Original Article

Antioxidant and Physicochemical Properties of Saffron (*Crocus sativus* L.) Nanoemulsion and Its Effect on Rainbow Trout Fillet Preservation

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ABSTRACT

Background: Fish spoils quickly due to oxidation, microbial activity, and high moisture content. Therefore, natural preservatives, such as saffron (*Crocus sativus* L.) nanoemulsions have the potential to extend the shelf life of seafood while reducing the use of synthetic preservatives.

Objectives: This study evaluated the effectiveness of saffron (*C. sativus*) nanoemulsions (SNE1–SNE8), via spontaneous or ultrasonic emulsification, in extending the shelf life of rainbow trout and enhancing antioxidant effects.

Methods: Saffron nanoemulsions were characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR), and their physical stability was monitored for 28 days. Eight nanoemulsion formulations were initially prepared and characterized; however, only SNE1–SNE4 were subsequently evaluated in the rainbow trout preservation study. Rainbow trout fillets coated with SNE1–SNE4 were stored at 4 °C and 8 °C for 21 days. Total volatile basic nitrogen, peroxide value, thiobarbituric acid, pH, antioxidant activity, and sensory properties were compared with those of uncoated fillets and fillets treated with pure saffron extract.

Results: DLS and TEM demonstrated that the saffron nanoemulsions exhibited spherical and homogeneous droplets with particle sizes ranging from 9.7 to 22.3 nm, while SNE4 (10.6 nm) showed the highest physical stability during 28 days of storage ($P<0.05$). FTIR confirmed the preservation of the characteristic functional groups of saffron following nanoemulsion formation. During refrigerated storage, biochemical parameters differed significantly among the treatments ($P<0.05$). After 21 days at 4 °C, treatment d (SNE4) showed the lowest TVB-N (27.16±0.28 mg/100 g), peroxide value (PV) (0.4±0.002 meq O_2/kg), hiobarbituric acid (TBA) (0.54±0.008 mg MDA/kg), and pH (6.45±0.02), while maintaining the highest overall sensory acceptance.

Conclusion: Saffron nanoemulsions effectively preserved rainbow trout by delaying protein degradation and lipid oxidation while maintaining sensory quality during refrigerated storage ($P<0.05$). These findings highlight their potential as natural alternatives to synthetic preservatives for seafood preservation.

Keywords: *Crocus sativus* L., Nanoemulsion, *Oncorhynchus mykiss*, Shelf life, Antioxidant activity

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Introduction

In recent decades, the expansion of the pharmaceutical and food industries has driven research into nanostructures, particularly nanoemulsions, aimed at enhancing antimicrobial and antioxidant properties (Donsì & Ferrari, 2011). The application of these nanosystems for the delivery of plant-derived bioactive compounds serves as an effective strategy to extend the shelf life of highly perishable products, such as rainbow trout fillets (Ozogul et al., 2017). Among natural bioactive sources, saffron (*Crocus sativus* L.) is widely recognized for its potent antioxidant and antimicrobial activities, making it an excellent candidate for such nano-formulations (Gohari et al., 2013). Its antioxidant activity is mainly attributed to bioactive compounds, such as crocin, picrocrocin, and safranal, which have attracted considerable interest for food preservation applications. Its antioxidant and antimicrobial properties have also attracted considerable attention for food preservation applications (Abu-Izneid et al., 2022).

Seafood, particularly fish, is highly susceptible to autolysis, oxidation, lipid hydrolysis, and microbial spoilage (Bagheri et al., 2008). Rainbow trout (*Oncorhynchus mykiss*) is a commercially important freshwater fish rich in polyunsaturated fatty acids, making it particularly susceptible to lipid oxidation and quality deterioration during refrigerated storage (Secci & Parisi, 2016). Changes due to oxidation lead to alterations in the taste of the fish, and due to the degradation of the muscle's protein system, the amount of fluid exudate (drip loss) from the fish increases (Secci & Parisi, 2016). Considering that the amount of fat in the bodies of different fish species varies, almost all of them have unsaturated fatty acids in their lipid structure that oxidize in the presence of air and cause undesirable changes in the fats (Secci & Parisi, 2016). Despite the belief that refrigerated and subzero temperatures should prevent lipid changes, it is practically observed that these changes still occur and cause a decrease in product quality (Secci & Parisi, 2016). Although the exact mechanisms can be complex, tissue damage caused by temperature fluctuations and the reduction in moisture content during storage enhance this oxidative reaction (Secci & Parisi, 2016).

Nanoemulsion-based edible coating technology, which operates at droplet sizes ranging from 20 to 200 nanometers, is recognized as a valuable alternative for enhancing seafood quality and physical stability (Ul Islam et al., 2024). This approach aligns with the criteria for healthy, minimally processed foods and enhances the bioavailability and absorption of active compounds (Ubbink & Levine, 2024). The primary components used in this technology include herbal extracts and essential oils, which possess antibacterial and antioxidant properties. Delivering these compounds at nanoscale sizes significantly boosts their effectiveness by increasing their surface-area-to-volume ratio (Patra et al., 2018).

For instance, nanoemulsions based on *Rosa damascena* essential oil have been produced via ultrasonic homogenization and demonstrated notable antibacterial activity relevant to food preservation (Sahraei et al., 2025).

The ultrasonic homogenization method is commonly employed to produce stable nanoemulsions (Abbas et al., 2013). This technique relies on mechanical vibrations that create micro-bubbles through cavitation, which improves droplet size reduction and stability (Abbas et al., 2013). Additionally, high-pressure homogenisers enhance emulsion stabilization efficiency (Heffernan et al., 2009).

Despite the well-documented antioxidant and antimicrobial properties of saffron (Abu-Izneid et al., 2022) and the proven efficiency of nanoemulsions in food preservation (Abbas et al., 2013; Patra et al., 2018), little information is available regarding the application of saffron-loaded nanoemulsions for the preservation of seafood. Most previous studies have either focused on the bulk extract of saffron or applied other essential oils. The synergistic effect of delivering saffron's active compounds at the nanoscale to control lipid oxidation and chemical spoilage in fish fillets has rarely been investigated. Therefore, to address this gap, the present study aimed to formulate and characterize a stable saffron nanoemulsion (SNE) using ultrasonic homogenization. Furthermore, this study evaluated the highly effective role of this novel nanoemulsion in extending the shelf life and maintaining the chemical quality (total volatile basic nitrogen [TVB-N], peroxide value [PV], thiobarbituric acid (TBA), pH, and antioxidant capacity using DPPH radical scavenging assay) of rainbow trout (*O. mykiss*) fillets during a 21-day storage period at 4 ˚C.

Materials and Methods

Materials

All analytical grade chemicals, including acetic acid, chloroform, potassium iodide, sodium thiosulfate, magnesium oxide, bromocresol green, methyl red, sulfuric acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol,

ethanol, Span® 80, Tween® 80, pectin powder, perchloric acid, trichloroacetic acid, thiobarbituric acid, 1,1,3,3-tetraethoxypropane (as malondialdehyde (MDA) standard), and n-hexane were purchased from Merck (Darmstadt, Germany). Boric acid was obtained from Sigma-Aldrich (St. Louis, MO, USA).

High-quality saffron stigmas (*C. sativus*) were procured from local markets in Khorasan (Iran). Refined coconut oil and extra virgin olive oil were supplied by Kourosh Agro-Industrial Company (Iran), while refined sunflower oil was obtained from Shomal Agro-Industrial Company (Iran). Fresh rainbow trout (*O. mykiss*) was purchased from Alborz Caspian fish farm (Karaj, Iran) and immediately transported to the laboratory in insulated boxes containing crushed ice.

Saffron extract preparation

To obtain saffron extract, 10 g of powdered saffron was placed in a porcelain mortar and ground using 100 mL of liquid nitrogen (10 mL/g). The liquid nitrogen was added slowly and gradually during grinding to facilitate the pulverization process while minimizing heat generation and preserving the bioactive compounds. Subsequently, 100 mL of distilled water (25 ˚C) was gradually added to prepare a 10% (w/v) saffron extract. The mixture was kept at room temperature for 2 h to allow sufficient extraction before being filtered through Whatman No. 42 filter paper.

Nanoemulsion formulation

As outlined in Table 1, eight distinct SNE systems were developed by varying the core lipid phase and emulsification technique.

Aqueous phase nanoemulsions: A 1% pectin solution was prepared by combining 1 g of pectin powder with 50 mL of distilled water. The solution was agitated using an IKA Alpha Silver magnetic stirrer (Germany) at 50 ˚C to promote complete pectin dissolution. Subsequently, 0.1 N citric acid was gradually added to adjust the solution to the target pH (pH 5.0). Next, 5 mL of glycerol was introduced under continuous stirring, followed by the addition of 4 mL of Tween 80.

Oil phase nanoemulsions

A preliminary nanoemulsion formulation was created using 3 mL of Span® 80 as the surfactant mixed with different vegetable oils, specifically 3% virgin olive oil, refined coconut oil, refined sunflower oil, and n-decane. The preparation of the SNE was performed through spontaneous emulsification. To start, the oil phase underwent magnetic stirring at 400 rpm for 10 minutes at 50 ˚C. After this step, 30 mL of saffron extract was gradually incorporated into the surfactant-oil blend while the stirring speed was increased to 600 rpm. This blended oil phase was then added to an aqueous phase, with distilled water introduced to reach a total volume of 100 mL. Stirring continued for another 30 minutes, creating an oil-in-water nanoemulsion. For ultrasonic homogenization, the resulting emulsion was sonicated for 30 minutes using a 7 mm diameter probe at 85% power (400 W), leading to a consistent and transparent mixture. Ultimately, both synthesis methods produced a stable 3% SNE, which was stored in a dark, dry environment to protect it from light exposure and preserve the formulation's integrity.

Characterization of nanoemulsions

Dynamic light scattering (DLS)

The nanoemulsion's droplet size, particle size distribution, and polydispersity index (PDI) were measured using a DLS instrument (Cordouan-Technologies, VASCO, France) following the protocol outlined in a previous study (Sugumar et al., 2014).

Fourier transform infrared spectroscopy (FTIR)

An infrared spectroscopy system from Perkin Elmer (USA) was used to identify chemical functional groups and observe changes during the formation of nanoemulsion particles. Measurements covered a wavenumber range of 400–4000 cm^{-1}, with samples placed between cells with thicknesses ranging from 0.1 to 0.01 mm (Ballesteros et al., 2017).

Transmission electron microscopy (TEM)

TEM was used to examine the structural characteristics, morphological properties, and droplet distribution of the selected nanoemulsion (Salvia-Trujillo et al., 2015). For TEM analysis, one drop of the diluted nanoemulsion (1:100, v/v, with distilled water) was placed onto a carbon-coated copper grid and allowed to stand for 2–3 min. Excess liquid was carefully removed using filter paper, and the grid was air-dried at room temperature before observation. The prepared sample was then examined using a JEOL JEM-2100 transmission electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 200 kV.

Nanoemulsion stability assay

To evaluate physical stability, exactly 10 mL of the nanoemulsion was placed into screw-capped tubes and tightly sealed to prevent evaporation. The samples were stored undisturbed at ambient temperature to monitor the phase separation (layering) process. The height of the upper layer was measured in millimeters on days 1, 14, and 28. Finally, the layering percentage was calculated from these values to determine the physical stability of the nanoemulsion over the storage period (Donsì et al., 2011).

Fish sample preparation and SNE application

A total of 20 live rainbow trout (*O. mykiss*), weighing approximately 700±50 g, were acquired from a local aquaculture facility in Karaj, Alborz, Iran. All animal handling procedures complied with the Arrive guidelines and received ethical approval from the Medical Sciences Ethics Committee of the Islamic Azad University, Science and Research Branch. The specimens were transported to the Sanjesh Pasargad Laboratory (Faculty of Natural Resources, University of Tehran) in insulated boxes packed with crushed ice. Following decapitation, evisceration, and filleting, the samples were extensively washed with chilled water and allowed to drain. The rainbow trout fillets were cut into portions weighing approximately 50 g with a thickness of approximately 2 cm. Each fillet was immersed in the coating solution at a fish-to-solution ratio of 1:2 (w/v) to ensure complete and uniform surface coverage.

The experimental setup comprised six distinct groups (two controls and four SNE-coated treatments), as detailed in Table 2. Eight SNE formulations (SNE1–SNE8) were initially prepared and comprehensively characterized by DLS, FTIR, TEM, and physical stability analysis. Based on the combined results of these characterization techniques, particularly droplet size, particle morphology, structural characteristics, and storage stability, four formulations (SNE1–SNE4) were selected for subsequent application to rainbow trout fillets.

The fillets were subjected to a 5 min immersion in their respective treatments, drained for 2 min to eliminate excess fluid, and packaged in low-density polyethylene (LDPE) bags. The packaged samples were incubated at 4 ˚C and 8 ˚C for up to 21 days. The 8 ˚C condition was deliberately chosen to mimic potential thermal abuse during commercial retail display. Periodic sampling for subsequent chemical and antioxidant analyses was conducted on days 1, 3, 7, 14, and 21. All experiments were performed using three biological replicates to guarantee statistical validity.

Chemical and antioxidant analysis of rainbow trout fillets coated with SNE

Peroxide value (PV)

The PV of the samples was determined according to the AOCS Official Method Cd 8b-90 (AOCS, 2017). Briefly, 1 g of oil sample extracted from rainbow trout was weighed in a 250 mL Erlenmeyer flask. Then, 25 mL of a chloroform-acetic acid solution (with a chloroform-to-acetic acid ratio of 3:2) was added to the flask. Next, 0.5 mL of saturated potassium iodide solution, 30 mL of distilled water, and 0.5 mL of a 1% starch solution as an indicator were added to the mixture. The amount of iodine liberated was titrated with a 0.01N standard sodium thiosulfate solution and the titration was maintained until the color vanished entirely. The PV (meq g O_2/kg fat) was calculated according to the Equation 1:

1. $$\text{Peroxidevalue (meq/kg)} = \frac{(S-B)\times F\times N\times 1000}{W}$$

Where, S is the sample titration volume (mL), B is the blank titration volume (mL), N is the normality of the sodium thiosulfate solution, and W is the weight of the oil sample (g).

Thiobarbituric acid reactive substances (TBARS)

The TBARS assay was performed to evaluate secondary lipid oxidation in the rainbow trout samples using a standard colorimetric method (Schmedes & Hølmer, 1989). Briefly, a 200 mg sample was transferred to a 25 mL volumetric flask and diluted with 1-butanol. Next, 5 mL of this mixture was placed into dry, capped tubes, and 5 mL of TBA reagent was added. The capped tubes were incubated in a water bath at 95 ˚C for 2 hours and then cooled to room temperature. Finally, the absorbance was measured at 532 nm, using distilled water as a control. The TBARS content (expressed as mg MDA/kg fish tissue) and MDA content were calculated.

pH determination

The pH of the samples was measured according to Iranian National Standard No. 1028 (ISIRI, 2007). A 10-g portion of each homogenized sample was added to 90 mL of distilled water and blended for 30 s. The pH of each mixture was then measured using a digital pH meter.

Antioxidant activity test (DPPH radical scavenging assay)

The antioxidant activity of the treated rainbow trout fillets was determined using extracts prepared from

the fish samples. DPPH free radical scavenging activity was evaluated according to the method described by Brand-Williams et al., (1995) with minor modifications. Briefly, fish extracts were prepared and diluted to concentrations ranging from 50 to 200 ppm. An aliquot of 100 µL of each fish extract was mixed with 4 mL of a methanolic DPPH solution (0.004%). The mixture was incubated in the dark at room temperature for 30 min. A control consisting of methanol and DPPH solution without fish extract was prepared under the same conditions. After incubation, the absorbance was measured at 515 nm using a UV–Vis spectrophotometer.

Total volatile basic nitrogen (TVB-N)

To quantify TVB-N, minced rainbow trout samples were mixed with magnesium oxide (MgO) and subjected to direct steam distillation (Goulas & Kontominas, 2005). The resulting volatile compounds were trapped in an Erlenmeyer flask containing a 2% aqueous boric acid solution, which was previously enriched with a mixed ethanolic indicator comprising 0.1% methyl red and 0.1% bromocresol green. Finally, the absorbed nitrogen was quantified through titration using a 0.1 N sulfuric acid (H_2SO_4) standard solution. The final values were calculated and reported as milligrams of nitrogen per 100 g of the fish sample (mg N/100 g).

Sensory evaluation

The sensory quality of the rainbow trout fillets was evaluated by a panel of 25 untrained consumer panelists (consisting of students and staff members of the department). Overall acceptability was assessed based on the panelists' overall perception of appearance, color, odor, texture, and general eating quality, using a 5-point hedonic scale (1: very undesirable, 2: undesirable, 3: desirable, 4: good, and 5: excellent). The evaluation was performed on each sampling day during the 21-day storage period at both 4 ˚C and 8 ˚C (Jouki et al., 2014). A score below 3 was defined as the sensory rejection point.

Statistical analysis

Quantitative variables were summarized using Mean±SD and frequency (%). The Kruskal-Wallis test was used to compare the means of the quantitative variables among the study groups. Further analysis was conducted on day 7, employing the Dunn-Bonferroni post hoc test to identify which nanoemulsions exhibited statistically significant differences compared to the SNE4 and treatment groups. Statistical analyses were performed using SPSS version 20.0 and GraphPad Prism version 6, with a significance level set at 0.05.

Results

DLS nanoparticle analysis

DLS analysis revealed the particle size distribution of the eight SNE formulations. The recorded particle dimensions for samples SNE1-SNE8 were 11.7 nm, 9.7 nm, 12.2 nm, 10.6 nm, 15.4 nm, 12.8 nm, 22.3 nm, and 15.4 nm, respectively. Overall, the particle sizes ranged from 9.7 to 22.3 nm, confirming the successful formation of nanoemulsions in the nanoscale range.

Fourier transform infrared spectroscopy (FTIR) nanoparticle analysis

FTIR spectroscopy was performed on the optimized nanoemulsion formulation (SNE4) to investigate the characteristic functional groups and possible interactions among the formulation components (Figure 4). The FTIR spectrum confirmed the presence of the characteristic absorption bands of saffron extract and the emulsion matrix, indicating the successful incorporation of saffron into the nanoemulsion system. No additional absorption bands or major shifts in the characteristic peaks were observed, suggesting that the nanoemulsification process did not induce significant chemical modifications and that the formulation was mainly stabilized through physical interactions.

TEM image analysis

TEM analysis was performed on the selected SNE to investigate its morphology and droplet size. Representative TEM micrographs at different magnifications are presented in Figure 2. Figure 2A (1 µm) provides an overall view of the nanoemulsion droplets, whereas Figure 2B and 2C (200 and 100, respectively) (nm) show higher-magnification images that reveal the spherical morphology and homogeneous distribution of the droplets in greater detail. The TEM observations confirmed that the nanoemulsion droplets were nearly spherical with a relatively uniform size distribution. Furthermore, the particle size observed by TEM was consistent with the results obtained by DLS, showing droplet diameters below 30 nm.

Nanoemulsion stability over time

The physical stability of the eight SNE formulations was monitored over a 28-day period, with measurements taken on days 1, 14, and 28, as depicted in Figure

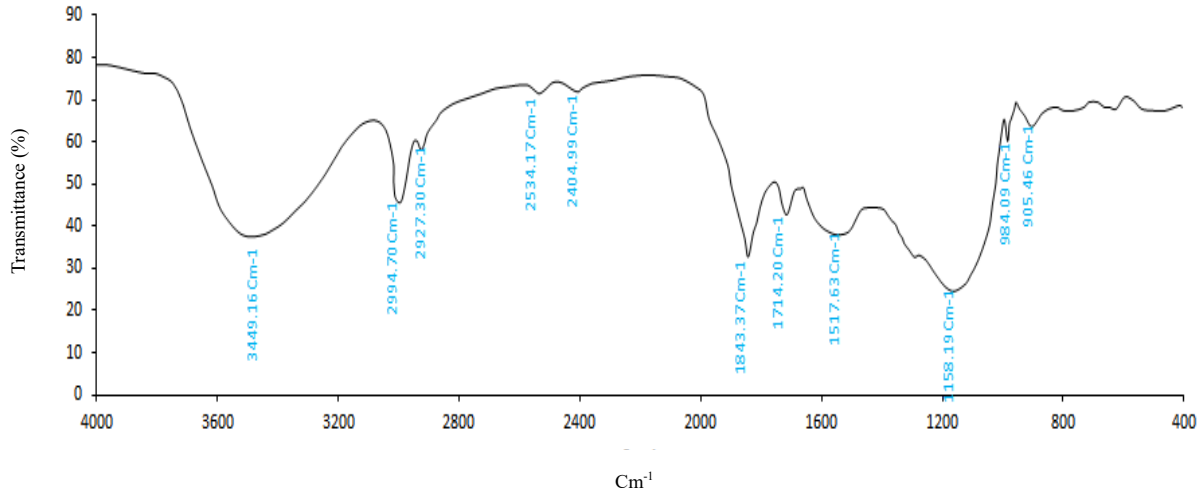


Figure 1. FTIR spectrum of the optimized saffron nanoemulsion (SNE4)

Note: The spectrum exhibited characteristic absorption bands at approximately 3449 cm^1 (O–H stretching), 2995 and 2927 cm^1 (C–H stretching of aliphatic chains), 1714 cm^1 (C=O stretching), 1518 cm^1 (aromatic ring vibrations), and 1158 cm^1 (C–O stretching), confirming the presence of the main functional groups of the saffron extract and emulsion components.

3. Statistical analysis confirmed that storage time had a significant impact on droplet size for all SNEs (Friedman test, $P<0.05$). Initially, on days 1 and 14, the formulations exhibited comparable stability with no significant differences among them ($P>0.05$). However, by the end of the storage period (day 28), significant divergence in performance was observed ($P=0.03$). Post-hoc analysis for day 28 revealed that SNE4 maintained the highest stability, differing significantly from SNE6 and SNE7, which were the least stable formulations ($P<0.05$). Among the tested formulations, SNE4 exhibited the highest physical stability throughout the storage period and remained significantly more stable than SNE6 and SNE7 on day 28 ($P<0.05$).

Based on the combined results of droplet size, TEM observations, and storage stability, SNE1–SNE4 demonstrated the most suitable physicochemical characteristics and were therefore selected for subsequent evaluation as edible coatings on rainbow trout fillets.

Comparison of antioxidant and physicochemical characteristics of coated fish fillets stored at 4 ˚C and 8 ˚C for 21 days

Table 3 presents the TVN (mg/100 g), DPPH (mg/g), PV (meq/kg O_2), TBA (mg/kg) values for rainbow trout fish in the control control a, control b, treatment a, treatment b, treatment c, and treatment d groups. These measurements were taken on day 21 of storage at 4 ˚C.

Significant differences in the mean values of TVN, PV, TBA, and pH were observed among the study groups (control a, control b, treatment a, treatment b, treatment c, and treatment d) on days 3, 7, 14, and 21 ($P<0.05$). This indicates that the observed variations in these parameters were statistically significant, highlighting the dynamics of the experimental conditions over time. The results indicated a significant difference in mean DPPH levels observed on days 1, 3, 7, 14, and 21 after treatment among the groups (control a, control b, treatment a, treatment b,

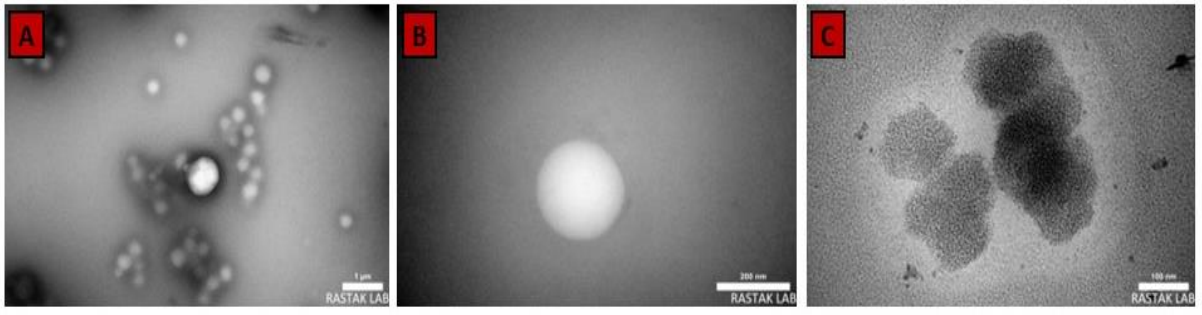


Figure 2. Representative TEM micrographs of the selected saffron nanoemulsion at different magnifications: (A) scale bar = 1 µm, (B) scale bar = 200 nm, and (C) scale bar = 100 nm, showing the morphology and droplet size of the nanoemulsion

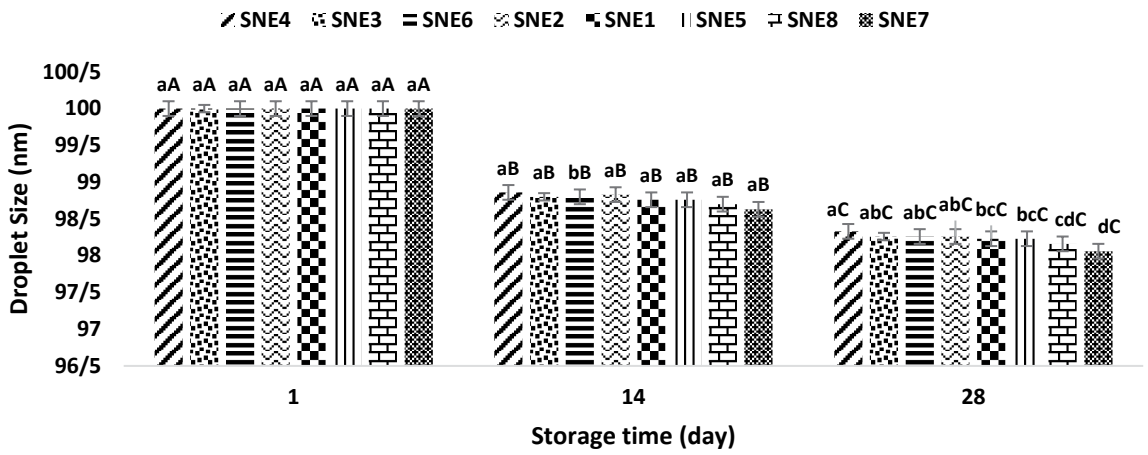


Figure 3. Variations in droplet size, an indicator of physical stability, for formulated saffron nanoemulsions (SNE1–SNE8) during storage

Note: Values were recorded at the beginning (day 1), middle (day 14), and end (day 28) of the study. Different uppercase letters indicate significant differences among storage times, whereas different lowercase letters indicate significant differences among formulations (P<0.05).

treatment c, and treatment d) (P<0.05). The mean values of all five chemical parameters (TVN, DPPH, PV, TBA, and pH) in the control a group were significantly higher than those in the treatment d group (P<0.05). Similarly, the mean values of TVN, DPPH, TBA, and pH in the Control b group were also significantly higher than in the Treatment d group (P<0.05). The Treatment a group had significantly higher mean TBA and pH values than the Treatment d group (P<0.05). Furthermore, the mean PV in the treatment b group was considerably higher than in the treatment d group (P<0.05).

Table 4 presents the TVN (mg/100 g), DPPH (mg/g), PV (meq/kg O_2), TBA (mg/kg), and pH values of rainbow trout fish for the control a, control b, treatment a, treatment b, treatment c, and treatment d groups. These measurements were taken on day 21 of storage at 8 ˚C.

The results indicated significant differences in the mean PV and TBA values among the groups studied (control a, control b, treatment a, treatment b, treatment c, and Treatment d) on days 3, 7, 14, and 21 (P<0.05). significant differences in the mean TVN, DPPH, and pH values among these groups were found only on days 3, 7, 14, and 21(P<0.05). On day 7, the mean values of TVN, DPPH, PV, TBA, and pH in the Control a and Control b groups were significantly higher than those in the Treatment d group (P<0.05). The mean TVN, PV, TBA, and pH values in the Treatment a group were significantly higher than those in the Treatment d group (P<0.05).

Comparison of various SNEs on TVN, DPPH, PV, TBA, and pH of rainbow trout fish during 21 days of storage (4 °C and 8 °C)

The mean TVN (Figure 4A), DPPH (Figure 4B), PV (Figure 4C), TBA (Figure 4D), and pH (Figue 4E) measurements on day 7 were assessed for the study groups (control a, control b, treatment a, treatment b, treatment c, and treatment d) at the two refrigeration temperatures under investigation (4 ˚C and 8 ˚C). Notably, the mean TVN, PV, TBA, and pH values at 8 ˚C were significantly higher than those at 4 ˚C in the control a, control b, treatment a, treatment b, treatment c, and treatment d groups. Also, the mean DPPH values at 8 ˚C were significantly higher than those at 4 ˚C in the control a, control b, treatment a, and treatment b groups. These differences were statistically significant (P<0.05), highlighting the impact of temperature on these key parameters.

Overall acceptance over the 21-day storage period at 4 ˚C

Overall acceptance scores decreased significantly across all groups over the 21-day storage period at 4C (P<0.05; Table 5). Despite this general decline, the treated samples consistently maintained higher acceptance levels than the control. Specifically, the control group exhibited significantly lower overall acceptance scores compared to treatments a, b, and d on day 7, treatment d on day 14, and treatments a and d at the end of the storage period (P<0.05).

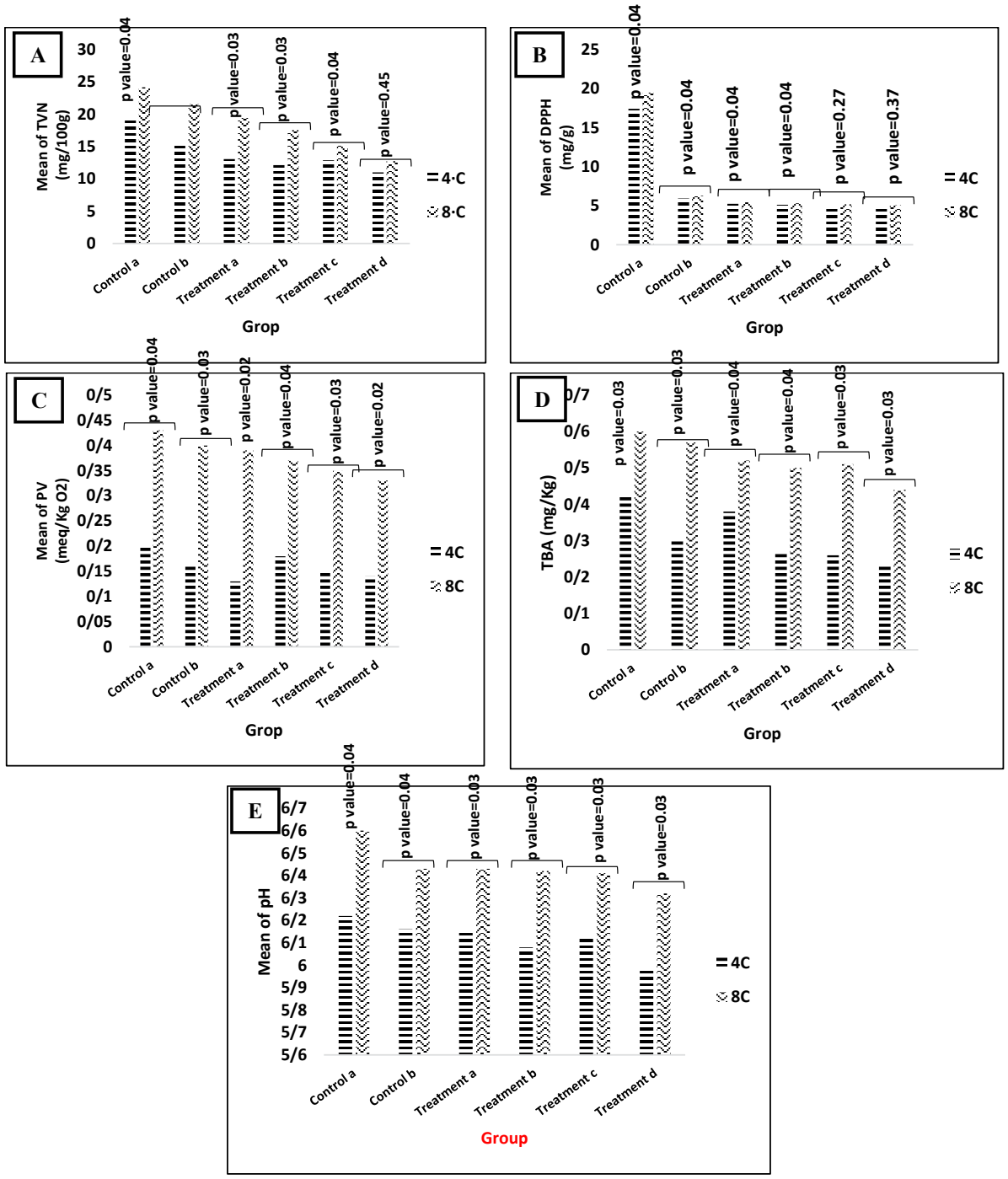


Figure 4. Comparison of various saffron nanoemulsions based on TVN (mg/100 g) (A), antioxidant activity level measured using DPPH (mg/g) (B), PV (meq/kg O) (C), TBA (mg/kg) (D), and pH (E) in rainbow trout during 21 days of storage at 4 ˚C and 8 ˚C

Overall acceptance over the 21-day storage period at 8 ˚C

Overall acceptance scores decreased significantly across all groups during the 21-day storage at 8 ˚C ($P<0.05$; Table 6). Significant differences were observed only on day 14, with treatments b and d showing higher acceptance than the control ($P<0.05$). By day 21, all samples reached their lowest scores (1.00–2.00), with no significant differences among treatments ($P>0.05$). Throughout storage, treatments b and d consistently maintained higher numerical scores than the control, indicating better preservation of sensory quality.

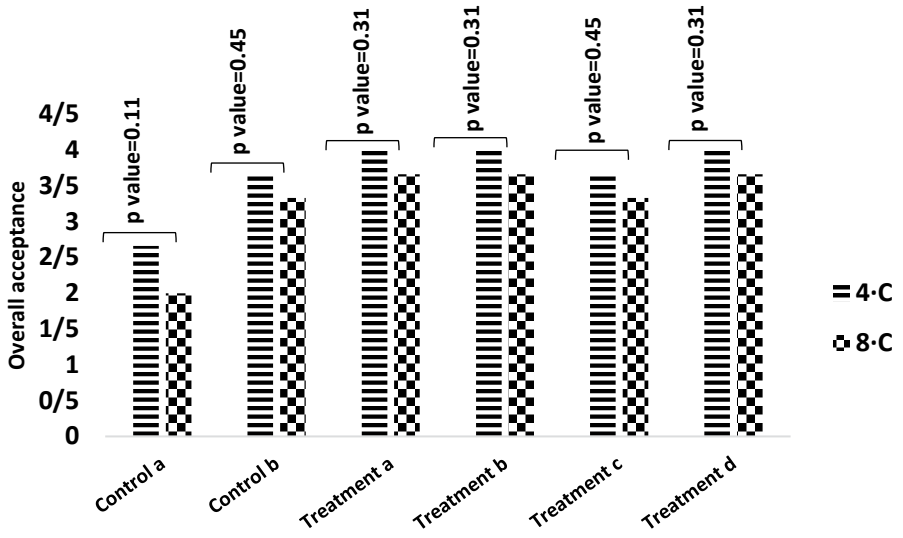


Figure 5. Comparison of sensory evaluation (overall acceptance) among the control a, control b, treatment a, treatment b, treatment c, and treatment d study groups on the 7th day of storage at 4 ˚C and 8 ˚C

Note: Values are reported as Mean±SD. Significance was set at the 0.05 level.

Comparison of overall acceptance results for rainbow trout coated with SNEs on day 7 of storage at 4 ˚C and 8 ˚C

Figure 5 shows overall acceptance at 4 ˚C and 8 ˚C on day 7. In the control a, control b, treatment a, treatment b, treatment c, and treatment d groups, the mean overall acceptance scores did not differ significantly between the two temperatures (P>0.05).

Discussion

This study assessed the antioxidant properties of a saffron (*C. sativus*) nanoemulsion formulated using spontaneous emulsification and ultrasonic homogenization. The nanoemulsion's stability and antioxidant effects were analyzed to assess its potential in preserving food quality. By coating rainbow trout with the optimized nanoemulsion and storing it at 4 ˚C and 8 ˚C, we evaluated its impact on shelf life. The results demonstrated that the SNE effectively slowed oxidation, enhancing the freshness and overall acceptability of the rainbow trout during the 21 days of storage.

Fresh meat is highly susceptible to oxidation, which affects lipids and proteins. This is a significant concern in food preservation (Gautam et al., 2023). Lipid peroxidation, in particular, leads to the formation of hydroperox-

Table 1. Composition and preparation methods of the formulated saffron nanoemulsions

Sample Code	Lipid Phase	Preparation Technique
SNE1	Refined coconut oil	Spontaneous emulsification
SNE2	Refined coconut oil	Ultrasonic homogenization
SNE3	Virgin olive oil	Spontaneous emulsification
SNE4	Virgin olive oil	Ultrasonic homogenization
SNE5	n-decane	Spontaneous emulsification
SNE6	n-decane	Ultrasonic homogenization
SNE7	Refined sunflower oil	Spontaneous emulsification
SNE8	Refined sunflower oil	Ultrasonic homogenization

Table 2. Classification of the rainbow trout fillet treatment groups

Group Designation	Applied Treatment	Corresponding Emulsion
Control a	Uncoated (negative control)	-
Control b	Pure saffron extract coating	-
Treatment a	Coating with saffron nanoemulsion 1	SNE1
Treatment b	Coating with saffron nanoemulsion 2	SNE2
Treatment c	Coating with saffron nanoemulsion 3	SNE3
Treatment d	Coating with saffron nanoemulsion 4	SNE4

ides (Gautam et al., 2023). As these compounds break down, they generate secondary oxidative products that contribute to the undesirable odors and flavors often associated with spoiled meat (Gautam et al., 2023).

One way to combat this oxidation and improve fresh meat's shelf life is through microemulsions and nanoemulsions, which can encapsulate active compounds, like saffron (Avila-Sosa et al., 2022). The controlled release characteristics of these emulsions allow for the gradual discharge of saffron's antioxidant compounds, ensuring prolonged bioactivity and functional benefits (Avila-Sosa et al., 2022). Beyond their use in functional food development, saffron-loaded microemulsions and nanoemulsions offer promising applications in food packaging systems (Al-Tayyar et al., 2020). The antimicrobial and antioxidant properties of saffron help inhibit microbial growth and slow oxidative processes, ultimately extending the shelf life of packaged foods, including meat products (Al-Tayyar et al., 2020). Encapsulation has been shown to improve antioxidant efficiency (Binazir et al., 2024). The antioxidant properties of saffron are linked to its carotenoids and phenolics, which may work synergistically with other antioxidants, and the NLC's antioxidant activity is attributed to phenolic compounds in essential oils (Binazir et al., 2024). Key saffron constituents, such as crocin, crocetin, safranal, and picrocrocin contribute significantly to its antioxidant potential by scavenging free radicals, inhibiting lipid peroxidation, and stabilizing reactive oxygen species (ROS) generated during fish storage (Kyriakoudi et al., 2015; Melnyk et al., 2010).

A previous study on sea bass fillets demonstrated that nanoencapsulation of saffron extract within zein nanofibers reduced TVB-N values by 30% (Najafi et al., 2022). The improved performance of SNEs compared with free extracts may be attributed to their small droplet size and larger surface area, which enhance the stability, dispersion, and bioavailability of bioactive compounds. Nanoemulsions also protect sensitive compounds, such as crocin from degradation during storage and allow their gradual release onto the fish surface (McClements, 2012; Tadros et al., 2004).

The evaluation of nanoemulsion stability on days 1, 14, and 28 revealed that formulation SNE4 exhibited the best performance. On day 1, all formulations demonstrated initial uniformity and structural integrity, and they remained highly stable up to day 14. However, significant differences emerged by day 28, where SNE4 significantly outperformed SNE6 and SNE7 in preventing droplet coalescence. The enhanced stability of SNE4 can be attributed to the combined effect of virgin olive oil as the lipid phase and ultrasonic homogenization as the preparation method. Ultrasonic homogenization has been reported to produce nanoemulsions with finer and more uniformly distributed droplets, resulting in improved physical stability by reducing droplet aggregation and coalescence during storage (Salvia-Trujillo et al., 2015). Moreover, the lipid composition of virgin olive oil, which is rich in monounsaturated fatty acids and natural antioxidant constituents, has been associated with improved oxidative and physical stability of food nanoemulsions (Mehmood et al., 2017). Therefore, the superior stability of SNE4 is likely the result of the synergistic effect of the oil phase and the emulsification technique rather than droplet size alone. This stability is vital for commercial food applications. Our results are comparable to the findings of Faridi Esfanjani et al. (2017), who utilized a whey protein-pectin complex to encapsulate saffron into 100 nm droplets, achieving high stability. However, the smaller droplets in our study may provide higher release rates for active compounds, like picrocrocin and crocin, which are essential for sustained antioxidant protection. In another study, the effectiveness of saffron essential oil nanoemulsions, produced through spontaneous emulsification and ultrasonic ho-

Table 3. Changes in TVN,antioxidantactivity measured using DPPH, PV, TBA,and pH values in rainbow trout treated with various saffron nanoemulsions during frozen storage,by storage time (days)

Variables	Group	Day 1	Day 3	Day 7	Day 14	Day 21
TVN (mg/100 g)	Control a	0.56 ± 0.00^{aE}	8.12 ± 0.56^{aD}	19.22 ± 0.42^{aC}	37.80 ± 1.12^{aB}	51.70 ± 0.42^{aA}
	Control b	0.28 ± 0.00^{abE}	3.82 ± 0.32^{dD}	15.12 ± 0.28^{bC}	29.49 ± 0.16^{bB}	46.10 ± 0.58^{bA}
	Treatment a	0.37 ± 0.16^{abE}	5.32 ± 0.56^{bD}	13.06 ± 0.42^{cC}	26.32 ± 0.74^{cB}	36.40 ± 0.28^{cA}
	Treatment b	0.37 ± 0.16^{abE}	4.57 ± 0.42^{bcD}	12.13 ± 0.42^{cdC}	23.70 ± 0.42^{dB}	33.60 ± 0.74^{dA}
	Treatment c	0.37 ± 0.16^{abE}	4.01 ± 0.42^{cdD}	12.88 ± 0.28^{cC}	25.48 ± 0.28^{cB}	33.04 ± 0.56^{dA}
	Treatment d	0.28 ± 0.00^{bE}	3.36 ± 0.28^{dD}	11.01 ± 0.58^{eC}	21.56 ± 0.28^{eB}	27.16 ± 0.28^{eA}
DPPH (mg/g)	Control a	13.99 ± 0.21^{aE}	16.13 ± 0.19^{aD}	17.43 ± 0.49^{aC}	22.37 ± 1.08^{aB}	26.46 ± 1.16^{aA}
	Control b	3.80 ± 0.02^{bE}	4.72 ± 0.12^{bD}	5.93 ± 0.10^{bC}	7.44 ± 0.09^{bB}	8.53 ± 0.12^{bA}
	Treatment a	3.96 ± 0.11^{bE}	4.55 ± 0.04^{bcD}	5.24 ± 0.04^{cC}	5.68 ± 0.08^{cB}	6.36 ± 0.10^{cA}
	Treatment b	3.88 ± 0.09^{bD}	4.47 ± 0.11^{bcC}	5.12 ± 0.06^{cdB}	5.51 ± 0.07^{cdAB}	5.94 ± 0.11^{cdA}
	Treatment c	3.93 ± 0.09^{bE}	4.51 ± 0.07^{bcD}	4.99 ± 0.14^{deC}	5.28 ± 0.02^{deB}	6.10 ± 0.09^{cdA}
	Treatment d	3.83 ± 0.04^{bD}	4.41 ± 0.09^{cC}	4.89 ± 0.13^{eB}	5.15 ± 0.03^{eAB}	5.25 ± 0.07^{dA}
PV (meq/kg O_2)	Control a	0.02 ± 0.001^{aE}	0.12 ± 0.002^{aD}	0.20 ± 0.003^{aC}	0.44 ± 0.001^{aB}	0.61 ± 0.005^{aA}
	Control b	0.02 ± 0.002^{aE}	0.06 ± 0.001^{dD}	0.16 ± 0.002^{cC}	0.37 ± 0.002^{bcB}	0.50 ± 0.001^{bcA}
	Treatment a	0.02 ± 0.001^{aE}	0.05 ± 0.002^{eD}	0.13 ± 0.001^{eC}	0.34 ± 0.001^{dB}	0.41 ± 0.002^{dA}
	Treatment b	0.02 ± 0.001^{aE}	0.09 ± 0.002^{bD}	0.18 ± 0.003^{bC}	0.41 ± 0.006^{bB}	0.53 ± 0.003^{bA}
	Treatment c	0.01 ± 0.003^{aE}	0.08 ± 0.02^{cD}	0.15 ± 0.001^{dC}	0.36 ± 0.002^{cB}	0.42 ± 0.001^{dA}
	Treatment d	0.01 ± 0.005^{aE}	0.07 ± 0.001^{cdD}	0.14 ± 0.002^{deC}	0.33 ± 0.001^{dB}	0.40 ± 0.002^{dA}
TBA (mg/kg)	Control a	0.08 ± 0.00^{aE}	0.25 ± 0.003^{aD}	0.42 ± 0.005^{aC}	0.74 ± 0.005^{aB}	0.92 ± 0.01^{aA}
	Control b	0.06 ± 0.007^{bE}	0.09 ± 0.003^{dD}	0.30 ± 0.003^{cC}	0.60 ± 0.004^{bB}	0.70 ± 0.003^{bA}
	Treatment a	0.06 ± 0.01^{bE}	0.21 ± 0.002^{bD}	0.38 ± 0.005^{bC}	0.69 ± 0.005^{aB}	0.82 ± 0.002^{abA}
	Treatment b	0.06 ± 0.005^{bE}	0.13 ± 0.005^{cD}	0.27 ± 0.003^{dC}	0.55 ± 0.01^{cB}	0.63 ± 0.001^{cA}
	Treatment c	0.06 ± 0.007^{bE}	0.13 ± 0.005^{cD}	0.26 ± 0.002^{dC}	0.58 ± 0.005^{bcB}	0.59 ± 0.005^{cdA}
	Treatment d	0.05 ± 0.005^{bE}	0.08 ± 0.003^{dD}	0.23 ± 0.003^{eC}	0.50 ± 0.006^{dB}	0.54 ± 0.008^{dA}
pH	Control a	5.88 ± 0.005^{aE}	6.08 ± 0.01^{aD}	6.22 ± 0.01^{aC}	6.64 ± 0.01^{aB}	6.89 ± 0.01^{aA}
	Control b	5.76 ± 0.01^{bE}	5.89 ± 0.005^{cD}	6.16 ± 0.02^{bcC}	6.53 ± 0.01^{bB}	6.68 ± 0.001^{bA}
	Treatment a	5.78 ± 0.01^{bE}	6.02 ± 0.01^{bD}	6.15 ± 0.005^{bcC}	6.45 ± 0.005^{cB}	6.59 ± 0.005^{cA}
	Treatment b	5.76 ± 0.02^{bE}	5.93 ± 0.005^{cD}	6.08 ± 0.004^{dC}	6.41 ± 0.01^{cB}	6.54 ± 0.004^{cA}
	Treatment c	5.77 ± 0.005^{bE}	5.95 ± 0.01^{cD}	6.13 ± 0.01^{cC}	6.40 ± 0.01^{cB}	6.52 ± 0.01^{cA}
	Treatment d	5.76 ± 0.01^{bE}	5.91 ± 0.01^{cD}	5.98 ± 0.005^{eC}	6.25 ± 0.02^{dB}	6.45 ± 0.02^{dA}

Note: Different lowercase letters in the same column indicate significant differences between treatments, and different uppercase letters in the same row indicate significant differences over storage time ($P<0.05$).

Table 4. Changes in TVN, antioxidant activity measured using DPPH, PV, TBA, and pH values in rainbow trout treated with various saffron nanoemulsions during 21 days of storage at 8 ˚C

Variables	Group	Time (Day)				
		1	3	7	14	21
TVN (mg/100 g)	Control a	0.84 ± 0.00^{aE}	11.94 ± 0.32^{aD}	24.17 ± 0.42^{aC}	51.05 ± 0.16^{aB}	70.28 ± 0.28^{aA}
	Control b	0.56 ± 0.00^{bE}	7.56 ± 0.28^{bD}	21.56 ± 0.28^{bC}	47.60 ± 0.28^{bB}	64.96 ± 0.28^{bA}
	Treatment a	0.65 ± 0.16^{abE}	8.49 ± 0.42^{abD}	19.41 ± 0.32^{cC}	40.88 ± 0.27^{cB}	57.77 ± 0.42^{cA}
	Treatment b	0.65 ± 0.16^{abE}	8.12 ± 0.28^{bD}	17.54 ± 0.32^{cdC}	38.17 ± 0.32^{dB}	55.62 ± 0.32^{dA}
	Treatment c	0.65 ± 0.16^{abE}	7.56 ± 0.28^{bD}	15.12 ± 0.56^{dC}	35.84 ± 0.28^{eB}	53.01 ± 0.32^{eA}
	Treatment d	0.56 ± 0.00^{bE}	6.44 ± 0.28^{cD}	12.78 ± 0.58^{eC}	33.04 ± 0.28^{fB}	49.65 ± 0.85^{fA}
DPPH (mg/g)	Control a	14.71 ± 0.37^{aE}	16.63 ± 0.29^{aD}	19.49 ± 0.58^{aC}	26.63 ± 0.79^{aB}	36.84 ± 1.67^{aA}
	Control b	4.07 ± 0.12^{bE}	4.86 ± 0.06^{bD}	6.26 ± 0.03^{bC}	7.83 ± 0.06^{bB}	9.90 ± 0.16^{bA}
	Treatment a	4.10 ± 0.13^{bE}	4.62 ± 0.07^{bCD}	5.44 ± 0.11^{cC}	5.96 ± 0.08^{cB}	6.96 ± 0.24^{cA}
	Treatment b	4.05 ± 0.09^{bE}	4.54 ± 0.13^{cD}	5.30 ± 0.10^{cdC}	5.73 ± 0.07^{cdB}	6.14 ± 0.08^{dA}
	Treatment c	4.08 ± 0.10^{bE}	4.58 ± 0.10^{bCD}	5.18 ± 0.22^{deC}	5.49 ± 0.06^{deB}	6.49 ± 0.07^{cdA}
	Treatment d	4.02 ± 0.09^{bE}	4.47 ± 0.11^{cD}	5.06 ± 0.21^{eC}	5.35 ± 0.05^{eB}	5.69 ± 0.10^{eA}
PV (meq/kg O_2)	Control a	0.04 ± 0.001^{aE}	0.18 ± 0.001^{aD}	0.43 ± 0.005^{aC}	0.67 ± 0.01^{aB}	0.89 ± 0.01^{aA}
	Control b	0.02 ± 0.002^{bE}	0.14 ± 0.01^{bD}	0.40 ± 0.003^{bC}	0.62 ± 0.003^{bB}	0.81 ± 0.003^{bA}
	Treatment a	0.03 ± 0.001^{abE}	0.16 ± 0.001^{abD}	0.39 ± 0.003^{bcC}	0.59 ± 0.003^{cB}	0.77 ± 0.003^{cA}
	Treatment b	0.03 ± 0.002^{abE}	0.15 ± 0.001^{bcD}	0.37 ± 0.005^{cdC}	0.58 ± 0.002^{cB}	0.74 ± 0.001^{dA}
	Treatment c	0.02 ± 0.003^{bE}	0.15 ± 0.002^{bcD}	0.35 ± 0.003^{deC}	0.55 ± 0.003^{dB}	0.70 ± 0.005^{eA}
	Treatment d	0.02 ± 0.005^{bE}	0.13 ± 0.001^{cD}	0.33 ± 0.005^{eC}	0.52 ± 0.001^{eB}	0.67 ± 0.005^{fA}
TBA (mg/kg)	Control a	0.13 ± 0.005^{aE}	0.32 ± 0.007^{aD}	0.60 ± 0.00^{aC}	0.93 ± 0.01^{aB}	1.22 ± 0.01^{aA}
	Control b	0.12 ± 0.003^{abE}	0.20 ± 0.001^{bD}	0.57 ± 0.00^{bC}	0.87 ± 0.003^{bB}	1.13 ± 0.005^{bA}
	Treatment a	0.12 ± 0.005^{abE}	0.25 ± 0.00^{cD}	0.52 ± 0.007^{cC}	0.75 ± 0.005^{cB}	1.00 ± 0.003^{cA}
	Treatment b	0.11 ± 0.003^{bcE}	0.20 ± 0.003^{bD}	0.50 ± 0.005^{cdC}	0.68 ± 0.007^{dB}	0.98 ± 0.001^{dA}
	Treatment c	0.10 ± 0.005^{cdE}	0.21 ± 0.003^{bcD}	0.51 ± 0.007^{cC}	0.69 ± 0.005^{dB}	0.95 ± 0.003^{eA}
	Treatment d	0.09 ± 0.005^{dE}	0.18 ± 0.003^{dD}	0.44 ± 0.003^{eC}	0.65 ± 0.003^{eB}	0.93 ± 0.005^{fA}
pH	Control a	5.91 ± 0.005^{aE}	6.22 ± 0.02^{aD}	6.60 ± 0.01^{aC}	7.06 ± 0.01^{aB}	7.66 ± 0.01^{aA}
	Control b	5.89 ± 0.01^{aE}	6.07 ± 0.02^{bD}	6.43 ± 0.01^{bC}	6.97 ± 0.01^{bB}	7.45 ± 0.01^{bA}
	Treatment a	5.89 ± 0.01^{aE}	6.19 ± 0.01^{aD}	6.43 ± 0.005^{bC}	6.92 ± 0.005^{cB}	7.35 ± 0.01^{cA}
	Treatment b	5.88 ± 0.00^{aE}	6.14 ± 0.005^{cD}	6.42 ± 0.01^{bcC}	6.86 ± 0.003^{dB}	7.22 ± 0.005^{dA}
	Treatment c	5.89 ± 0.005^{aE}	6.15 ± 0.005^{bcD}	6.41 ± 0.005^{cC}	6.78 ± 0.01^{eB}	7.28 ± 0.01^{eA}
	Treatment d	5.89 ± 0.01^{aE}	6.09 ± 0.005^{dD}	6.32 ± 0.005^{dC}	6.64 ± 0.01^{fB}	7.16 ± 0.02^{fA}

Note: Different lowercase letters in the same column indicate significant differences between treatments, and different uppercase letters in the same row indicate significant differences over storage time ($P<0.05$).

Table 5. Changes in overall acceptance scores of rainbow trout fillets during 21 days of storage at 4 ˚C

Group	Time (Day)				
	1	3	7	14	21
Control a	4.00±0.00^{aA}	3.33±0.57^{aB}	2.66±0.57^{bC}	1.33±0.57^{bD}	1.00±0.00^{cE}
Control b	5.00±0.00^{aA}	4.66±0.57^{aB}	3.66±0.57^{abC}	2.66±0.57^{bD}	1.66±0.57^{bcE}
Treatment a	4.33±0.57^{aA}	4.33±0.57^{aA}	4.00±0.00^{aB}	3.00±0.00^{abC}	2.66±0.57^{aD}
Treatment b	4.66±0.57^{aA}	4.33±0.57^{aA}	4.00±0.00^{aB}	3.00±0.00^{abC}	2.00±0.00^{abD}
Treatment c	4.66±0.57^{aA}	4.33±0.57^{aA}	3.66±0.57^{abB}	3.00±0.00^{abC}	2.00±0.00^{abD}
Treatment d	4.66±0.57^{aA}	4.66±0.57^{aA}	4.00±0.00^{aB}	3.33±0.57^{aC}	2.66±0.57^{aD}

Note: Different lowercase letters in the same column indicate significant differences between treatments, and different uppercase letters in the same row indicate significant differences over storage time (P<0.05).

mogenization, in extending the shelf life of shrimp (*Litopenaeus vannamei*) was investigated (Aboutorab et al., 2021). Shrimp samples were treated with either 3.0% or 5.0% SNEs and then stored at 4 ˚C or 8 ˚C. The lowest pH levels were recorded in the US/5%/4 ˚C and SP/5%/4 ˚C treatments. By days 7 and 14, all treatments had a pH of approximately 8±0.008. PV increased across all subgroups; however, samples treated with the 5% emulsions showed reduced peroxide levels. TBA values displayed a similar trend. Overall, the 5% SNE prepared using ultrasonic homogenization provided the most effective protection for the shrimp (Aboutorab et al., 2021).

Rainbow trout treated with saffron extract had lower bacterial counts than controls, with NSPE samples showing the lowest TBARS and TVB-N after nine days at 4 ˚C. Shelf life was 5–8 days for controls, 8–13 for free saffron-petal extract, and 9–13 for nano capsulate (Khalili et al., 2021). Encapsulation improved nano capsulate's protective effects, enhancing rainbow trout preservation (Khalili et al., 2021). According to Commission Regulation (EC) No. 2074/2005, the acceptable TVB-N limit for fresh fish generally ranges between 25 and 35 mg N/100 g, depending on fish species. In the present study, all SNE-treated samples stored at 4 ˚C remained within this acceptable range throughout the 21-day storage period, while Treatment d exhibited the lowest TVB-N value (27.16 mg/100 g). Conversely, at 8 ˚C, the control groups exceeded the acceptable limit by day 14, whereas the nanoemulsion-treated samples remained below the regulatory limit until day 14. Although TVB-N values increased in all treatments by day 21 at 8 ˚C, treatment d showed the lowest final value, demonstrating that SNE effectively delayed protein degradation and spoilage (European Commission, 2005). The degree of oxidation can be assessed by measuring PV or using the TBARS method. In one study, shrimp samples treated with 3% and 5%

Table 6. Changes in overall acceptance scores of rainbow trout fillets during 21 days of storage at 8 ˚C

Treatment	Time (Day)				
	1	3	7	14	21
Control a	4.00±0.00^{aA}	3.00±0.00^{aB}	2.00±0.00^{aC}	1.00±0.00^{dD}	1.00±0.00^{aD}
Control b	5.00±0.00^{aA}	4.33±0.57^{aB}	3.33±0.57^{aC}	2.00±0.00^{cdD}	1.33±0.57^{aE}
Treatment a	4.33±0.57^{aA}	4.33±0.57^{aA}	3.66±0.57^{aB}	2.66±0.57^{abC}	1.66±0.57^{aD}
Treatment b	4.66±0.57^{aA}	4.33±0.57^{aB}	3.66±0.57^{aBC}	3.00±0.00^{aCD}	2.00±0.00^{aD}
Treatment c	4.66±0.57^{aA}	4.33±0.57^{aB}	3.33±0.57^{aC}	2.33±0.57^{bcD}	1.66±0.57^{aE}
Treatment d	4.66±0.57^{aA}	4.33±0.57^{aB}	3.66±0.57^{aBC}	3.00±0.00^{aCD}	2.00±0.00^{aD}

Note: Different lowercase letters in the same column indicate significant differences between treatments, and different uppercase letters in the same row indicate significant differences over storage time (P<0.05).

saffron essential oil emulsions showed significantly lower PV compared to untreated samples, particularly by the 14th day of the study. Similarly, TBARS analysis indicated a notable difference in TBA values between the emulsion-coated samples and the uncoated ones (Aboutorab et al., 2021). In another study, a nanoemulsion made from oregano essential oil and resveratrol in pectin emulsion coating was tested on pork loin. The coated samples reached near the spoilage threshold by day 20, while the uncoated samples exceeded this value by day 5 (Xiong et al., 2020). PV is a widely accepted indicator of primary lipid oxidation, and values below approximately 10 meq O_2/kg lipid are generally considered indicative of acceptable oxidative quality in fish and fishery products (Shahidi & Zhong, 2010). In the present study, PV values for all treatments remained substantially below this limit throughout storage at both temperatures, indicating that primary lipid oxidation was effectively controlled. Furthermore, Treatment d consistently exhibited the lowest PV values, demonstrating that SNE effectively inhibited the initiation of lipid oxidation (Shahidi & Zhong, 2010).

A study on chicken meat coated with rosemary extract and ε-poly-L-lysine revealed significantly lower TBARS values (1.523–1.97 mg MDA/kg) compared to uncoated samples (2.27 mg MDA/kg) (Gautam et al., 2023). For chicken fillets coated with barley straw germ (BSG)-based coatings, PV was 5 meq active O_2/kg lipid after 12 days, while the uncoated samples had a value of 8.07 meq active O_2/kg lipid. This was attributed to the antioxidant properties of thyme and summer savory EO, with thyme's higher phenolic content (including carvacrol and thymol) enhancing its antioxidant effects (Gautam et al., 2023). TBA values are commonly used as indicators of secondary lipid oxidation, and fish products with TBA values below 1–2 mg MDA/kg are generally considered acceptable from a sensory perspective (Sallam, 2007). In the present study, all samples stored at 4 ˚C remained below this limit throughout storage. At 8 ˚C, the control groups exceeded 1 mg MDA/kg by day 21, whereas Treatment d remained below this threshold (0.93 mg MDA/kg), indicating that SNE effectively retarded secondary lipid oxidation and preserved product quality during refrigerated storage (Sallam, 2007).

A study investigated the impact of sage seed gum edible coating, enhanced with a nanoemulsion of lemon verbena (*Aloysia citrodora*) essential oil, on the chemical, microbial, and sensory qualities of fresh turkey meat. The coating formulation with SSG and 3% nanoemulsion showed the highest antioxidant activity compared to other treatments (Sayadi et al., 2024). The control group experienced a notable increase in peroxide and TBA values during storage, while the sage seed gum-3% nanoemulsion group maintained the lowest peroxid and TBA levels. Additionally, the inclusion of essential oil and nanoemulsion led to a dose-dependent reduction in bacterial growth, boosting the antimicrobial effectiveness of the sage seed gum coating against Bacillus cereus, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The sage seed gum-essential oil/nanoemulsion coatings also improved the sensory attributes of the turkey meat, with the sage seed gum-3% nanoemulsion-coated group receiving the highest sensory ratings after 16 days. These results indicate that the sage seed gum-nanoemulsion active coating holds potential as a powerful antimicrobial and antioxidant solution for meat preservation (Sayadi et al., 2024).

Building on the previous studies, we also demonstrated a significant reduction in TVN, PV, TBA, and pH levels in the treatment groups compared to the control groups, particularly in treatment d, which showed the lowest values across all measured parameters ($P<0.05$). The reductions observed in PV and TBARS values in this study are consistent with previous reports on nanoemulsion-based coatings applied to seafood products, where lipid oxidation was reduced by approximately 30–50% during refrigerated storage (Ozogul et al., 2017; Donsì & Ferrari, 2016).

As measured by DPPH, the antioxidant activity was also significantly higher in the treatment groups, confirming the protective effect of SNEs against oxidative damage. The significant difference in DPPH values among groups over time ($P<0.05$) indicates that nanoencapsulation effectively preserved the antioxidant activity of saffron during storage. The nanoemulsion matrix effectively shielded the electron-donating antioxidants of saffron from the aqueous environment of the fish, allowing them to remain active and neutralize DPPH radicals long after the control groups had undergone severe oxidative degradation. These findings are in agreement with those of Ahmadi et al. (2024), who reported that incorporating natural bioactive compounds into food systems significantly enhanced antioxidant capacity and improved oxidative stability during refrigerated storage. Although their study was conducted on dairy products enriched with phycocyanin and phycoerythrin, both studies demonstrate that bioactive-rich formulations effectively preserve antioxidant activity and protect food matrices against oxidative deterioration during storage.

The gradual increase in pH during storage is mainly attributed to the accumulation of alkaline compounds, including ammonia, trimethylamine, and other volatile basic nitrogen compounds produced during microbial growth and proteolytic activity. Fresh fish generally exhibit pH values ranging from 6.0 to 6.8, whereas a progressive increase in pH is considered an indicator of quality deterioration during refrigerated storage (Ozogul & Ozogul, 2012). In the present study, all samples stored at 4 ˚C maintained pH values below 7.0 throughout the 21-day storage period, indicating acceptable freshness. In contrast, all groups stored at 8 ˚C exceeded pH 7.0 by day 21, confirming accelerated spoilage under higher storage temperature. Nevertheless, treatment d consistently exhibited the lowest pH values at both storage temperatures, demonstrating that SNE effectively delayed the accumulation of alkaline spoilage compounds and better preserved fish quality compared with the untreated controls (Ozogul & Ozogul, 2012).

Storage temperature plays a crucial role in the preservation of rainbow trout. The results indicated that samples stored at 8 ˚C exhibited significantly higher TVN, PV, TBA, and pH levels than those stored at 4 ˚C, The acceleration of oxidative reactions at higher temperatures can be attributed to increased lipid oxidation kinetics and enhanced microbial metabolism, which promote the formation of volatile nitrogenous compounds and secondary oxidation products (Huss, 1995; Gram & Dalgaard, 2002). Additionally, DPPH values were higher at 8 ˚C in control a, control b, treatment a, and treatment b, suggesting that antioxidant activity decreases rapidly at higher temperatures. This further underscores the necessity of maintaining lower storage temperatures to maximize the protective effects of SNEs. The increase in pH during storage is mainly attributed to the production of alkaline compounds, including ammonia and volatile amines, resulting from microbial activity and protein degradation (Huss, 1995). Fresh fish generally exhibit pH values between 6.0 and 6.8, whereas values exceeding approximately 7.0 are considered indicative of advanced spoilage (Huss, 1995). In the present study, all samples stored at 4 ˚C maintained pH values below 7.0 throughout the 21-day storage period, indicating acceptable freshness. Conversely, all groups stored at 8 ˚C exceeded pH 7.0 by day 21, suggesting accelerated quality deterioration at the higher storage temperature. Among the treated groups, treatment d consistently exhibited the lowest pH values at both temperatures, indicating that SNE effectively delayed the formation of alkaline spoilage compounds and preserved fish quality for a longer period. On day 7, the differences in biochemical parameters between the treatment and control groups were particularly evident. The significantly lower TVN, PV, TBA, and pH levels in treatment d compared to control a and control b ($P<0.05$) demonstrate that SNEs provide a strong protective effect, delaying spoilage and oxidation. The variations observed across different SNE formulations indicate that the composition and concentration of active compounds influence their efficacy in preserving fish quality.

These findings align with recent studies on lipid-based nanocarriers for seafood preservation. For instance, Pourmoghadam et al. (2023) demonstrated that essential oil nanoemulsions effectively extend the shelf life of aquatic products by ensuring superior penetration and uniform distribution of active compounds. Compared to synthetic preservatives or metallic nanomaterials, plant-based nanoemulsions act as safe, clean-label alternatives that efficiently delay biochemical spoilage.

Our work supports these findings to further suggest that saffron-coated samples scored significantly higher in terms of overall acceptance at 4 ˚C, probably due to increased moisture retention, reduced proteolytic degradation, and the establishment of a semi-permeable barrier limiting oxygen diffusion, as supported by Pourmoghadam et al. (2023). Treatment d, by far the best, was characterized by its high overall acceptance, which resulted from the protective action of the nanoemulsion against enzymatic degradation and drip loss (Pourmoghadam et al., 2023). In the same way, Ozogul et al. (2017) noted that the shelf life of trout fillets stored on ice rose from 14 days in the control to 17 days when coated with nanoemulsions from rosemary, thyme, sage, and laurel essential oils (Najafi et al., 2021). Based on sensory evaluations, fish treated with such coatings retained better freshness and overall acceptance. At 8 ˚C; however, these benefits were diminished. Rapid microbial proliferation and increased enzymatic activity likely compromised the protective effects of nanoemulsion, as previously reported (Chuesiang et al., 2021; Ghosh et al., 2024). These results underscored the critical role of maintaining storage temperatures at or below 4 ˚C to maximize the overall acceptance benefits of nanoemulsion coatings. Besides, SNEs—particularly SNE4—offer notable advantages over other preservation methods due to their small droplet size, efficient bioactive delivery, and the ease of application. While some systems, such as multilayer coatings combining Farsi gum and Persian lime oil nanoemulsions, can extend shelf life up to 20 days, they require complex packaging technologies (Heydari-Farsani et al., 2025). On the other hand, SNE4 offers similar results through a simple and scalable approach. Chitosan and alginate coatings also possess an-

timicrobial properties, but most of them require higher active concentrations and might negatively affect overall acceptance (Sáez et al., 2021; Metekia & Ulusoy, 2023). Nevertheless, hurdles include high production cost and the need for specialized equipment, which challenge commercial exploitation. Future work should explore the combination of SNEs with biodegradable films to enhance effectiveness and industrial applicability.

Overall, this study confirms that SNEs, particularly treatment d, effectively extend the shelf life and maintain the quality of rainbow trout. The results suggest that SNE coatings could be considered a natural preservation strategy for the seafood industry, particularly as an alternative to synthetic antioxidants in chilled fish products (Shahidi & Zhong, 2010). However, further studies are needed to evaluate the sensory threshold of SNEs and their economic feasibility for large-scale industrial applications.

Conclusion

This study presents strong evidence that SNEs, especially treatment d, significantly contribute to preserving the quality and extending the shelf life of rainbow trout. By effectively reducing protein degradation and lipid oxidation while maintaining antioxidant capacity and sensory quality, SNEs help mitigate spoilage. This is evident from the lower levels of TVN, PV, TBA, and pH in the treatment groups compared to the controls. Furthermore, the higher antioxidant activity, measured through DPPH values, reinforces their protective effects against oxidative damage. Storage temperature also played a crucial role, with lower temperatures (4 ˚C) enhancing the effectiveness of nanoemulsions in maintaining fish freshness. These findings highlight the potential of SNEs as a natural and effective preservation strategy in the seafood industry. Future research should optimize nanoemulsion formulations, evaluate their long-term effects, and explore their use in other perishable food items. By combining these innovative preservation methods with proper refrigeration, the seafood industry can significantly improve product quality and shelf life, reduce waste, and ensure safer consumption.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were conducted in accordance with the ARRIVE guidelines. This study was approved by the Research Ethics Committee of the Islamic Azad University-Science and Research Branch, Tehran, Iran (Code: IR.IAU.SRB.REC.1401.267). Rainbow trout (*O. mykiss*) were handled and euthanized in accordance with institutional ethical requirements to minimize animal suffering.

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Authors' contributions

Conceptualization: Homa Alvandi and Seyed Amir Ali Anvar; Investigation, data curation, and writing of the original draft: Homa Alvandi; Formal analysis and validation: Homa Alvandi and Amir Eghbal Khajeh Rahimi; Supervision and project administration: Seyed Amir Ali Anvar; Resources: Seyed Amir Ali Anvar, Sara Allahyaribeik, Hamed Ahari, and Amir Eghbal Khajeh Rahimi; Methodology, review and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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