The SLNs were successfully prepared in microscopy properties. Therefore, lipid nanoparticles are used as promising nanocarriers to develop functional characteristics such as the antimicrobial and scavenging activity of PEO. Meanwhile, PEO–SLN showed boosted antibacterial activity than free PEO, representing enhanced antimicrobial activity via the encapsulation system both in vitro and food model.

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AUTHOR CONTRIBUTIONS

Sara Safaeian laein: Investigation; Methodology; Writing – original draft. **Saeid Khanzadi**: conceptualization; funding acquisition; project administration; supervision; validation; writing – original draft; writing – review & editing. **Mohammad Hashemi**: methodology; resources; visualization; writing – review & editing. **Fatemeh Gheybi**: methodology; resources; visualization; writing – review & editing. **Mohammad Azizzadeh**: data curation; formal analysis; software; validation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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