



Improving quality of trout fillet using gelatin coating-contain peppermint essential oil loaded solid lipid nanoparticles (PEO-SLN)

Sara Safaeian Laein¹ · Saeid Khanzadi¹ · Mohammad Hashemi^{2,3} · Fatemeh Gheybi⁴ · Mohammad Azizzadeh⁵

Received: 1 July 2023 / Accepted: 10 September 2023

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2023

Abstract

The aim of the present study was to design a delivery system of peppermint essential oil (PEO loaded solid lipid nanoparticles (PEO-SLN) for evaluating the chemical and sensory properties of trout fillet during cold storage. PEO-SLN was provided through a bath sonication method and characterized by several techniques. Different formulations including glycerol monostearate (1.39×10^{-2} – 5.56×10^{-2} mM), Tween 80 (3.18×10^{-3} – 25.44×10^{-2} mM), and PEO (5.28×10^{-3} mM) were applied to optimize the PEO-SLN. The optimized PEO-SLN sample showed a spherical morphology with droplet size of 139.46 ± 0.32 nm and 0.26 ± 0.01 PDI under transmission electron microscopy (TEM). Encapsulation efficiency (EE) and zeta potential were around 55.5% and -30.64 ± 0.59 mV, respectively. Moreover, the effect of gelatin-coating containing of PEO-SLN was measured on Chemical (pH, TVB-N, TBARS, PV, and FFA) and organoleptic properties of trout fillet over a 12-day storage period. The most effective treatment against chemical deterioration was found to be gelatin-coating fortified with PEO-SLN (Gel + PEO-SLN). Furthermore, fillets which was coated with PEO-SLN had the acceptable score in all sensory attributes during storage period. The obtained results demonstrate that the incorporation of Gel + PEO-SLN into trout fillets may improve, or at least not adversely affect, their sensory properties and suggests this coating is an effective strategy to delay chemical degradation of trout fillets. In addition, the FRAP and DPPH radical scavenging of PEO-SLN exhibited a higher antioxidant activity than free PEO. This study provides useful insights into the preparation of a lipid-based delivery system enriched with essential oils for food safety applications.

Keywords Fish fillet · Gelatin coating · Peppermint · Essential oil · SLNs

Introduction

SLNs contain fully crystallized lipid droplets, in which the bioactive components are part of the lipid matrix and have a highly ordered crystal structure introduced in the early 1990s [1]. These particles are made from solid lipids or mixtures of solid lipids that generally are stabilized with surfactants, and are presented as sub-micron particles (40–1000 nm). They are selected based on food ingredients, matrix properties, and required processing parameters. Due to their high stability and loading capacity, they are widely used in the pharmaceutical and cosmetics fields. Nonetheless, the use of SLNs in the food industry has expanded significantly [2]. SLNs are suitable delivery systems for lipophilic nutrients, being able to increase their stability, bioavailability, and functionality. For various nutritional applications, different bioactive compounds have been tested and incorporated into these versatile nano-delivery systems, such as *Zataria multiflora* essential oil in GMS and precirol® ATO 5 [3], carvacrol loaded in

✉ Saeid Khanzadi
khanzadi@um.ac.ir

¹ Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

² Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

³ Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

⁴ Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

⁵ Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

propylene glycol monopalmitate and GMS [4], and curcumin loaded in Compritol® 888 ATO [5]. These studies reflect an increase in the levels of bioavailability and stability of the active compounds entrapped in SLNs.

Mentha piperita, commonly known as peppermint, belongs to the mint family. Peppermint essential oil (PEO) has various antibacterial and antioxidant compounds, such as limonene, menthone, and menthol [6]. The hydrophobic and volatile natures of essential oils, as well as their sensitivity to light and oxygen, diminish their stability during the storage period [7]. Therefore, lipid-based nanocarriers can be considered an alternative solution to this problem. Such an approach makes it possible to overcome physicochemical instability problems, adjust distribution profiles, and provide specific organoleptic properties. Recently, there has been much research into the potential of essential oils (EOs) to develop edible coating as an alternative to extending the shelf life of sensitive products [5]. Gelatin as an edible coating derived from the hydrolysis of collagen has multiple nutritional properties. The main benefit of gelatin-coating is to prevent oxygen penetration, delay microbial spoilage, and prevent fat oxidation in meat. The most notable feature of fish gelatin is that it is widely accepted in all cultures and is not associated with the risk of bovine spongiform encephalopathy (BSE).

Numerous studies have been conducted to evaluate the effectiveness of EOs in maintaining the quality and extending the shelf life of sea food [8, 9, 10]. Mehraie et al. [11] investigated the effect of chitosan coating containing *Hysosopus Officinalis* EO on preservation of shrimp from chemical and sensory alterations. Lipid stability during the frozen storage of fillets from silver catfish exposed in vivo to the EO of *Lippia alba* was also studied by Veeck et al. [12]. Several studies have also been conducted with the target of potential use of different nanoemulsions on fish quality [13–15]. Although, as per our knowledge, there is no report on the preservation of fish fillet quality applying SLNs. Therefore, the objective of the present research was to design and characterize PEO loaded SLN and evaluate the effect of gelatin coating containing PEO-SLN as a new edible active coating on chemical and sensory properties of rainbow trout fillets during the cold storage.

Material and methods

Materials

Glycerol monostearate (GMS; Alfa Aesar, USA), tween 80 (Merck, Germany), gelatin (cold water fish skin; Sigma-Aldrich, Germany), peppermint essential oil (PEO; Nader agro-industry, Mashhad, Iran), thiobarbituric acid (TBA; Merck, Germany), glycerol, and

2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich (St. Louis, MO, USA) were purchased.

Preparation of SLNs

High-shear homogenization and bath sonication methods were applied to prepare PEO-loaded SLNs. The composition of SLN formulation is presented in Table 1 [16]. In brief, GMS was heated at 5 °C to melt, and then PEO as a lipid phase was added. The aqueous phase (tween 80 dissolved in double-distilled water) was added to the lipid phase at the end of the melting process to prevent PEO evaporation. The emulsion was then prepared using a bath sonication (Power sonic 505, Hwashin Technology, Gyeonggi-do, South Korea) at 3 cycles of 15 min with 15-s intervals.

Characterization of SLNs

Particle size and zeta potential

The average particle size (Z diameter), zeta potential, and polydispersity index (PDI) of PEO-SLN evaluated by Dynamic Light Scattering (DLS) (ZetaSizer NanoZS; Malvern Instruments Ltd., UK) [3].

Encapsulation efficacy (EE)

Dialysis tubing cellulose membrane with a pore size of 12–14 kDa (Sigma-Aldrich Co., Madrid, Spain) was used to measure the EE of PEO-SLN [17]. First, dialysis bags were filled with a known amount of PEO-SLN. Then, it was put on a magnetic stirrer (IKA, C-MAG HS 10, Germany) at 75 rpm for 24 h to be completely purified. Thereafter, 50 µL of the sample was mixed with 1950 µL of methanol. Finally, the absorbance of the final solution was recorded at 235.1 nm wavelength with the spectrophotometry method (CE 9500, UK). The amount of PEO was calculated using a suitable calibration curve of pure PEO in methanol with an R² of

Table 1 Production methods and formulation composition of peppermint essential oil-loaded SLN

Formulation code	GMS (mM)	Tween 80 (mM)	PEO (mM)	Method
1	1.39×10^{-2}	3.18×10^{-3}	5.28×10^{-3}	Bath sonication
2	2.78×10^{-2}	3.18×10^{-3}	5.28×10^{-3}	Bath sonication
3	2.78×10^{-2}	3.18×10^{-3}	5.28×10^{-3}	Prob sonication
4	2.78×10^{-2}	6.36×10^{-3}	5.28×10^{-3}	Bath sonication
5	2.78×10^{-2}	3.18×10^{-3}	5.28×10^{-3}	Bath sonication
6	2.78×10^{-2}	12.72×10^{-3}	5.28×10^{-3}	Bath sonication
7	2.78×10^{-2}	25.44×10^{-2}	5.28×10^{-3}	Bath sonication
8	5.56×10^{-2}	25.44×10^{-2}	5.28×10^{-3}	Bath sonication

0.99. Methanol was used as a blank. This process was carried out in triplicate. The amount of encapsulation efficacy of PEO-SLN were obtained using the following equation:

$$EE (\%) = \frac{\text{total amount of PEO} - \text{initial amount of PEO}}{\text{initial amount of PEO}} \times 100 \quad (1)$$

Transmission electron microscopy (TEM)

Internal matrix and morphology of individual nanoparticles were monitored by transmission electron microscopy (TEM; LEO 912AB, Germany). First, a drop of sample, which was completely diluted, was placed on a Formvar/Carbon 400-mesh copper grid (Ted Pella, CA). It was then dried at the room temperature (15 min), forming a thin layer. Before analysis by means of TEM, the prepared sample was equilibrated overnight at the room temperature. The mode of phase contrast was used to obtain the TEM images (Sadat Khadem et al., 2021).

In-vitro antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of PEO and PEO-SLN were measured using DPPH radical scavenging assay [18]. In brief, 50 μL of different concentrations (5.5, 2.75, 1.38, 0.69, and 0.34 mg/mL) of free PEO, PEO-SLN, and ascorbic acid (standard solution) were mixed with 2 mL of fresh DPPH solution (0.004% w/v). Next, the absorbance was read at the wavelength of 517 nm after their incubation at room temperature (1 h). The blank sample was methanol. Each reaction was performed in triplicate. The radical scavenging activity was determined by the following equation:

$$\text{DPPH scavenging effect (\%)} = [1 - (Ac - As)/Ac] \times 100 \quad (2)$$

Ac is the absorbance of control and As is the absorbance of sample.

Ferric reducing antioxidant power (FRAP)

The ferric reducing capacity of PEO and PEO-SLN were examined according to Benzie and Strain [19] method with some modifications. The FRAP solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ, and 2.5 mL of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. Briefly, 30 μL of different concentrations of samples (5.5, 2.75, 1.38, 0.69, and 0.34 mg/mL) were added to 900 μL of FRAP reagent, and their absorbance was recorded at the wavelength of 593 nm. The antioxidant capacity of PEO and PEO-SLN was obtained using a standard curve of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (100–1000 $\mu\text{M/L}$).

Preparation of trout sample and treatments

Fresh rainbow trout (*Oncorhynchus mykiss*) fillets with an average weight of 600–700 g were purchased from a local seafood store in Mashhad, Iran and immediately transferred to ice flasks in polystyrene isolation at the food hygiene laboratory of the Ferdowsi University of Mashhad, Iran. After washing off the slime and blood from the fish in the laboratory, the fillets were dried and cut into cut into $2 \times 4 \times 1$ cm pieces and weighing 10 g. Next, trout fillets were randomized into four groups with different treatments shown in Table 2. Fillets without any coating served as group 1 (control). To prepare gelatin-coating, cold fish gelatin (5% w/v) was dissolved in sterilized distilled water, and placed on a hotplate magnetic stirrer at 75 rpm and 70 °C until completely solved (group 2). Then, 0.2% (w/v) PEO as the oily phase, and 0.2% (w/v) tween 80 as the surfactant were mixed with gelatin to obtain a homogenous mixture (group 3). Group 4 was prepared by mixing gelatin and PEO-SLN 0.2% (W_{PEO}/V). The samples were coated with desired treatments for 2 min, and after draining, all the samples (control and treatment groups) were placed in sterile zip packs. Finally, they were stored under refrigeration conditions (4 ± 1 °C) for the chemical analysis on days 0, 1, 2, 4, 6, 8, and 12.

Chemical changes of trout fillet

pH value

Trout fillets pH values were determined using a pH meter (Martini, Mi 151, pH/ORP/Temperature Bench Meter). 10 g of treated fillets was well blended with distilled water in a ratio (1:10) for 1 min. The blended sample was used to quantify the pH value [20].

Total volatile basic nitrogen (TVB-N)

Trout fillet TVB-V was studied by a distillation-titration method using sulfuric acid (Hashemi et al., 2021). The amount of TVB-N was given as a sample of mg N/100 g.

Table 2 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_{PEO}/V)

$$\text{TVB-N} = \frac{1.4 \times \text{usedH}_2\text{SO}_4 \times \text{sample amount} \times 100}{1000\text{mg}} \quad (3)$$

Peroxide value (PV)

The peroxide value (PV) of the fat extract was analyzed according to Keykhosravi et al. [21] method and expressed as the uptake of milliequivalents (meq) of reactive oxygen species per kg of fat.

Thiobarbituric acid reactive substance (TBARs)

The TBARs values were assessed using the colorimetric method [22]. Its content was presented as mg of malondialdehyde (MDA) per kg of the fillet. The sample absorbance (A_s) was determined at 532 nm against the blank absorption (A_b). The values of TBARs were obtained using the following equation:

$$\text{TBARs} = (A_s - A_b) \times 0.25 \quad (4)$$

Free fatty acids (FFA)

The method of Ozogul et al. [23] was applied to assess free fatty acid (FFA) content, and the percentage of oleic acid was used to express its values. The FFA content was determined based on a titration method with 0.1 N NaOH and the use of phenolphthalein as an indicator. The FFA value was assessed based on equation no. 4:

$$\text{Acid value} = 56.1 \times N \times V$$

$$\text{FFA (\%)} = \text{Acid value} \times 12 \quad (5)$$

Fatty acids profile

Fatty acids trans-esterification was analyzed on the basis of Ehsani et al. [24] method using methanolic KOH and n-heptane. Fatty acid profiles of trout fillet were obtained using of gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA).

Sensory evaluation

Raw trout fillet

Twenty one panellists were recruited from the staff of the food hygiene laboratory, Ferdowsi university of Mashhad, Iran. Next, panel participants were trained with elementary sensory evaluation methods (four basic taste thresholds:

sweet, salty, sour and bitter). Trained panellists scored for sensory characteristics such as colour, odour, texture, overall acceptability, using a nine-point hedonic scale (1 very unpleasant, 9 very pleasant). The evaluation of the samples were carried out in separate booths under natural day light by the panellists. For each sample, three replicates were randomly tested. The fillets were cut into $2 \times 4 \times 1$ cm pieces and weighing 10 g and then randomized into four groups with different treatments (Table 2). The uncoated and coated fillets were individually blind coded, labeled with a 3-digit number, and placed on clean plates. Samples were served at a temperature of $25^\circ\text{C} \pm 2^\circ\text{C}$. Mineral water was provided for mouth-rinsing. Samples that scored > 4 by 50% or more of the judges are considered acceptable, treatments scored below 4 were not accepted and rejected. The maximum shelf life for trout samples were defined as the last sampling day where the treatment scored ≥ 4 [25].

Cooked trout fillet

Sensory evaluation of the cooked fillets were performed in the same way as in the former section and the following attributes were analysed at zero time: color, smell, taste, texture, and overall acceptability. For taste assessment, the samples were placed in a 700 W microwave oven with the addition of salt (1.5%) for 10 min. Moreover, To maintain the assessor's health, the assessment does not continue for the remaining days [26].

Statistical analysis

In this research, all the experiments were performed in triplicates. Statistical data analysis was executed via SPSS V21.0 software (SPSS, Inc. Chicago, IL, USA). Repeated measure analysis of variance and Tukey test were applied to evaluate significant differences at $P \leq 0.05$ level. Sensory scores were compared using the non-parametric Friedman Test. The pairwise comparison of the experimental groups was carried out using the test of Wilcoxon signed-rank.

Result and discussion

SLNs characterization

Particle size (PS), polydispersity index (PDI), and zeta potential (ZP)

Factors like the levels of emulsifier and lipid are regarded as important elements influencing SLNs quality dispersions. Eight runs were performed to consider the surfactant effects and lipid on PS, PDI, and ZP. Ingredient values, methods, and measured responses are presented in Tables 1 and 3.

Table 3 Characterization of peppermint essential oil encapsulated in SLN using different method

Characterization				
Formulation code	Particle size (nm)	Zeta potential (Mv)	Poly dispersity (PDI)	Encapsulation efficiency (%)
1	173.20±0.74	- 18.56±0.056	0.32±0.01	42.65
2	166.75±0.29	- 25.25±0.66	0.31±0.01	42.95
3	298.67±1.63	- 13.67±0.46	0.35±0.01	44.07
4	169.07±0.85	- 19.78±0.84	0.32±0.03	38.98
5	179.64±0.74	- 17.68±0.49	0.34±0.01	49.73
6	175.08±0.79	- 19.46±0.09	0.32±0.01	48.38
7	139.46±0.32	- 30.64±0.59	0.26±0.01	55.55
8	171.21±0.41	- 25.71±0.53	0.35±0.01	49.85

Based on the results, the formulation containing 60 mg of GMS and 0.2% of surfactant was selected to produce the optimal SLN. An optimized formulation was chosen based on minimizing particle size below 200 nm, minimizing PDI below 0.25, maximizing encapsulation efficiency, and maximizing ZP to avoid particle agglomeration. As indicated in Table 1, the amounts of lipid and surfactant ranged from 1.39×10^{-2} to 5.56×10^{-2} mM and 3.18×10^{-3} to 25.44×10^{-2} mM, respectively. Furthermore, PS, PDI, and ZP values changed from 139 to 298 nm, 0.26 to 0.32, and - 13 to - 30 mV, respectively. To validate the experimental model, the optimized formulation was prepared in triplicate. The experimental responses to PS, PDI, ZP, and EE of the optimized sample was determined to be 139 ± 0.32 nm, 0.26 ± 0.01 , $- 30 \pm 0.56$ Mv, and 55.5%, respectively (formulation no. 7). As the lipid level increased, the nanoparticles and PDI also increased. This is mainly due to the dispersion increased viscosity and the inadequate quantity of surfactant to coat the particle's surface, which requires higher shear strength [27]. In our study, SLNs were deployed using glycerol lipid monostearate (GMS) as the core and fixed with Tween 80 as the surfactant. It has been shown that the selection of HMS as a lipid compound has the advantage of stable dispersion with smaller particles, and improved release and loading properties compared with other lipid compounds [3]. As can be seen from Table 3, increasing the amount of surfactant generally reduces the lipid nanoparticles size.

The Zeta potential of all formulations showed a negative charge, ranging from - 13 to - 30 Mv. Therefore, it can be concluded that the negative charge of ZP is correlated to the presence of GMS and tween 80 on the lipid matrix surface. Our results exhibited that all SLNs fabricated had PDI values less than 0.5, referring to their narrow size distribution. Zeta potential values of SLNs revealed that formulation no. 7 had the highest surface charge. Above 30 mV zeta potential, proper electrostatic balance, and good physical stability occurred; however, this regulation cannot be useful to colloidal systems with non-ionic stabilizers like Tween

80 [28]. Tween 80 influence on the particle-water interface and the electric double layer can make the particles more stable. Moreover, the differential adsorption of hydroxyl and hydrated oxonium ions at neutral pH via nonionic surfactants causes the generation of a negative charge at the interface. Therefore, Tween 80 is a suitable nonionic surfactant as it is non-toxic.

Encapsulation efficiency (EE)

To determine EE, a PEO calibration curve was created in the concentration range of 14.4–924.9 µg/mL in methanol, and the amount of EE was evaluated using the UV–Vis method. As summarized in Table 3, EE values of PEO-SLN ranged from 38.9 to 55.5%. These results are in agreement with the findings of Fathi et al. [29]. They reported that hesperetin-loaded SLNs with different amounts of GMS and stearic acid have an EE range of approximately 39–63%. Several factors have significant impacts on the EE values, such as the solubility of core materials in the lipid matrix, the compression of lipid structures, the types and concentrations of surfactant, and the environmental variables [30]. In another study, Shetta et al. [31] evaluated the encapsulation efficiency of two essential oils in chitosan nanoparticles. They reported different EE values for encapsulated PEO (25–78%) and green tea oil (22–81%). The different patterns of EE between these EOs are probably caused by differences in the compositions of green tea oil and PEO. Likewise, Barzegar et al. [32] reported that the EE value of thyme loaded in chitosan nanoparticles is 26.6%. They indicated that the EE of thyme is affected by the initial weight ratio of chitosan to thyme oil. Also, they pointed out that EE tends to decrease, as initial thyme oil content increases and particle size decreases.

Transmission electron microscopy (TEM)

The morphological properties of PEO-SLN are illustrated in Fig. 1. The results revealed that the shape of SLNs is

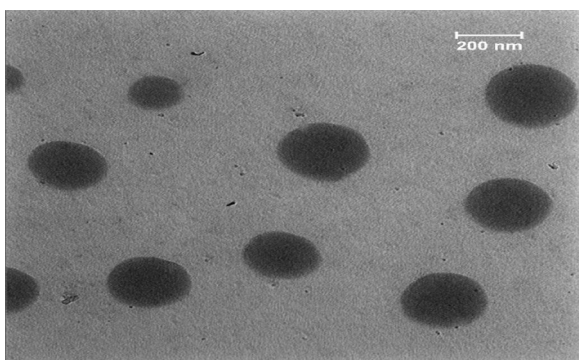


Fig. 1 Electron microscopy images of TEM from PEO-SLN

spherical with a size of 200 nm and also corresponds to previous studies [3, 33]. The sphericity of SLNs gives them the greatest ability for controlled release and protection of the encapsulated essential oil. This is because the spherical shape provides the longest route for the movement of essential oil encapsulated in the nanoparticles and the lowest contact area with the aqueous medium of the dispersed phase compared to the other nanoparticle shapes. Comparing the mean size results ascertained by TEM (for smaller particles) and DLS (for greater particles), that are consistent with the previous studies [31]. It can be seen that the difference is connected to the physical properties of materials which are used. The measurement of the average size by TEM was conducted on dry materials with limited particle movement. In DLS, the experiments were done on the suspension condition, and the size is associated with the sample hydrodynamic diameter in the solvated status.

In-vitro antioxidant activity

In our study, PEO and PEO-SLN antioxidant potential was investigated using DPPH and FRAP methods. The DPPH

radical scavenging assay is an excellent method for evaluating the antioxidant potency of various compounds. DPPH is a stable free radical with a violet colour that turns yellow upon the reduction in the presence of antioxidant compounds. Figure 2 depicted the radical scavenging activity of free PEO and PEO-loaded SLNs prepared by bath sonication at different concentrations. Ascorbic acid was also used as the positive control and showed 88.56% DPPH scavenging at the highest concentration. The scavenging activity of free PEO and encapsulated PEO ranged from 7.56 to 32.12% and 12.98 to 49.78%, respectively. The highest concentration (5.5 mg/mL) showed maximum scavenging efficiency in free (32.12%) and encapsulated PEO (49.78%). These results indicated that increasing the EO concentration enhanced the DPPH scavenging activity in PEO and PEO-SLN. Obtained results are confirmed by the study was performed by Barzegar et al. [32]. They reported that the scavenging effect of free and encapsulated thyme on DPPH radicals showed a concentration-dependent activity and ranges between 17.2–53.4 and 23.7–64.54%, respectively. The antioxidant power of free PEO and encapsulated PEO was also defined as IC_{50} which was expressed as the concentration (mg/mL) of PEO require to scavenge DPPH radicals by 50%. The IC_{50} value of the encapsulated PEO (0.54 mg/mL) was lower than that of free PEO (1.26 mg/mL). The enhancement in PEO antioxidant activity after the encapsulation process is likely caused by the protective impact of encapsulation that diminished the rate of evaporation by managed release of EOs during the experiment. These results are consistent with those obtained by Talón et al. [34] who reported a similar enhanced antioxidant activity for encapsulated eugenol in nanoparticles compared to free eugenol.

FRAP assay determines a compound's reducing capacity as its antioxidant power. This method is based on the reduction of ferric ion (Fe^{3+} -TPTZ) to the ferrous form (Fe^{2+} -TPTZ), a blue color complex in the presence of antioxidant agents. The concentration of Fe^{2+} in the sample is

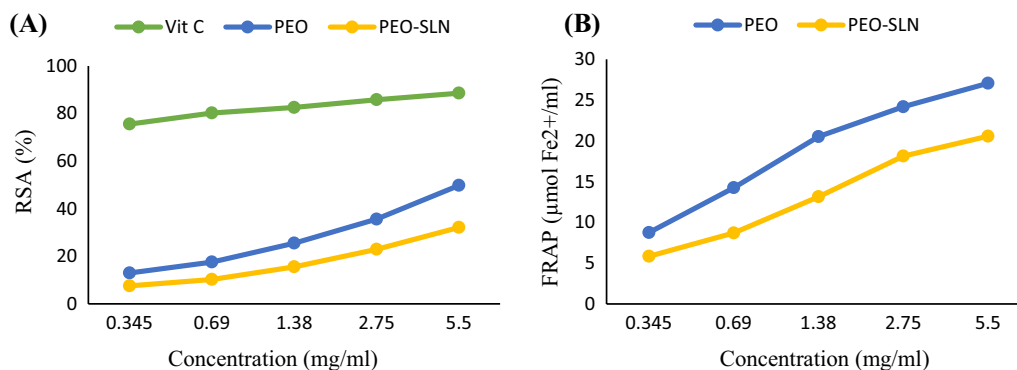


Fig. 2 Antioxidant activity (radical scavenging activity (DPPH; **A**) and ferric reducing antioxidant power (FRAP; **B**) of PEO and PEO-SLN in various concentrations

measured by the increase in absorbance that can be related to its antioxidant capacity. The antioxidant power of PEO and PEO-SLN was also illustrated in the FRAP assay (Fig. 2). PEO-loaded SLNs displayed higher antioxidant activity (27.06 mM Fe²⁺/mL) than free PEO (20.57 mM Fe²⁺/mL) at the concentration of 5 mg/mL. The results indicated a significant improvement in their antioxidant capacity after incorporating the essential oil into solid lipid nanoparticles. Mazzarino et al. [35] exhibited that the free jaboticaba extract and loaded in nanoemulsion exhibited similar antioxidant activity, in a concentration-dependent manner. The FRAP values of the free jaboticaba and jaboticaba nanoemulsion were reported about 24.18 ± 0.49 mM Fe²⁺/mL and 22.17 ± 0.99 mM Fe²⁺/mL, respectively. The slight difference between the results displayed by jaboticaba nanoemulsion and free extract can be explained by the encapsulation of antioxidant compounds inside the droplets of nanoemulsion, which avoid their optimal contact with the reagent. In another study, Damasceno et al. [36] reported that after the encapsulation of *Lippia origanoides* essential oil by the material, there is a significant increase in the antioxidant activity of the resulting material. They observed that droplets of essential oil adhere effectively to the inside of the matrix.

Chemical analysis of trout fillet

The pH values of the trout fillets during 12 days of cold storage have been represented in Table 4. The initial control group pH was 6.17, which reached 6.93 at the end of the storage period. As shown in Table 4, the pH value of sample without coating was considerably higher than other groups of treatment ($P < 0.05$). Similar results have been obtained by Keykhosravi et al. [21] and may be associated with the presence of phenolic compounds and acetic acid in the coating. The inhibitory effect of phenolic compounds on bacterial growth and later degradation of amino complexes during storage can indicate a slower tendency to improve pH in EO-containing samples. In the current study, the pH of the uncoated fillets was significantly higher than the coated samples with PEO and PEO-SLN during cold storage. It can be concluded that coating treatment was effective on the pH values of fillets storage. The increases in the pH indicated the accumulation of alkaline compounds, such as ammonia mainly derived from microbial action during fish muscle spoilage. No significant differences were found between the pH of the fillets coated with Gel + PEO and Gel + PEO-SLN at the end of the cold storage time. These results are in complete agreement with Özyurt et al. [37] demonstrated that the pH value of trout fillets coated with a protein-based biodegradable coating was significantly lower than that of uncoated samples.

In this study, TVB-N concentration notably increased ($P < 0.05$), and the accumulation rate of TVB-N was also higher in the control group than in the treated samples during the refrigerated storage. The initial TVB-N value (12.69 mg N/100 g) increased to 27.31 mg N/100 g (Table 4). The TVB-N contents observed in different treated groups did not exceed the limits recommended in the literature for fish fillets (25 mg N / 100 g) [23]. Previous studies can support our obtained results [10]. In control and Gel samples, the TVB-N values exceeded the acceptable limit after 12 days, while the final TVB-N values in Gel + PEO and Gel + PEO-SLN treated samples were below 25 mg N/100 g. After the storage period, the minimum levels of TVB-N were observed in the trout fillets treated with Gel + PEO (23.98 mg N/100 g) and Gel + PEO-SLN (23.04 mg N/100 g). A possible reason for the lower rate of TVB-N increase in a coating containing PEO can be the greater potential of these edible coatings to decrease the number of proteolytic bacteria or decrease the ability of the bacteria for the oxidative deamination of non-protein nitrogen compounds. Similar results have been reported for trout fillet treated with an active coating containing carrageenan and lemon essential oil [38].

The PV content of trout samples is represented in Table 4. Acceptable PV values are regarded to be between 10 and 20 meq kg⁻¹ [39]. During the first steps of storage, The PV values were between 3.98 and 4.04 meq kg⁻¹. In all treatment groups, PV values showed a gradual increase during storage. This is probably due to the decomposition of the primary oxidation products into the secondary oxidation products and, the reaction of hydroperoxide with the proteins. A similar pattern of hydroperoxide levels was reported in rainbow trout fillets during refrigerated storage [23]. During storage, samples coated with gelatin containing PEO and PEO-SLN had lower values of PV than the control sample. The samples treated with Gel + PEO-SLN had the most effective treatment to reduce the lipid degradation compared to other samples. Similarly, Yazgan (2017) reported lower PV values in nanoemulsion-treated samples than the control group in sea bream and sea bass. As the nanoemulsion droplet size is reduced, its oxidation stability is improved. As a result, the progress of the preliminary oxidation step and peroxide formation is slowed [40]. The treatments containing Gel + PEO-SLN was more effective compared to the samples covered with Gel + PEO. Previous studies have also shown that high levels of EO application can lead to additional antioxidant effects. Therefore, due to the higher concentration of antioxidant compounds such as phenolics, lipids are well protected from degradation.

TBAR is a method to measure the levels of secondary metabolites produced when meat is oxidized by fatty acids [24]. As shown in Table 4, the maximum amount of TBAR was observed in the control sample (3.19 ± 0.04 mg MDA/

Table 4 Changes in the chemical parameters of trout fillet during 12-day cold storage

Attributes	Treatments				
	Sampling time (day)	Con	Gel	Gel + PEO	Gel + PEO-SLN
Ph	0	6.17 ± 0.01 ^{a,B}	6.15 ± 0.01 ^{b,A}	6.13 ± 0.01 ^{b,A}	6.13 ± 0.01 ^{b,A}
	1	6.28 ± 0.01 ^{a,AB}	6.23 ± 0.03 ^{b,A}	6.23 ± 0.02 ^{b,A}	6.22 ± 0.02 ^{b,A}
	2	6.36 ± 0.05 ^{a,AB}	6.30 ± 0.04 ^{a,A}	6.23 ± 0.04 ^{b,A}	6.21 ± 0.01 ^{b,A}
	4	6.48 ± 0.01 ^{a,AB}	6.38 ± 0.01 ^{b,A}	6.29 ± 0.02 ^{b,A}	6.29 ± 0.01 ^{b,A}
	6	6.57 ± 0.05 ^{a,AB}	6.49 ± 0.02 ^{c,A}	6.47 ± 0.02 ^{c,AC}	6.44 ± 0.01 ^{c,A}
	8	6.79 ± 0.01 ^{a,AB}	6.67 ± 0.05 ^{d,AB}	6.52 ± 0.13 ^{d,AC}	6.46 ± 0.10 ^{d,AB}
	12	6.93 ± 0.04 ^{a,A}	6.81 ± 0.06 ^{b,AB}	6.75 ± 0.04 ^{bc,AC}	6.68 ± 0.01 ^{c,AB}
TVB-N (mg/100g)	0	12.69 ± 0.49 ^{a,E}	11.41 ± 0.51 ^{a,C}	8.58 ± 0.95 ^{a,B}	8.48 ± 0.42 ^{a,B}
	1	15.61 ± 0.34 ^{a,D}	14.04 ± 0.52 ^{a,C}	11.05 ± 0.52 ^{b,D}	10.06 ± 0.05 ^{b,C}
	2	16.98 ± 0.21 ^{a,D}	16.42 ± 0.86 ^{a,C}	12.88 ± 0.50 ^{b,D}	12.48 ± 0.23 ^{b,G}
	4	20.33 ± 0.85 ^{a,B}	20.41 ± 0.12 ^{a,B}	17.83 ± 0.59 ^{b,A}	16.76 ± 0.69 ^{b,D}
	6	22.19 ± 0.06 ^{a,A}	21.53 ± 0.42 ^{a,B}	18.51 ± 0.60 ^{c,A}	18.02 ± 0.20 ^{c,A}
	8	25.58 ± 0.35 ^{a,F}	24.76 ± 0.46 ^{a,F}	21.31 ± 0.12 ^{b,F}	20.33 ± 0.11 ^{b,F}
	12	27.31 ± 0.34 ^{a,C}	26.51 ± 0.14 ^{a,G}	23.98 ± 0.12 ^{c,G}	23.04 ± 0.25 ^{c,G}
PV (meq O ₂ /Kg)	0	4.04 ± 0.05 ^{a,C}	4.00 ± 0.04 ^{a,C}	3.92 ± 0.02 ^{a,B}	3.98 ± 0.02 ^{a,B}
	1	4.60 ± 0.05 ^{a,B}	4.54 ± 0.02 ^{a,B}	4.50 ± 0.03 ^{b,D}	4.44 ± 0.06 ^{b,E}
	2	4.88 ± 0.08 ^{a,B}	4.85 ± 0.12 ^{a,B}	5.54 ± 0.11 ^{b,D}	5.53 ± 0.14 ^{b,E}
	4	8.56 ± 0.32 ^{b,A}	8.19 ± 0.09 ^{b,A}	6.58 ± 0.09 ^{c,C}	5.98 ± 0.01 ^{c,E}
	6	10.46 ± 0.21 ^{c,D}	10.13 ± 0.06 ^{c,G}	7.55 ± 0.01 ^{b,G}	6.94 ± 0.28 ^{b,C}
	8	11.88 ± 0.30 ^{c,D}	11.16 ± 0.19 ^{d,G}	8.86 ± 0.18 ^{b,E}	8.56 ± 0.06 ^{b,D}
	12	15.00 ± 0.65 ^{d,E}	14.97 ± 0.66 ^{a,E}	10.41 ± 0.20 ^{a,F}	9.35 ± 0.0 ^{a,F}
TBARs (mg MDA/100 g)	0	0.93 ± 0.02 ^{a,D}	0.92 ± 0.02 ^{a,D}	0.91 ± 0.01 ^{a,D}	0.91 ± 0.01 ^{a,D}
	1	1.20 ± 0.04 ^{a,D}	1.17 ± 0.04 ^{a,D}	1.13 ± 0.28 ^{a,B}	1.12 ± 0.25 ^{a,B}
	2	1.77 ± 0.10 ^{b,A}	1.72 ± 0.12 ^{b,A}	1.52 ± 0.07 ^{c,A}	1.38 ± 0.15 ^{d,A}
	4	1.93 ± 0.03 ^{a,A}	1.90 ± 0.05 ^{a,C}	1.67 ± 0.09 ^{b,A}	1.54 ± 0.05 ^{b,E}
	6	2.27 ± 0.15 ^{a,E}	2.13 ± 0.01 ^{a,C}	1.88 ± 0.05 ^{b,C}	1.79 ± 0.07 ^{b,C}
	8	2.87 ± 0.17 ^{a,F}	2.84 ± 0.26 ^{a,F}	2.25 ± 0.05 ^{b,E}	2.16 ± 0.04 ^{c,G}
	12	3.19 ± 0.04 ^{a,G}	3.09 ± 0.10 ^{b,G}	2.53 ± 0.01 ^{c,G}	2.43 ± 0.11 ^{c,F}
FFA (% oleic acid)	0	3.61 ± 0.08 ^{a,D}	3.56 ± 0.09 ^{a,D}	3.54 ± 0.03 ^{a,D}	3.52 ± 0.01 ^{a,D}
	1	3.98 ± 0.07 ^{a,D}	3.92 ± 0.02 ^{a,D}	3.73 ± 0.10 ^{b,D}	3.70 ± 0.05 ^{b,D}
	2	4.67 ± 0.07 ^{a,B}	4.45 ± 0.04 ^{a,C}	4.32 ± 0.13 ^{b,C}	4.26 ± 0.16 ^{b,C}
	4	6.41 ± 0.16 ^{a,E}	5.65 ± 0.03 ^{b,A}	4.92 ± 0.08 ^{c,C}	4.63 ± 0.08 ^{d,C}
	6	8.37 ± 0.22 ^{a,A}	8.20 ± 0.05 ^{b,B}	6.11 ± 0.01 ^{c,B}	5.96 ± 0.02 ^{d,B}
	8	9.12 ± 0.09 ^{a,A}	9.09 ± 0.23 ^{a,E}	6.54 ± 0.09 ^{b,A}	6.13 ± 0.06 ^{c,A}
	12	10.35 ± 0.17 ^{a,F}	10.21 ± 0.05 ^{a,F}	7.61 ± 0.24 ^{b,G}	7.14 ± 0.06 ^{b,G}

TVB-N, total volatile basic nitrogen; FFA, free fatty acid; PV, peroxide value; TBARs, thiobarbituric reactive substance

^{a-d}Different lowercase letters in the same row indicate significant differences ($p < 0.05$)

^{A-G}Different uppercase letters in the same column indicate significant differences ($p < 0.05$)

Date is expressed as the mean ± SD ($n = 3$)

kg) during 12 days of storage. At the beginning of the storage, TBARs values were determined as 0.93 mg MDA/kg for trout fillet and slightly increased ($p < 0.05$) during cold storage period. Although the TBAR values of all samples increased with storage time, these levels were below the acceptable limit (3 mg MDA/kg) according to Connell et al. [41]. After 12 days of storage, the TBAR values of

Gel + PEO and Gel + PEO-SLN were 2.53 and 2.43 mg MDA/kg, respectively. A significant difference in TBAR values ($P < 0.05$) was observed between the control and treatment groups during the storage. These results are proved by values that have been reported in other studies [23, 42].

Table 4 shows the FFA content as an indicator of the lipid hydrolysis progression in trout fillets. The formation

of FFA reduces the nutritional value of meat by impacting on proteins denaturation and lipids oxidation. Overall, no considerable difference in FFA concentrations was observed at the beginning of the storage period. The increase in FFA levels during refrigeration storage is likely due to lipid degradation of meat tissue by hydrolyzing enzymes, like phospholipase and lipase, corresponding with the observations of Ehsani et al. [39]. In this research, the initial amount of FFA was 3.61 ± 0.08 , which enhanced to the highest level of 10.35 ± 0.17 in the control sample at the end of the storage period. Comparing the levels of FFA between the control and treatment groups, it can be concluded that due to the inactivation of associated enzymes, the effect of gelatin-coatings containing PEO and PEO-SLN on FFA production decreases. Likewise, Shadman et al. [43] found that the FFA value of the trout fillet treated with nanoemulsion with *Zataria multiflora* EO remained lower than that in the control sample.

The main fatty acids content of the trout fillets are listed in Table 5. Among all identified fatty acids, palmitic (16:0), oleic (18:1n9), linoleic (18:2n6), and docosahexaenoic acid (22:6n3) revealed significant amounts. Our results showed that the fatty acid composition of trout fillet notably affected due to cold storage. However, the SFA and MUFA levels increased significantly in all treatments, the amount of PUFA decreased, during the storage time ($p < 0.05$). Similar results were found by Keykhosravy et al. [44]. Hence, the EPA and DHA that are important of fatty acid in nutrition; reduced dramatically during the storage period in all groups, and no significant differences were seen among control and coated fillets. During storage, a slight reduction in PUFA levels ($P < 0.05$) was observed in the samples coated with

gelatin containing PEO and PEO-SLN compared to fillet treated with gelatin-coating alone. The same pattern of changes was obtained in MUFA and SFA. Hence, obtained result revealed that both of PEO and PEO-loaded SLN was effectively delayed changes in the FA composition of the trout during cooling.

Sensory evaluation

A summary of the sensory analysis of raw rainbow trout samples over 12 days of cold storage can be seen in Table 6. Trout samples were considered acceptable for human consumption until the sensory score reached 4 [25]. Obtained result demonstrated that the scores of the panellists reduced dramatically ($P < 0.008$) with increasing storage time, which had a relatively slow trend in the coated samples ($P < 0.008$). The results showed that all sensory attributes of control samples were acceptable by the 6th day of the storage period; while it was acceptable by the 12th day of storage for texture and colour attributes, and by the 8th day of storage for odour and overall acceptability in coated samples. In all sensory evaluations, the maximum grade was given to the sample treated with Gel + PEO-SLN. Besides, Gel + PEO improves the sensory properties of trout fillets and prolongs their shelf life under refrigeration. It has been reported that the EOs reduced oxidative stress and thus production of lipid oxidation secondary metabolites compared with control group and samples coated with gelatin [45]. These results were similar to the results obtained from Keykhosravy et al. [21] as well, in which *Zataria multiflora* and *Bunium persicum* EOs improved the sensory properties of turkey fillets for 15 days. Furthermore, the current results showed that the

Table 5 Fatty acid (FA) content (% of total FAs) of trout fillets during refrigerated storage

FA (%)	Day 0				Day 12			
	Con	Gel	Gel + PEO	Gel + PEO-SLN	Con	Gel	Gel + PEO	Gel + PEO-SLN
16:0	15.84 ± 1.1	14.30 ± 0.9	14.39 ± 0.04	14.29 ± 0.01	18.38 ± 0.1	18.30 ± 0.10	17.42 ± 0.18	17.75 ± 0.11
18:0	5.84 ± 0.1	5.01 ± 0.05	5.66 ± 0.06	5.90 ± 0.06	6.98 ± 0.04	6.18 ± 0.09	6.87 ± 0.04	6.90 ± 0.09
SFA	21.99 ± 1.2^a	21.08 ± 1.1^a	20.64 ± 1.1^a	21.37 ± 1.0^a	25.03 ± 0.1^a	26.83 ± 0.08^b	24.91 ± 0.18^c	24.60 ± 0.51^c
18:1w9tr	20.01 ± 0.2	20.01 ± 0.2	20.03 ± 0.02	20.01 ± 0.03	26.01 ± 0.0	26.21 ± 0.01	27.03 ± 0.01	27.05 ± 0.03
18:1w9cis	18.95 ± 0.6^a	15.08 ± 0.4^b	16.84 ± 0.23^c	18.78 ± 0.32^a	20.64 ± 0.1	20.48 ± 0.08	20.57 ± 0.13	20.78 ± 0.12
MUFA	39.11 ± 0.8^a	37.73 ± 0.5^b	37.57 ± 0.52^b	39.82 ± 0.8^{ab}	47.86 ± 0.1^a	46.89 ± 0.09^a	48.17 ± 0.05^b	48.58 ± 0.08^b
18:2w6cis	18.47 ± 0.5	$18.20 \pm .02$	19.80 ± 0.9	18.87 ± 0.9	14.18 ± 0.1^a	12.60 ± 0.03^b	14.85 ± 0.54^a	14.89 ± 0.08^a
18:3w6	0.24 ± 0.08	0.16 ± 0.01	0.25 ± 0.03	0.34 ± 0.05	0.10 ± 0.08	0.16 ± 0.07	0.18 ± 0.05	0.18 ± 0.02
18:3w3	1.04 ± 0.4^a	0.56 ± 0.23^b	0.75 ± 0.02^b	1.16 ± 0.21^a	0.75 ± 0.08	0.43 ± 0.03	0.51 ± 0.04	0.81 ± 0.01
20:3w6	0.41 ± 0.12	0.23 ± 0.12	0.26 ± 0.11	0.38 ± 0.11	0.21 ± 0.09	0.26 ± 0.13	0.38 ± 0.18	0.75 ± 0.09
20:5w3	2.12 ± 0.05	2.02 ± 0.01	2.09 ± 0.05	2.32 ± 0.01	1.09 ± 0.03	1.12 ± 0.10	1.14 ± 0.04	1.74 ± 0.11
22:6w3	9.87 ± 0.12	8.45 ± 0.54	8.02 ± 0.23	8.94 ± 0.04	4.21 ± 0.04	4.25 ± 0.08	5.12 ± 0.05	5.65 ± 0.04
PUFA	31.92 ± 0.2^a	29.65 ± 0.2^b	30.71 ± 0.45^b	30.85 ± 0.28^b	20.76 ± 0.2^a	18.15 ± 0.14^b	21.37 ± 0.15^{ac}	21.74 ± 0.15^{ac}

^{a-d}Different lowercase letters in the same row indicate significant differences ($p < 0.05$)

Date is expressed as the mean \pm SD ($n = 3$)

Table 6 Sensory evaluations of raw trout fillet during 12 days of cold storage

Attributes	Treatments	Sampling time (days)						
		D	D1	D2	D4	D6	D8	D12
Color	Control	9 (9,9) ^a	8 (8,9) ^a	8 (7.5,8) ^a	7 (7,8) ^{ab}	6 (6,8) ^b	6 (6,7) ^b	5 (5,6) ^c
	Gel	9 (9,9) ^a	9 (8,9) ^a	8 (8,8) ^a	8 (7,8) ^a	7 (7,7) ^{ab}	7 (6,7) ^b	6 (5,6) ^c
	Gel + PEO	9 (9,9) ^a	9 (8,9) ^a	8 (8,8) ^a	8 (7,8) ^a	7 (7,7.5) ^{ab}	7 (6.5,7) ^b	6 (5, 6.5) ^c
	Gel + PEO-SLN	9 (9,9) ^a	9 (8,9) ^a	8 (8,8) ^a	8 (7,8) ^a	7 (7,8) ^{ab}	7 (6,8) ^b	6 (5, 6.5) ^c
odor	Control	9 (9,9) ^a	8 (8,9) ^a	6 (6,7) ^{ab}	5 (5,5) ^b	4 (4,5) ^{bc}	3 (3,4) ^d	2 (2,3) ^d
	Gel	9 (9,9) ^a	9 (8,9) ^a	6 (6,7) ^{ab}	5 (5,6) ^b	5 (4,5) ^{bc}	4 (4,4) ^d	3 (3,3) ^d
	Gel + PEO	9 (9,9) ^a	9 (8,9) ^a	8 (7,7) ^b	6 (5,6) ^c	5 (5,5) ^{dc}	4 (3,4) ^d	3 (3,4) ^d
	Gel + PEO-SLN	9 (9,9) ^a	9(8,9) ^a	8 (7,9) ^b	6 (5,6) ^c	5 (5,5) ^{dc}	4 (3,4) ^d	4 (3,4) ^d
Texture	Control	9 (9,9) ^a	9 (8,9) ^a	7 (8,7) ^a	6 (6,6) ^{ab}	4 (4,4) ^{bc}	4 (4,3) ^{bc}	4 (2,4) ^c
	Gel	9 (9,9) ^a	9 (8,9) ^a	8 (8,8) ^a	6 (6,5) ^{ab}	5 (5,5) ^{bc}	4 (4,4) ^{bc}	4 (4,4) ^c
	Gel + PEO	9 (9,9) ^a	9 (8,9) ^a	8 (8,8) ^a	8 (7,8) ^b	5 (4,6) ^{bc}	5 (4,5) ^{bc}	5 (5,5) ^c
	Gel + PEO-SLN	9 (9,9) ^a	9 (8,9) ^a	8 (8,8.5) ^a	8 (7,8) ^b	5 (5,6) ^{bc}	5 (4,6) ^{bc}	5 (5,5) ^c
General acceptability	Control	9 (9,9) ^a	9 (8,9) ^a	7 (7,8) ^{ab}	6 (6,6) ^b	4 (5,5) ^{bc}	3 (3,4) ^{dc}	3 (2,3) ^d
	Gel	9 (9,9) ^a	9 (8,9) ^a	7 (7,8) ^{ab}	6 (6,7.5) ^{ab}	5 (5,5.5) ^{bc}	3 (3,4) ^c	3 (3,3) ^c
	Gel + PEO	9 (9,9) ^a	9 (8,9) ^a	8 (7.5,8) ^a	8 (7,8) ^{ab}	5 (5,6) ^c	4 (4,4) ^{dc}	4 (3,4) ^c
	Gel + PEO-SLN	9 (9,9) ^a	9 (8,9) ^a	8 (8,8) ^a	8 (7,8) ^{ab}	5 (5,6) ^c	4 (4,5) ^{dc}	4 (4,4) ^c

^{a-c}Different lowercase letters in the same row indicate significant differences ($p < 0.008$)

Date is expressed as the median (min, max) ($n=3$)

Gel + PEO and Gel + PEO-SLN treatments improved the odor, texture, and general acceptability of trout fillet during storage time. It is also noted that encapsulation of essential oil into gelatin coating reduced the fishy odor of gelatin, enhanced its acceptability and prolonged the shelf life of fillets, which is in line with other studies that reported different essential oils can improved the sensory properties of fish fillets, as well as increased their shelf life [23, 42]. This may be attributed to functional properties of gelatin-coating loaded with PEO-SLN as a barrier against moisture transfer, oxygen uptake, and also antioxidant and antibacterial potential of encapsulated PEO. Regarding to the use of PEO in low concentration (0.2%), it had a significant sensory effect according to the scores of panellist, which were completely consistent with previous reports [14]. Likewise, color data showed that there were no significant differences between different experimental groups during the storage time. Therefore, it can be concluded that there were no significant effects of gelatin-coating containing PEO and PEO-SLN on trout fillet's color during cold storage. Similar results for pink salmon were also reported by Sathivel [46].

Table 7 shows the results obtained from the sensory evaluation of cooked fillet at zero time. The color, odor, and texture of the cooked fillets were not significantly influenced by the use of the coating. The obtained result showed that gelatin-coating with PEO and PEO-SLN improved the flavor of the cooked fillet. It has been suggested that EO's aromatic compounds play a crucial role in enhancing the flavor of trout fillet [23]. Overall, these studies suggest that the using

Table 7 Sensory evaluations of cooked trout fillet at day 0 cold storage

Treatments	Attributes				
	Color	Odor	Texture	Taste	General acceptability
Control	9 (7,9) ^a	8 (7,7) ^a	8 (8,7.5) ^a	8 (5,6) ^a	8 (7,7) ^a
Gel	9 (8,9) ^a	8 (8,7) ^a	8 (8,8) ^a	8 (5,6) ^a	8 (7,6) ^a
Gel + PEO	9 (8,9) ^a	9 (8,9) ^a	8 (8,8.5) ^a	9 (8,9) ^{a,b}	9 (8,9) ^{a,b}
Gel + PEO-SLN	9 (8,9) ^a	9 (8,9) ^a	8 (8,8) ^a	9 (8.5,9) ^{a,b}	9 (8,9) ^{a,b}

^{a-b}Different lowercase letters in the same column indicate significant differences ($p < 0.008$)

Date is expressed as the median (min, max) ($n=3$)

gelatin-coating loaded with PEO-SLN in trout fillets may improve, or at least not negatively affect, their sensory properties. However, this will likely depend on the compatibility of the specific bioactive used with the particular product. For example, curcumin will impart a yellow-orange color to the product, while many essential oils have distinct flavours (such as peppermint, lemon, or thyme). Consequently, it may be necessary to optimize the delivery system used to the food product that is being fortified. It should be noted that one disadvantage of using EOs is their strong aroma and taste, which can significantly affect the organoleptic properties of fresh products. Many studies have shown that using high concentrations of essential oils can have a negative impact

on the flavour of food products. For instance, Keykhosravi et al. (2021) reported that using high concentration (1%) of Eos (*Zataria multiflora Boiss and Bunium persicum*) produced bitterness and reduced organoleptic quality of cooked turkey fillets.

Conclusion

In the present study, the oxidative stability of trout fillet was maximized with gelatin-loaded SLN, which contained PEO. Furthermore, sensory analysis indicated that gelatin-coating loaded with PEO-SLN can improve the organoleptic quality of trout fillets and also extend the shelf life of fillets during cold storage. Moreover, encapsulated PEO exhibited remarkable antioxidant activity compared to free PEO. Overall, gelatin-coating in combination with PEO-SLN is proposed as a suitable choice to improve the preservation as well as the quality of different food products.

Acknowledgements This study was supported by the Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran (Grant No.: 3/53613).

Author contribution SSL: Investigation; Methodology; Writing—original draft. SK: conceptualization; funding acquisition; project administration; supervision; validation; writing—original draft; writing—review and editing. MH: methodology; resources; visualization; writing—review and editing. FG: methodology; resources; visualization; writing—review and editing. MA: data curation; formal analysis; software; validation.

Declarations

Conflict of interest The authors declare no conflicts of interests.

References

1. D.R.B. Oliveira, G. de Figueiredo Furtado, R.L. Cunha, Solid lipid nanoparticles stabilized by sodium caseinate and lactoferrin. *Food hydrocoll.* **90**, 321–329 (2019)
2. G.I. Sakellari, I. Zafeiri, A. Pawlik, D. Kurukji, P. Taylor, I.T. Norton, F. Spyropoulos, Independent co-delivery of model actives with different degrees of hydrophilicity from oil-in-water and water-in-oil emulsions stabilised by solid lipid particles via a Pickering mechanism: a proof-of-principle study. *J. Colloid Interface Sci.* **587**, 644–649 (2021)
3. M. Nasser, S. Golmohammadzadeh, H. Arouiee, M.R. Jaafari, H. Neamati, Antifungal activity of *Zataria multiflora* essential oil-loaded solid lipid nanoparticles in-vitro condition. *Iran. J. Basic Med. Sci.* **19**(11), 1231 (2016)
4. J. He, S. Huang, X. Sun, L. Han, C. Chang, W. Zhang, Q. Zhong, Carvacrol loaded solid lipid nanoparticles of propylene glycol monopalmitate and glyceryl monostearate: preparation, characterization, and synergistic antimicrobial activity. *Nanomater.* **9**(8), 1162 (2019)
5. M. Alanchari, M. Mohammadi, F. Yazdian, H. Ahangari, N. Ahmadi, Z. Emam-Djomeh, A. Homayouni-Rad, A. Ehsani, Optimization and antimicrobial efficacy of curcumin loaded solid lipid nanoparticles against foodborne bacteria in hamburger patty. *J. Food Sci.* **86**(6), 2242–2254 (2021)
6. M.L. Badea, S.L. Iconaru, A. Groza, M.C. Chifriuc, M. Beuran, D. Predoi, Peppermint essential oil-doped hydroxyapatite nanoparticles with antimicrobial properties. *Molecules* **24**(11), 2169 (2019)
7. L.F. Cossetin, Q.I. Garlet, M.C. Velho, S. Gündel, A.F. Ourique, B.M. Heinzmann, S.G. Monteiro, Development of nanoemulsions containing *Lavandula dentata* or *Myristica fragrans* essential oils: Influence of temperature and storage period on physical-chemical properties and chemical stability. *Ind. Crops Prod.* **160**, 113115 (2021)
8. S. Khanzadi, K. Keykhosravi, M. Hashemi, M. Azizzadeh, Alginate coarse/nanoemulsions containing *Zataria multiflora Boiss* essential oil as edible coatings and the impact on microbial quality of trout fillet. *Aquac. Res.* **51**(3), 873–881 (2020)
9. S.S. Laein, S. Khanzadi, M. Hashemi, F. Gheybi, M. Azizzadeh, 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. *J. Food Sci.* **87**(7), 2920–2931 (2022)
10. M. Raeisi, M. Hashemi, M. Aminzare, F. Ghorbani Bidkorphe, M. Ebrahimi, B. Jannat, B. Tepe, S.M.A. Noori, Effects of sodium alginate and chitosan coating combined with three different essential oils on microbial and chemical attributes of rainbow trout fillets. *J. Aquat. Food Prod. Technol.* **29**(3), 253–263 (2020)
11. A. Mehraie, S. Khanzadi, M. Hashemi, M. Azizzadeh, New coating containing chitosan and *Hyssopus officinalis* essential oil (emulsion and nanoemulsion) to protect shrimp (*Litopenaeus vannamei*) against chemical, microbial and sensory changes. *Food Chem. X* **19**, 100801 (2023)
12. A.P.L. Veeck, B. Klein, L.F. Ferreira, A.G. Becker, C.G. Heldwein, B.M. Heinzmann, B. Baldisserotto, T. Emanuelli, Lipid stability during the frozen storage of fillets from silver catfish exposed in vivo to the essential oil of *Lippia alba* (Mill.) NE Brown. *J. Sci. Food Agric.* **93**(4), 955–960 (2013)
13. P.A. Popescu, L.M. Palade, I.C. Nicolae, E.E. Popa, A.C. Mitelut, M.C. Drăghici, M.E. Popa, Chitosan-based edible coatings containing essential oils to preserve the shelf life and postharvest quality parameters of organic strawberries and apples during cold storage. *Foods* **11**(21), 3317 (2022)
14. M.A. Sani, A. Ehsani, M. Hashemi, Whey protein isolate/cellulose nanofibre/TiO₂ nanoparticle/rosemary essential oil nanocomposite film: its effect on microbial and sensory quality of lamb meat and growth of common foodborne pathogenic bacteria during refrigeration. *Int. J. Food Microbiol.* **251**, 8–14 (2017)
15. M. Zhang, W. Luo, K. Yang, C. Li, Effects of sodium alginate edible coating with cinnamon essential oil nanocapsules and Nisin on quality and shelf life of beef slices during refrigeration. *J. Food Prot.* **85**(6), 896–905 (2022)
16. B.S.F. Bazzaz, B. Khameneh, H. Zarei, S. Golmohammadzadeh, Antibacterial efficacy of rifampin loaded solid lipid nanoparticles against *Staphylococcus epidermidis* biofilm. *Microb. Pathog.* **93**, 137–144 (2016)
17. C. Deka, D. Deka, M.M. Bora, D.K. Jha, D.K. Kakati, Synthesis of peppermint oil-loaded chitosan/alginate polyelectrolyte complexes and study of their antibacterial activity. *J. Drug Deliv. Sci. Technol.* **35**, 314–322 (2016)
18. K. Sridhar, A.L. Charles, In vitro antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: estimation methods for EC50 using advanced statistical programs. *Food Chem.* **275**, 41–49 (2019)
19. I.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem.* **239**(1), 70–76 (1996)

20. N.B. El-Borai, M.A.E.-G. Mohamed, M.A. Khalaf, M. Hamada, H.S. Heikal, H.D. Mahboub, Protective effect of propolis against chromium toxicity in *Nile tilapia*: evaluation of fillet quality, geno-oxidative and pathological alterations in liver. *IOSR JESTFT* **12**, 34–41 (2018)
21. K. Keykhosravi, S. Khanzadi, M. Hashemi, M. Azizzadeh, Protective effect of chitosan-loaded nanoemulsion containing *Zataria multiflora* Boiss and *Bunium persicum* Boiss essential oils as coating on lipid and protein oxidation in chill stored turkey breast fillets. *J. Food Sci.* **87**(1), 251–265 (2022)
22. S. Noori, F. Zeynali, H. Almasi, Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. *Food Control* **84**, 312–320 (2018)
23. Y. Ozogul, İ Yuvka, Y. Ucar, M. Durmus, A.R. Kösker, M. Öz, F. Ozogul, Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets during ice storage. *LWT* **75**, 677–684 (2017)
24. A. Ehsani, M. Hashemi, M. Aminzare, M. Raeisi, A. Afshari, A. Mirza Alizadeh, M. Rezaeigolestani, Comparative evaluation of edible films impregnated with sage essential oil or lactoperoxidase system: Impact on chemical and sensory quality of carp burgers. *J. Food Process. Preserv.* **43**(9), e14070 (2019)
25. W. Fan, J. Sun, Y. Chen, J. Qiu, Y. Zhang, Y. Chi, Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food Chem.* **115**(1), 66–70 (2009)
26. S.M.T. Gharibzahedi, S. Mohammadnabi, Effect of novel bioactive edible coatings based on jujube gum and nettle oil-loaded nanoemulsions on the shelf-life of *Beluga sturgeon* fillets. *Int. J. Biol. Macromol.* **95**, 769–777 (2017)
27. J. Araujo, E. Gonzalez-Mira, M.A. Egea, M.L. Garcia, E.B. Souto, Optimization and physicochemical characterization of a triamcinolone acetonide-loaded NLC for ocular antiangiogenic applications. *Int. J. Pharm.* **393**(1–2), 168–176 (2010)
28. A.N. Oktay, S. Ilbasimis-Tamer, A. Karakucuk, N. Celebi, Screening of stabilizing agents to optimize flurbiprofen nanosuspensions using experimental design. *J. Drug Deliv. Sci. Technol.* **57**, 101690 (2020)
29. M. Fathi, J. Varshosaz, M. Mohebbi, F. Shahidi, Hesperetin-loaded solid lipid nanoparticles and nanostructure lipid carriers for food fortification: preparation, characterization, and modeling. *Food Bioprocess Technol.* **6**(6), 1464–1475 (2013)
30. F.K. Nahr, B. Ghanbarzadeh, H. Hamishehkar, H.S. Kafil, Food grade nanostructured lipid carrier for cardamom essential oil: preparation, characterization and antimicrobial activity. *J. Funct. Foods* **40**, 1–8 (2018)
31. A. Shetta, J. Kegere, W. Mamdouh, Comparative study of encapsulated peppermint and green tea essential oils in chitosan nanoparticles: encapsulation, thermal stability, in-vitro release, antioxidant and antibacterial activities. *Int. J. Biol. Macromol.* **126**, 731–742 (2019)
32. M. Barzegar, M. Ghaderi Ghahfarokhi, M.A. Sahari, M.H. Azizi, Enhancement of thermal stability and antioxidant activity of thyme essential oil by encapsulation in chitosan nanoparticles. *J. Agric. Sci. Technol.* **18**(7), 1781–1792 (2016)
33. B. Fazly Bazzaz, B. Khameneh, N. Namazi, M. Iranshahi, D. Davoodi, S. Golmohammadzadeh, Solid lipid nanoparticles carrying *Eugenia caryophyllata* essential oil: the novel nanoparticulate systems with broad-spectrum antimicrobial activity. *Lett. Appl. Microbiol.* **66**(6), 506–513 (2018)
34. E. Talón, M. Vargas, A. Chiralt, C. González-Martínez, Eugenol incorporation into thermoprocessed starch films using different encapsulating materials. *Food Packag. Shelf Life* **21**, 100326 (2019)
35. L. Mazzarino, H. da Silva Pitz, A.P. Lorenzen Voytena, A.C. Dias Trevisan, R.M. Ribeiro-Do-Valle, M. Maraschin, Jaboticaba (*Plinia peruviana*) extract nanoemulsions: development, stability, and in vitro antioxidant activity. *Drug Dev. Ind. Pharm.* **44**(4), 643–651 (2018)
36. E.T. Damasceno, R.R. Almeida, S.Y. de Carvalho, S.S. Vieira, V. Mano, L.G.D.L. Guimarães, Nano-encapsulation of Lippia organoides kunth. Essential oil by chitosan-caffeic acid nanogel. *Quim. Nova* **43**, 16–23 (2020)
37. G. Özyurt, A.S. Özkütük, A. Şimşek, A.F. Yeşilsu, M. Ergüven, Quality and shelf life of cold and frozen rainbow trout (*Oncorhynchus mykiss*) fillets: effects of fish protein-based biodegradable coatings. *Int. J. Food Prop.* **18**(9), 1876–1887 (2015)
38. M.G. Volpe, F. Siano, M. Paolucci, A. Sacco, A. Sorrentino, M. Malinconico, E. Varricchio, Active edible coating effectiveness in shelf-life enhancement of trout (*Oncorhynchus mykiss*) fillets. *LWT - Food Sci. Technol.* **60**(1), 615–622 (2015)
39. A. Ehsani, M. Hashemi, M. Raeisi, S.S. Naghibi, A. Afshari, Rainbow trout fillet biopreservation by edible chitosan-based coating containing egg yolk antibody (IgY) and lycopene. *J. Food Sci. Technol.* **57**(1), 282–292 (2020)
40. E. Amiri, M. Aminzare, H.H. Azar, M.R. Mehrasbi, Combined antioxidant and sensory effects of corn starch films with nanoemulsion of *Zataria multiflora* essential oil fortified with cinnamaldehyde on fresh ground beef patties. *Meat Sci.* **153**, 66–74 (2019)
41. J.J. Connell, Methods of assessing and selecting for quality. *Control Fish Qual* **2**, 122–150 (1990)
42. H. Yazgan, Y. Ozogul, M. Durmuş, E. Balıkcı, S. Gökdoğan, Y. Uçar, E.T. Aksun, Effects of oil-in-water nanoemulsion based on sunflower oil on the quality of farmed sea bass and gilthead sea bream stored at chilled temperature (2±2 C). *J. Aquat. Food Prod. Technol.* **26**(8), 979–992 (2017)
43. S. Shadman, S.E. Hosseini, H.E. Langroudi, S. Shabani, Evaluation of the effect of a sunflower oil-based nanoemulsion with *Zataria multiflora* Boiss. essential oil on the physicochemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets during cold storage. *LWT-Food Sci. Technol.* **79**, 511–517 (2017)
44. K. Keykhosravi, S. Khanzadi, M. Hashemi, M. Azizzadeh, Chitosan-loaded nanoemulsion containing *Zataria Multiflora* Boiss and *Bunium persicum* Boiss essential oils as edible coatings: its impact on microbial quality of turkey meat and fate of inoculated pathogens. *Int. J. Biol. Macromol.* **150**, 904–913 (2020)
45. B. Bazargani-Gilani, J. Aliakbarlu, H. Tajik, Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. *Innov. Food Sci. Emerg. Technol.* **29**, 280–287 (2015)
46. S. Sathivel, Chitosan and protein coatings affect yield, moisture loss, and lipid oxidation of pink salmon (*Oncorhynchus gorbuscha*) fillets during frozen storage. *J. Food Sci.* **70**(8), e455–e459 (2005)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.