Research Article

www.dergipark.org.tr/en/pub/mediterranean

MEDITERRANEAN AGRICULTURAL SCIENCES (2026) 39: 3
DOI: 10.29136/mediterranean.1798160

Nanoemulsion with comprehensive protective effects for fish quality protection

Ayşe KARA

Recep Tayyip Erdoğan University, Faculty of Fisheries, Department of Fisheries Processing Technology, Rize, Türkiye

Corresponding author: A. Kara, e-mail: ayse.kara1@erdogan.edu.tr

ARTICLE INFO

Received: October 6, 2025
Received in revised form: February 4, 2026
Accepted: March 9, 2026

***Keywords*:**

Nanoemulsion-based edible coating
Seafood shelf-life
Moringa oleifera oil
Sea buckthorn oil

ABSTRACT

This study evaluated the effects of fish gelatin (FG) and chitosan (Ct) based nanoemulsion enriched with moringa (*Moringa oleifera*) oil (MO) and sea buckthorn (*Hippophae rhamnoides* L.) oil (HrO) on the quality preservation of rainbow trout (*Oncorhynchus mykiss*) fillets during cold storage (4 ± 1°C). The prepared nanoemulsion exhibited high physicochemical stability, as evidenced by its low pH (3.61 ± 0.02), high conductivity (218 ± 1.00 μS·cm^{-1}), moderate viscosity (216 ± 3.61 mPa·s), small droplet size (225.24 ± 1.10 nm), negative zeta potential (–8.25 ± 0.18 mV) and low polydispersity index (0.18 ± 0.02). Throughout the storage period, increases in pH and the accumulation of total volatile basic nitrogen (TVB-N) were significantly slower in the nanoemulsion-coated group compared with the control group. Moreover, the water-holding capacity was maintained at 88.64% at the end of day 12, which was significantly higher than that of the control group (86.88%) (P<0.05). Overall, these findings demonstrate that this approach represents a promising and innovative preservation strategy for extending shelf life and maintaining the quality of fish products in the seafood industry.

1. Introduction

In recent years, increasing consumer awareness of food quality and health benefits has encouraged researchers to develop new techniques and products that can enhance food quality and shelf life while maintaining nutritional value and safety. The use of essential oils as natural preservatives in the food industry has attracted considerable interest (Seow et al. 2014; Sharma et al. 2022). However, the hydrophobic nature and high reactivity of these compounds limit their direct incorporation into food systems. To overcome these challenges, nanoencapsulation has been recognized as an effective approach for preserving the biological activity of essential oils, enhancing their compatibility with food matrices, and reducing undesirable sensory effects. In recent years, nanotechnology has become an important tool for the delivery of natural bioactive compounds in food applications; within this context, nanoemulsions have emerged as promising nanocarrier systems due to their non-toxic nature and their ability to maintain structural stability under high temperature and pressure conditions (Bolouri et al. 2022; Reis et al. 2022). They are dispersed systems with droplet sizes typically around 100–500 nm (Azmi et al. 2022). Composed of oil, water, and surfactant, nanoemulsions appear as a single phase and exhibit high thermodynamic stability, along with antioxidant, antimicrobial, and hydrocolloid properties (İlyasoğlu and El 2010). The type of oil used plays a crucial role in determining formation, stability, and functionality, as it affects viscosity, density, interfacial tension, and phase behavior. Over time, nanoemulsions may undergo Ostwald ripening, characterized by an increase in droplet size due to aggregation or gravitational separation. This can be minimized using surface-active stabilizers such as emulsifiers, ripening inhibitors, and texture modifiers (Tontul 2020; Wooster et al. 2008). Emulsifiers are particularly important as they lower interfacial tension between oil and water, form smaller droplets, and enhance kinetic stability (Mason et al. 2006). Emulsifiers are classified as synthetic or natural. Synthetic emulsifiers include anionic, cationic, nonionic, and amphoteric types. Common examples in food systems are acetic acid esters of mono- and diglycerides, sodium dodecyl sulfate (SDS), and Tween 20, 40, 60, and 80 (Sarathchandraprakash et al. 2013; Aswathanarayan and Vittal 2019; Marhamati et al. 2021). Natural emulsifiers are derived from plants, animals, and microorganisms, including proteins, polysaccharides, phospholipids, and glycolipids (Öztürk and McClements 2016). Gelatin, a biodegradable and water-soluble polymer, is widely used in pharmaceuticals, food, and cosmetics due to its gelling, film-forming, and emulsifying properties (Vanin et al. 2014; Cozmuta et al. 2015; Steyaert et al. 2016). These properties depend on its source, type, extraction method, and molecular weight (Ding et al. 2020; Kan et al. 2023; Zhang et al. 2020). Gelatin is produced from the partial hydrolysis of collagen from animal tissues, mainly pig skin (46%), bovine hide (29.4%), bone (23.1%), and to a lesser extent, fish (1.5%) (Sultana et al. 2018).

To promote the widespread commercial application of nanoemulsions, further studies are required to identify suitable carrier systems, optimize production processes, and overcome formulation-related challenges. Although biocompatible and biodegradable biopolymers are widely used as edible coating materials in the food industry, their limited water barrier and mechanical properties restrict their effectiveness and necessitate further improvement. In this context, gelatin stands out as a promising carrier matrix due to its excellent film-forming ability, emulsifying capacity, and established safety in food applications.

© Akdeniz University Faculty of Agriculture

However, despite these advantages, studies focusing on gelatin-based nanoemulsion coatings, particularly those incorporating essential oils, remain limited in the literature (Wu et al. 2024). This study aims to address this gap in existing literature. In this study, sea buckthorn (*Hippophae rhamnoides* L.) and moringa (*Moringa oleifera*) oils were encapsulated using fish gelatin and chitosan as wall materials. To the best of our knowledge, no research has been conducted on the nanoencapsulation and characterization of these oils using fish gelatin and chitosan. Therefore, this study aimed to prepare a nanoemulsion via ultrasonication, characterize its physicochemical properties, and evaluate its bioactivity potential during cold storage of rainbow trout (*Oncorhynchus mykiss*) fillets.

2. Materials and Methods

2.1. Nanoemulsion formulation and characterization studies

Sea buckthorn (*Hippophae rhamnoides* L.) oil (HrO), moringa (*Moringa oleifera*) oil (MO), fish gelatin (FG) and chitosan (Ct) loaded nanoemulsion was prepared by modifying the method proposed by Kara et al. (2024a) (Table 1). Chitosan obtained from a commercial supplier (Sigma-Aldrich) was dissolved in acetic acid at a concentration of 1.5% (w-v). Fish gelatin (FishProJel), procured from a commercial source, was dissolved in distilled water at a concentration of 2% and left to hydrate overnight at room temperature, according to the method of Alak et al. (2023). Tween 80 was selected as the emulsifier due to its non-ionic nature, GRAS status, and high HLB value suitable for oil-in-water nanoemulsions. Subsequently, purified water was added to the beaker. The prepared solution was homogenized using an HG-15D digital homogenizer (2.000–27.000 rpm; Witeg Labortechnik, Germany) at 400 rpm for 3 min, while HrO and MO were simultaneously added dropwise using a syringe (Kara et al. 2024a). To reduce the particle size and obtain a nanoscale droplet, the emulsion was temperature treated at 25°C with an ultrasound probe (SONICS, vibra-cell, 500) at a power of 500 W and a frequency of 20 kHz for 15 min (2 s on 2 s off) at a distance of 1 cm from the bottom of the beaker. To minimize the heat released in this high-energy method and to preserve the structure of the nanoemulsion, the sample container was placed in a larger beaker containing ice. At the end of the process, an oil-in-water (O/W) nanoemulsion loaded with sea buckthorn (*Hippophae rhamnoides* L.) oil (HrO), moringa (Moringa oleifera) oil (MO), fish gelatin (FG), and chitosan (Ct) was obtained. Subsequently, the nanoemulsion was characterized in terms of pH, viscosity, conductivity, and particle size distribution, and its morphological features were examined by Transmission Electron Microscopy (TEM) after negative staining with uranyl acetate.

Table 1. Component content of nanoemulsion

Component	Percentage (%)
Fish gelatin (FG)	0.6
Chitosan (Ct)	1.4
Sea buckthorn oil (HrO)	3
Moringa oil (MO)	3
Tween 80	24
Distilled water	68

The pH value of the formulations was measured at room temperature (25 ± 2°C) with a pH meter (HACH TitraLab AT1000) according to the method of Curran (1980). The changes in viscosity were determined with a viscosity device (SOIF NDJ-8S (10^{-2}*10^{6} mPa.s, 30rpm-3s^{-1} min - rotor number 3) according to Talegaonkar et al. (2010) and measured with spindle number 3 at 30 rpm under a shear rate of 3 s^{-1} (10^{-2}*10^{6} mPa.s measurement interval). Electrical conductivity was measured at 25 ± 2°C by direct immersion of a HACH, HQ40d probe into the nanoemulsion. Particle size, zeta potential, polydispersity index (PdI) of the nanoemulsion were determined with a zetasizer, Malvern Zetasizer Nano ZSP (United Kingdom). The formulations were diluted 1:9 with ultrapure water. Zetasizer was measured in a DTS1070 cell at 25°C at 633 nm at a measurement angle of 173° (Zhang et al. 2017). This analysis was conducted at Atatürk University Eastern Anatolia High Technology Research and Application Center (DAYTAM). A transmission electron microscope (TEM, Hitachi HighTech HT7700, Japan) at Atatürk University Eastern Anatolia High Technology Research and Application Center (DAYTAM) was used for the morphology of the nanoemulsions. For TEM detection, the nanoemulsions were dispersed in ultrapure water and a drop of diluted nanoemulsion was placed on a 400 mesh carbon-coated copper grid. The grid was then dried overnight at room temperature. TEM imaging was performed at 120 kV.

2.2. *Nanoemulsion coating of fillets and storage analysis*

A total of 60 rainbow trout (Oncorhynchus mykiss) fillets, with an average weight of 285 ± 25 g, obtained from the Inland Fish Research and Production Center, Faculty of Fisheries, Atatürk University, were used in this study. After sterile filleting was performed on-site, the fillets were transported to the laboratory within a maximum of 15 min under cold-chain conditions prior to the onset of rigor mortis. Fillets immersed in distilled water were used as the control group (C), while the treatment group consisted of fillets coated with the nanoemulsion (HrO–MO NE). The prepared coating solutions were applied to the fillets by the dipping method, and the fillets were kept in the solution for at least 5 minutes under sterile conditions for an effective surface coating. They were then left to dry for 5 minutes in a sterile environment. After drying, the fillets were placed on plastic plates, wrapped in ordinary packaging with stretch film and subjected to cold storage for 12 days under refrigerator conditions (4 ± 1°C). During this storage period, the following chemical test were performed at certain intervals (on days 0, 3, 6, 9, and 12) to determine fillet quality and shelf life.

The following parameters, which give results in a short time and are widely used in monitoring the chemical changes occurring in fish muscle during degradation, were taken into consideration.

After the mixture was homogenized in Ultra Turrax for 1 min, the pH value was determined with a pH meter. Water holding capacity for 5 g of sample was weighed and centrifuged (4500 g-min, 10°C, 30 min), after which the pellet was weighed and the water holding capacity was calculated according to the following formula (Parnell-Clunies et al. 1986).

$$\text{Water Holding Capacity (\%)} = [1-(\text{pellet weight}/\text{first weight of the sample})] \times 100$$

Total volatile basic nitrogen (TVB-N) analysis for following the method described by Malle and Tao (1987), 40 g of tissue was homogenized with 80 ml of TCA solution in Ultra Turrax (IKA WerkTp 18-10 20.000 rpm) for 5 min and the mixture was filtered with filter paper (Filter Papers Ø125 mm, fast filtering). The resulting solution was distilled by adding 10% NaOH and

© Akdeniz University Faculty of Agriculture

TVB-N value was calculated as mg 100 g^{-1} (Alak et al. 2023). Statistical analysis: Data obtained in the study are presented as the mean ± standard deviation (SD) of parallel samples (n= 2–3). To determine differences between samples, groups with homogeneous variances were analyzed using One-Way ANOVA. Statistical significance was considered at $P<0.05$. All statistical analyses were performed using the JMP 5.0.1 software package (SAS Institute Inc., NC, USA) (Sümbüloğlu and Sümbüloğlu 2000).

3. Results and Discussion

3.1. Characterization of nanoemulsion data

The pH, conductivity, viscosity, droplet size, zeta potential, and polydispersity index (PDI) values of the nanoemulsion are presented in Table 2.

In this study, a nanoemulsion was prepared using sea buckthorn (*Hippophae rhamnoides* L.) oil (HrO) and moringa (*Moringa oleifera*) oil (Mo) as the oil phase and fish gelatin (FG) and chitosan (Ct) as emulsifiers. The pH value of the formulated nanoemulsion was measured as 3.61 ± 0.02 (Table 2). The conductivity value was 218 ± 1.00 µS cm^{-1}, confirming that the formulation exhibited the characteristics of an O-W-type nanoemulsion. Furthermore, an increase in the emulsifier and oil ratio was found to elevate the viscosity, which reached 216 ± 3.61 mPa·s (Table 2).

The average droplet size, zeta potential, and PDI values are also shown in Table 2. The mean droplet size was 225.24 ± 1.10 nm, indicating the formation of a microstructurally stable system with a high specific surface area. The zeta potential was recorded as −8.25 ± 0.18 mV, suggesting that the system exhibited partial electrostatic stability. In addition, the PDI value (0.18 ± 0.02) demonstrated a narrow droplet size distribution and homogeneous particle morphology. Figure 1 illustrates the TEM images of the nanoemulsion, showing spherical particle morphology with sizes distributed around 100 nm. The denser regions observed in the particle cores indicate the accumulation of the oil phase in the central area, stabilized by a surrounding layer of fish gelatin.

3.2. *Fillet storage data*

The statistical changes in the pH values of the fillet groups during storage are shown in Figure 2. Initially, the pH values were 6.56 ± 0.02 for the control (C) group and 6.50 ± 0.01 for the HrO–Mo NE group. By the end of storage, pH levels increased in both groups, reaching 7.11 ± 0.03 in the control and 6.78 ± 0.01 in the nanoemulsion-coated group ($P<0.05$). The water-holding capacity (WHC) of the control fillets decreased from 89.34 ± 0.34% at the beginning to 86.88 ± 0.90% on day 12. In contrast, the HrO–Mo NE group retained a significantly higher WHC value of 88.64 ± 0.26% at the end of storage ($P<0.05$). These findings indicate statistically significant differences between groups and overtime ($P<0.05$). The total volatile basic nitrogen (TVB-N) value is a widely used indicator to assess the degree of spoilage in seafood. Fresh fish typically have TVB-N levels ranging from 5–25 mg 100 g^{-1}, and the upper acceptability limit for consumption is 35 mg 100 g^{-1} (Ebadi et al. 2019; Valizadeh et al. 2020). On day 0, TVB-N values were 10.05 ± 0.99 mg 100 g^{-1} for the control group and 9.70 ± 0.14 mg 100 g^{-1} for the HrO–Mo NE group. By day 12, these values increased to 32.9 ± 0.8 mg 100 g^{-1} and 28.0 ± 0.7 mg 100 g^{-1}, respectively. A gradual increase in TVB-N levels was observed in all groups throughout the storage period ($P<0.05$), indicating ongoing protein degradation and volatile amine formation.

3.3. *Discussion*

In this study, the fundamental physicochemical properties of the nanoemulsion formulated with *Sea buckthorn* (*Hippophae rhamnoides* L.) oil (HrO) and *Moringa oleifera* oil (MO) as the oil phase, and fish gelatin (FG) and chitosan (Ct) as emulsifiers, were investigated. The findings indicated that the system exhibited an oil-in-water (O-W) type structure, which was microstructurally stable and optically clear.

Table 2. Some characteristic properties of the nanoemulsions used in the study.

pH	Conductivity (µS cm^{-1})	Viscosity (mPa.s)	Droplet size (nm)	Zeta (ζ) potential (mV)	Polydispersity index (PDI)
3.61±0.02	218±1.00	216±3.61	225.24±1.10	-8.25±0.18	0.18±0.02

Data are expressed as mean (n= 3) ± standard deviation (SD).

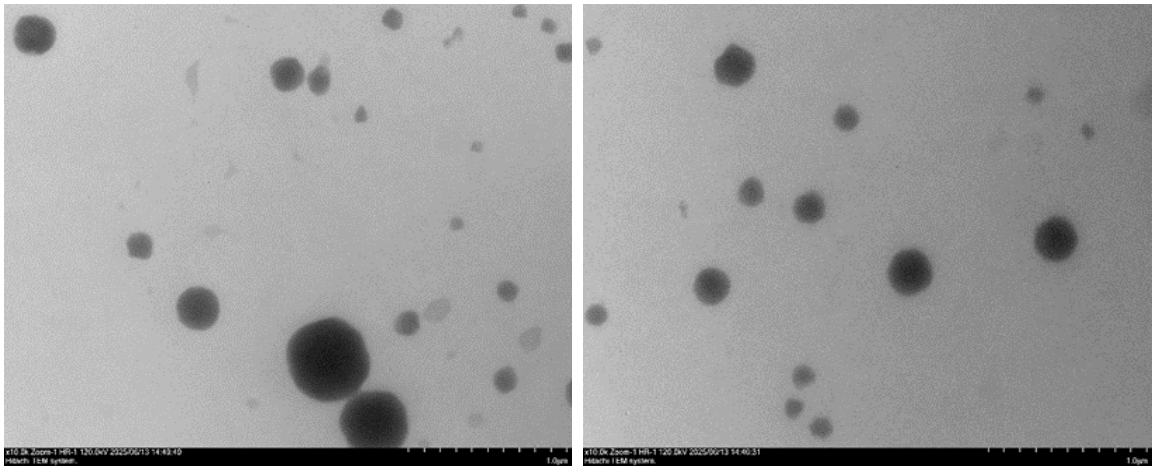

Figure 1. TEM images of the nanoemulsion.

© Akdeniz University Faculty of Agriculture

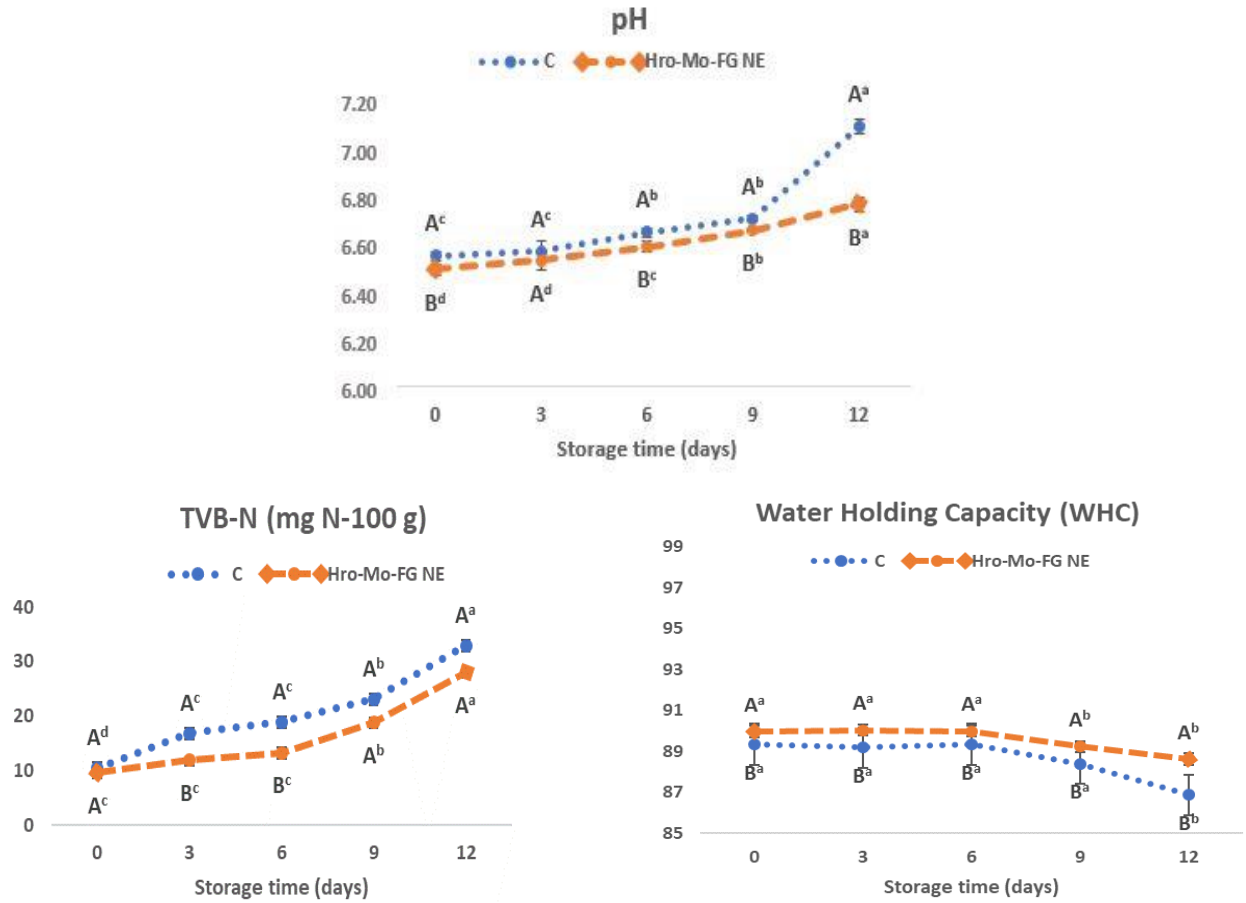

Figure 2. C: Control group immersed in distilled water, Hro-Mo NE: Group coated with nanoemulsion. Data are expressed as mean (n= 3) ± standard deviation (SD). Different lowercase letters (a, b, c) within the same row indicate statistically significant differences between groups on the same storage day ($P<0.05$). Different uppercase letters (A, B, C…) within the same column indicate statistically significant differences within the same group across different storage days ($P<0.05$).

The pH value of the nanoemulsion was 3.61 ± 0.02, confirming the acidic nature of the system and indicating conditions favorable for protonation, although pH measurement alone does not directly demonstrate molecular interactions. The conductivity value was determined as 218 ± 1.00 µS cm^{-1}, confirming the O-W type structure of the nanoemulsion (Patel and Joshi 2012). High conductivity reflect the presence of ionic species and protonated amino groups. When chitosan is dissolved in a mildly acidic medium, protonated amino groups ($–NH_3^+$) and acetate ions (CH_3COO^-) are generated, thereby increasing the total conductivity and electrostatic repulsion forces in the system (Mukurumbira et al. 2022; Maleki et al. 2022; Şimşek and Keyf 2018; Song et al. 2023; Yang et al. 2023). These results suggest that the measured pH and conductivity values are consistent with each other, and that the degree of chitosan protonation contributed to the microstructural stability of the system.

Viscosity is a useful parameter for assessing the physical stability of nanoemulsions in combination with other characterization techniques. It is not only an indicator of system stability but also plays an essential role in identifying emulsion type; low viscosity is generally characteristic of O-W emulsions, whereas high viscosity is typical of water-in-oil (W-O) emulsions (Richa and Choudhury 2021; Kara et al. 2024a; McClements 2013; Jiang et al. 2013). Emulsion viscosity is directly influenced by factors such as the chemical structure and concentration of the emulsifier, emulsifier–oil phase interactions, as well as the viscosity, amount, and distribution of the oil phase. The relatively low viscosity observed in this study confirms that the nanoemulsion possessed an O-W type and stable structure, consistent with previous reports (Richa and Choudhury 2021; Kildaci et al. 2021; Kara et al. 2024a).

The droplet size of nanoemulsions typically ranges from 20 to 500 nm (Gazolu-Rusanova et al. 2020), and an inverse relationship exists between droplet size and viscosity. Increasing the emulsifier concentration enhances the resistance to flow, preventing droplet aggregation and consequently reducing the average droplet size (Yu et al. 2025). Accordingly, the mean droplet size obtained in this study aligns with literature data on nanoemulsions prepared under similar formulation conditions and emulsifier ratios, confirming that the system achieved the targeted dimensional properties (Kara et al. 2024b; Mattar et al. 2024; Lyu et al. 2022; Zheng et al. 2020). Similarly, other studies utilizing *Hippophae rhamnoides* oil have reported comparable size ranges. For instance, Gore et al. (2024) reported a mean droplet size of 152.86 nm for nanoemulsions containing *H. rhamnoides*, while Zheng et al. (2020), observed an average particle size of 282 nm in sea buckthorn oil–water emulsions prepared by high-pressure homogenization, highlighting the influence of oil content and emulsifier type on both microstructure and antioxidant activity. These findings support

© Akdeniz University Faculty of Agriculture

that droplet size in nanoemulsions is strongly affected by formulation parameters and preparation conditions, and that a strong correlation exists between microstructural stability and functional performance.

Zeta potential represents the electric potential at the interface between dispersed droplets and the continuous phase and is a key indicator of surface charge density and polarity in nanoemulsion systems. It plays a pivotal role in determining emulsification efficiency and overall physicochemical stability. High zeta potential values generate strong electrostatic repulsion among droplets, inhibiting instability mechanisms such as coalescence and flocculation, whereas low zeta potential values increase the risk of phase separation (Sharif et al. 2017; Pazyar et al. 2014; Gurpreet and Singh 2018). The zeta potential of the developed nanoemulsion was measured as -6.53 ± 0.36 (Table 2), indicating relatively low electrostatic stability. This could be attributed to the limited adsorption of cationic, anionic, or nonionic surface-active components on the droplet surface. Previous studies have reported that increasing chitosan concentration enhances zeta potential and strengthens electrostatic stability (Silva et al. 2019). Therefore, the relatively low zeta potential observed in this study may be due to the limited amount of chitosan, reflecting a moderate level of electrostatic stabilization.

The polydispersity index (PDI) is a quantitative indicator of droplet size distribution, serving as a crucial parameter in evaluating the homogeneity and stability of nanoemulsions. PDI values range between 0 and 1, where values approaching 0 indicate a monodisperse (uniform and homogeneous) system, while values nearing 1 reflect a polydisperse (broad and heterogeneous) distribution (Baboota et al. 2007; Sugumar et al. 2014). The PDI value of the nanoemulsion developed in this study was 0.24 ± 0.01, suggesting a narrow droplet size distribution and high structural homogeneity. Similarly, Đorđević et al. (2015), reported PDI values below 0.15 for risperidone-loaded nanoemulsions and emphasized that both the type of active compound and formulation parameters significantly influence PDI. Thus, the present results are consistent with previous reports regarding microstructural integrity and physical stability (Uçar 2023; Özakar et al. 2022; Farzami et al. 2025; Hashem et al. 2024).

Transmission electron microscopy (TEM) is widely used to characterize the morphological features of nanoemulsions, particularly enabling direct visualization of lipid particles (Klang et al. 2012). In this study, the clearly defined boundaries observed in the TEM images indicate a uniform particle distribution, consistent with similar O-W nanoemulsion studies (Kumar and Mandal 2018). The morphological results are also in agreement with findings by Khan et al. (2022), who reported that lornoxicam-loaded chitosan nanoemulsions exhibited small, monodisperse, and spherical particles. Likewise, Abd-Elsalam and Khokhlov (2015) observed spherical morphology in eugenol-based nanoemulsions, while Shehabeldine et al. (2023) reported similar structures for clove oil (*Syzygium aromaticum*) nanoemulsions. Singh et al. (2008) also demonstrated smooth-surfaced spherical particles in primaquine-loaded nanoemulsions via TEM analysis.

During storage, the observed increase in pH values can be attributed to microbial metabolism of amino acids and low-molecular-weight compounds in fish muscle, resulting in the production of alkaline substances—a well-known indicator of spoilage and microbial activity (Dong et al. 2023). Although both groups exhibited an upward pH trend during storage, the nanoemulsion-coated group (Hro-Mo NE) showed a lower increase compared to the control group (C). Nanoemulsion application may limit microbial proliferation, slowing down the rise in pH and thereby contributing to chemical stability (da Silva Santos et al. 2017). This effect likely stems from the presence of phenolics and tocopherols with strong antioxidant and antimicrobial activities in moringa and sea buckthorn oils, which suppress protein degradation and microbial growth (Zhang et al. 2024; Rather et al. 2024; Song et al. 2023).

In the meat industry, meat with a high water-holding capacity (WHC) are preferred due to both economic considerations and their suitability for processing technologies (Alak et al. 2025). Nanoemulsions are also proposed to mitigate autolysis and microbial-induced deterioration in fish muscle, preventing protein degradation and myofibrillar disruption, thus contributing to the preservation of the water-holding capacity (WHC) (Dong et al. 2023; Gulzar et al. 2023; Kara et al. 2025). The high water-binding ability of gelatin combined with the oxidative stability conferred by moringa and sea buckthorn oils helped maintain the functional integrity of muscle proteins and reduced WHC loss. During cold storage, degradation and aggregation of myofibrillar proteins can alter WHC (Esquerdo et al. 2018; da Silva Santos et al. 2017). In a previous study, grass carp fillets coated with agarose-based films showed only a 3.40% loss in water content after 9 days of storage, demonstrating the effectiveness of this preservation strategy (Fu et al. 2023).

Wu et al. (2016) reported that citrus oil-based nanoemulsions effectively delayed increases in pH and TVB-N values in *P. argenteus* during refrigerated storage, extending shelf life. Similarly, Durmuş et al. (2020) found that olive oil-based nanoemulsion coatings on rainbow trout fillets slowed TVB-N accumulation over storage. Khedri et al. (2019) also observed that increasing concentrations of *Zataria multiflora* essential oil nanoemulsions reduced TVB-N levels in silver carp fillets. These findings are consistent with the present study, in which TVB-N accumulation was lower in the Hro-Mo NE group compared to the control, indicating that nanoemulsions suppressed microbial activity and delayed nitrogenous compound formation. Overall, the increases in pH and TVB-N values were less pronounced in the nanoemulsion-coated group than in the control, while WHC was better preserved in the Hro-Mo NE group (88.64%) compared to the control (86.88%). These effects are attributed to the combined action of the antioxidant and antimicrobial compounds in moringa and sea buckthorn oils and the high water-binding capacity of gelatin, which collectively helped limit protein degradation and maintain myofibrillar integrity.

4. Conclusion

This study evaluated the effects of a fish gelatin (FG) and chitosan (Ct)-based nanoemulsion, enriched with *Moringa oleifera* (MO) oil and Sea buckthorn (*Hippophae rhamnoides* L.) oil (HrO), on the quality preservation of rainbow trout (*Oncorhynchus mykiss*) fillets during cold storage. The prepared nanoemulsion exhibited an O-W type system with high stability and homogeneous particle distribution, characterized by low pH (3.61 ± 0.02), high conductivity (218 ± 1.00 μS cm^{-1}), moderate viscosity (216 ± 3.61 mPa·s), small droplet size (225.24 ± 1.10 nm), negative zeta potential (–8.25 ± 0.18 mV) and low polydispersity index (0.18 ± 0.02).

Although the small droplet size and low PDI values indicate a homogeneous nanoemulsion structure, time-dependent stability measurements were not performed in this study. Therefore, the evaluation of long-term physical stability is recommended for

© Akdeniz University Faculty of Agriculture

future studies. The application of the nanoemulsion coating significantly slowed the increase in pH and the accumulation of TVB-N, while maintaining the water-holding capacity (WHC) at higher levels compared to the control group. On day 12 of storage, WHC was retained at 88.64% in the nanoemulsion-coated fillets, whereas the control group measured 86.88%. These results indicate that the bioactive compounds in MO and HrO, together with fish gelatin, effectively limited protein degradation and myofibrillar structural breakdown, thereby reducing quality loss in the fillets.

Overall, the findings demonstrate that the nanoemulsion coating is effective in preserving both the chemical and physical quality of fish fillets. Future studies are recommended to optimize the ratios of bioactive oils and gelatin-chitosan, extend storage periods, and explore the potential application in other fish species. Moreover, detailed investigations on the effects of nanoemulsions on microstructural integrity and sensory properties would contribute to the development of effective strategies for enhancing food safety and shelf life.

References

Abd-Elsalam KA, Khokhlov AR (2015) Eugenol oil nanoemulsion: Antifungal activity against Fusarium oxysporum f. sp. vasinfectum and phytotoxicity on cottonseeds. Applied Nanoscience 5(2): 255-265.

Alak G, Ucar A, Parlak V, Turkez H, Kocaman EM, Atamanalp M, Abd El-Aty AM (2023) Effect of coating with chitosan enriched with different borates on the shelf life of fish fillet. Journal of the Science of Food and Agriculture 103(9): 4340-4350.

Alak G, Yavaş RN, Kara A, Gelen SU, Ucar A, Esenbuğa N, Chowdhury R (2025) A Comprehensive Multicriteria Assessment of Frozen Storage Systems on Quality of Benthic or Pelagic Fish. Journal of Food Quality 1: 5557956.

Aswathanarayan JB, Vittal RR (2019) Nanoemulsions and their potential applications in food industry. Frontiers in Sustainable Food Systems 3: 95.

Azmi NAN, Elgharbawy AA, Salleh HM, Moniruzzaman M (2022) Preparation, characterization and biological activities of an oil-in-water nanoemulsion from fish by-products and lemon oil by ultrasonication method. Molecules 27(19): 6725.

Baboota S, Shakeel F, Ahuja A, Ali J, Shafiq S (2007) Design development and evaluation of novel nanoemulsion formulations for transdermal potential of Celecoxib. Acta Pharmaceutica 57: 315-332.

Bolouri P, Salami R, Kouhi S, Kordi M, Asgari Lajayer B, Hadian J, Astatkie T (2022) Applications of essential oils and plant extracts in different industries. Molecules 27(24): 8999. doi:10.3390/molecules27248999.

Cozmuta AM, Turila A, Apjok R, Ciocian A, Cozmuta LM, Peter A, Benković T (2015) Preparation and characterization of improved gelatin films incorporating hemp and sage oils. Food Hydrocolloids 49: 144-155.

Curran PM (1980) The effect of temperature, pH, light and dark on the growth of fungi from Irish coastal waters. Mycologia 72(2): 350-358.

da Silva Santos FM, da Silva AIM, Vieira CB, de Araújo MH, da Silva ALC, Carneiro-da-Cunha MDG, de Souza Bezerra R (2017) Use of chitosan coating in increasing the shelf life of liquid smoked Nile tilapia (Oreochromis niloticus) fillet. Journal of Food Science and Technology 54(5): 1304-1311.

Ding M, Zhang T, Zhang H, Tao N, Wang X, Zhong J (2020) Gelatin-stabilized traditional emulsions: Emulsion forms, droplets, and storage stability. Food Science and Human Wellness 9(4): 320-327.

Dong Q, Yang L, Xiang L, Zhao Y, Li L (2023) Development of electrospun fish gelatin film containing lauroyl arginate ethyl and its application in large yellow croaker (Pseudosciaena crocea) preservation. Food Control 153: 109959.

Đorđević SM, Cekić ND, Savić MM, Isailović TM, Ranđelović DV, Marković BD (2015) Parenteral nanoemulsions as promising carriers for brain delivery of risperidone: Design, characterization and in vivo pharmacokinetic evaluation. International Journal of Pharmaceutics 493: 40-54.

Durmuş M, Ozogul Y, Köşker AR, Ucar Y, Boğa EK, Ceylan Z, Ozogul F (2020) The function of nanoemulsion on preservation of rainbow trout fillet. Journal of Food Science and Technology 57(3): 895-904.

Ebadi Z, Khodanazary A, Hosseini SM, Zanguee N (2019) The shelf life extension of refrigerated Nemipterus japonicus fillets by chitosan coating incorporated with propolis extract. International Journal of Biological Macromolecules 139: 94-102.

Esquerdo VM, Silva PP, Dotto GL, Pinto LA (2018) Nanoemulsions from unsaturated fatty acids concentrates of carp oil using chitosan, gelatin, and their blends as wall materials. European Journal of Lipid Science and Technology 120(2): 1700240.

Farzami SP, Kaplan ABU, Kılınboz YF, Çetin M (2025) Development and in vitro characterization of nanoemulsion and nanoemulsion-based gel formulations containing Heracleum persicum ethanol extract. Pharmata 5(1): 22-27.

Fu L, Ru Y, Hong Q, Zhang Y, Weng H, Yang Q, Xiao A (2023) Active packaging coatings based on agarose caffeate: Preparation, characterisation, and application in grass carp (Ctenopharyngodon idellus) preservation. Food Packaging and Shelf Life 37: 101089.

Gazolu-Rusanova D, Lesov I, Tcholakova S, Denkov N, Ahtchi B (2020) Food grade nanoemulsions preparation by rotor-stator homogenization. Food Hydrocolloids 102: 105579.

Gore DD, Sharma N, Mishra N, Parmar PK, Ranjana S, Kumar D, Singh IP (2024) Wound-healing effect of topical nanoemulsion-loaded cream and gel formulations of Hippophae rhamnoides L. (sea buckthorn) fruit oil and their acute dermal toxicity study on female SD rats. Indian Journal of Pharmacology 56(2): 120-128.

Gulzar S, Tagrida M, Prodpran T, Li L, Benjakul S (2023) Packaging films based on biopolymers from seafood processing wastes: Preparation, properties, and their applications for shelf-life extension of seafoods—A comprehensive review. Comprehensive Reviews in Food Science and Food Safety 22(6): 4451-4483.

Gurpreet K, Singh SK (2018) Review of nanoemulsion formulation and characterization techniques. Indian Journal of Pharmaceutical Sciences 80(5).

Hashem AH, Doghish AS, Ismail A, Hassanin MM, Okla MK, Saleh IA, Shehabeldine AM (2024) A novel nanoemulsion based on clove and thyme essential oils: Characterization, antibacterial, antibiofilm and anticancer activities. Electronic Journal of Biotechnology 68: 20-30.

İlyasoğlu H, El SN (2010) Nanoemülsiyonlar: Oluşumları, yapıları ve kollodial salınım sistemleri olarak gıda sektöründe kullanım alanları. Gıda 35(2): 143-150.

Jiang J, Zhu B, Liu Y (2013) Effects of sucrose on the physical stability of whey protein-stabilized emulsions during storage. Food Hydrocolloids 33: 195-203.

Kan G, Zi Y, Li L, Gong H, Peng J, Wang X, Zhong J (2023) Curcumin-encapsulated hydrophilic gelatin nanoparticle to stabilize fish oil-loaded Pickering emulsion. Food Chemistry: X 17: 100590.

Kara A, Caglak E, Karsli B (2024a) Optimization and bioactive properties of nanoemulsion formulated with hemp (Cannabis sativa L.) seed oil and aloe vera (Barbadensis miller Stockton) gel. ChemistrySelect 9(17): e202305110.

Kara A, Demirci AS, Şahin S (2024b) The influence of chitosan-coated nanoemulsions on the oxidative stability of plant oils. Journal of Food Engineering 345: 111398.

Kara A, Çağlak E, Karslı B (2025) Protective effects of hydrocolloid nanoemulsion coatings against myofibrillar protein oxidation in frozen trout. Frontiers in Nutrition 12: 1663357.

© Akdeniz University Faculty of Agriculture

Khan RU, Shah SU, Rashid SA, Naseem F, Shah KU, Farid A, Alamoudi SA (2022) Lornoxicam-loaded chitosan-decorated nanoemulsion: Preparation and in vitro evaluation for enhanced transdermal delivery. Polymers 14(9): 1922.

Khedri N, Roomiani L (2019) Effects of Zataria multiflora essential oil nanoemulsion on chemical, microbial and sensory properties of silver carp fillets. Iranian Journal of Nutrition Sciences and Food Technology 14(3): 63-74.

Kildaci I, Budama-Kilinc Y, Kecel-Gunduz S, Altuntas E (2021) Linseed oil nanoemulsions for treatment of atopic dermatitis disease: Formulation, characterization, in vitro and in silico evaluations. Journal of Drug Delivery Science and Technology 64: 102652.

Klang V, Haberfeld S, Hartl A, Valenta C (2012) Effect of γ-cyclodextrin on the in vitro skin permeation of a steroidal drug from nanoemulsions: Impact of experimental setup. International Journal of Pharmaceutics 423(2): 535-542.

Kumar N, Mandal A (2018) Thermodynamic and physicochemical properties evaluation for formation and characterization of oil-in-water nanoemulsion. Journal of Molecular Liquids 266: 147-159.

Lyu X, Wang Y, Gao S, Wang X, Cao W, Cespedes-Acuña CL (2022) Sea buckthorn leaf extract on the stability and antioxidant activity of microencapsulated sea buckthorn oil. Food Bioscience 48: 101818.

Maleki G, Woltering EJ, Mozafari MR (2022) Applications of chitosan-based carrier as an encapsulating agent in food industry. Trends in Food Science & Technology 120: 88-99.

Malle P, Tao SH (1987) Rapid quantitative determination of trimethylamine using steam distillation. Journal of Food Protection 50(9): 756-760.

Marhamati M, Ranjbar G, Rezaie M (2021) Effects of emulsifiers on the physicochemical stability of oil-in-water nanoemulsions: A critical review. Journal of Molecular Liquids 340: 117218.

Mason TG, Graves SM, Wilking JN, Lin MY (2006) Extreme emulsification: Formation and structure of nanoemulsions. Condensed Matter Physics, doi:10.5488/CMP.9.1.193.

Mattar AA, El-Desouky S, Elrefaey AAEA, Abd El-Raouf W, Soliman TN (2024) Preparing and evaluating a caseinate-based Moringa oleifera seed oil nanoemulsion to be incorporated into functional ice cream. Egyptian Journal of Chemistry 67(9): 173-184.

McClements DJ (2013) Edible nanoemulsions: Fabrication, properties, and functional performance. Soft Matter 7(6): 2297-2316.

Mukurumbira AR, Shellie RA, Keast R, Palombo EA, Jadhav SR (2022) Encapsulation of essential oils and their application in antimicrobial active packaging. Food Control 136: 108883.

Özakar E, Kaplan M, Yıldız S (2022) Nanoemulsions for food applications: Characterization, stability, and bioavailability. Trends in Food Science & Technology 126: 123-135.

Öztürk B, McClements DJ (2016) Progress in natural emulsifiers for utilization in food emulsions. Current Opinion in Food Science 7: 1-6.

Parnell-Clunies EM, Kakuda Y, Mullen K, Arnott DR, Deman JM (1986) Physical properties of yogurt: A comparison of vat versus continuous heating systems of milk. Journal of Dairy Science 69(10): 2593-2603.

Patel RP, Joshi JR (2012) An overview on nanoemulsion: A novel approach. International Journal of Pharmaceutical Sciences and Research 3(12): 4640.

Pazyar N, Yaghoobi R, Bagherani N, Kazerouni A (2014) A review of applications of tea tree oil in dermatology. International Journal of Dermatology 52(7): 784-790.

Rather SA, Hussain PR, Suradkar P (2024) Evaluating the effects of chitosan incorporated with seabuckthorn leaf extract composite edible coatings on the shelf life of peach (*Prunus persica* L.) fruits. Food and Humanity 2: 100280.

Reis DR, Ambrosi A, Di Luccio M (2022) Encapsulated essential oils: A perspective in food preservation. Future Foods 5: 100126.

Richa R, Choudhury SR (2021) Role of viscosity in emulsion stability: A comprehensive review. Colloids and Surfaces A: Physicochemical and Engineering Aspects 610: 125740.

Sarathchandraprakash NK, Mahendra C, Prashanth SJ, Manral K, Babu UV, Gowda DVS (2013) Emülsiyonlar ve emülgatörler. Asya Deneysel Kimya Dergisi 8: 30-45.

Seow YX, Yeo CR, Chung HL, Yuk HG (2014) Plant essential oils as active antimicrobial agents. Critical Reviews in Food Science and Nutrition 54(5): 625-644.

Sharif M, Madni A, Rehman M (2017) Formulation and evaluation of self-nanoemulsifying drug delivery system for curcumin. Journal of Drug Delivery Science and Technology 39: 274-282.

Sharma K, Babaei A, Oberoi K, Aayush K, Sharma R, Sharma S (2022) Essential oil nanoemulsion edible coating in food industry: A review. Food and Bioprocess Technology 15(11): 2375-2395.

Shehabeldine AM, Doghish AS, El-Dakroury WA, Hassanin MM, Al-Askar AA, AbdElgawad H, Hashem AH (2023) Antimicrobial, antibiofilm, and anticancer activities of Syzygium aromaticum essential oil nanoemulsion. Molecules 28(15): 5812.

Silva HD, Beldíková E, Poejo J, Abrunhosa L, Serra AT, Duarte CM, Vicente AA (2019) Evaluating the effect of chitosan layer on bioaccessibility and cellular uptake of curcumin nanoemulsions. Journal of Food Engineering 243: 89-100.

Singh KK, Vingkar SK (2008) Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. International Journal of Pharmaceutics 347(1-2): 136-143.

Song H, Liu C, Gui D, Sha Y, Song Q, Jia P, Lin Y (2023) Sustainable and mechanically robust epoxy resins derived from chitosan and tung oil with proton conductivity. Journal of Applied Polymer Science 140(20): e53857.

Steyaert I, Rahier H, Van Vlierberghe S, Olijve J, De Clerck K (2016) Gelatin nanofibers: Analysis of triple helix dissociation temperature and cold-water-solubility. Food Hydrocolloids 57: 200-208.

Sugumar S, Clarke SK, Nirmala MJ, Tyagi BK, Mukherjee A, Chandrasekaran N (2014) Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. Bulletin of Entomological Research 104(3): 393-402.

Sultana N, Hassan MI, Ridzuan N, Ibrahim Z, Soon CF (2018) Fabrication of gelatin scaffolds using thermally induced phase separation technique. International Journal of Engineering 31(8): 1302-1307.

Sümbüloğlu V, Sümbüloğlu K (2000) Sağlık bilimlerinde araştırma yöntemleri. Hatiboğlu Yayınları.

Şimşek T, Keyf S (2018) Alginat-kitosan bazlı biofilm sentezi için deneysel tasarım uygulaması. Karaelmas Fen ve Mühendislik Dergisi 8(2): 482-490.

Talegaonkar S, Mustafa G, Akhter S, Iqbal ZI (2010) Design and development of oral oil-in-water nanoemulsion formulation bearing atorvastatin: In vitro assessment. Journal of Dispersion Science and Technology 31(5): 690-701.

Tontul İ (2020) Gıda bileşenlerinin enkapsülasyonunda nanoemülsiyonların kullanımı. Turkish Journal of Agriculture-Food Science and Technology 8(1): 130-138.

Uçar T (2023) Encapsulation of bioactive compounds in food nanoemulsions: Recent advances and future prospects. Food Research International 165: 112427.

Valizadeh S, Naseri M, Babaei S, Hosseini SMH (2020) Shelf life extension of fish patty using biopolymer-coated active paper sheets. Food Packaging and Shelf Life 26: 100603.

Vanin FM, Hirano MH, Carvalho RA, Moraes ICF, Bittante AMQB, do Amaral Sobral PJ (2014) Development of active gelatin-based

© Akdeniz University Faculty of Agriculture

nanocomposite films produced in an automatic spreader. Food Research International 63: 16-24.

Wooster TJ, Golding M, Sanguansri P (2008) Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir24(22): 12758-12765.

Wu C, Wang L, Hu Y, Chen S, Liu D, Ye X (2016) Edible coating from citrus essential oil-loaded nanoemulsions: Physicochemical characterization and preservation performance. RSC Advances 6(25): 20892-20900.

Wu Y, Wu H, Hu L (2024) Recent Advances of Proteins, Polysaccharides and Lipids-Based Edible Films/Coatings for Food Packaging Applications: a Review. Food Biophysics 19: 29-45. doi:10.1007/s11483-023-09794-7.

Yang Y, Aghbashlo M, Gupta VK, Amiri H, Pan J, Tabatabaei M, Rajaei A (2023) Chitosan nanocarriers containing essential oils as a green strategy to improve the functional properties of chitosan: A review. International Journal of Biological Macromolecules 236: 123954.

Yu Q, Zhang M, Mujumdar AS, Rui L (2025) Extending shelf life of chilled pork pretreated with high-voltage electrostatic field in modified atmosphere packaging by cinnamaldehyde nanoemulsion at non-contact mode. Meat Science 225: 109802.

Zhang R, Zhang Z, McClements DJ (2020) Nanoemulsions: An emerging platform for increasing the delivery of hydrophobic bioactive compounds into foods. Food Hydrocolloids 96: 105589.

Zhang S, Zhang M, Fang Z, Liu Y (2017) Preparation and characterization of blended cloves/cinnamon essential oil nanoemulsions. LWT 75: 316-322.

Zhang Z, Chen Y, Chen Z, Gao Z, Cheng Y, Qu K (2024) Quality analysis and assessment of representative sea buckthorn fruits in northern China. Food Chemistry: X 24: 101828.

Zheng H, Mao L, Yang J, Zhang C, Miao S, Gao Y (2020) Effect of oil content and emulsifier type on the properties and antioxidant activity of sea buckthorn oil-in-water emulsions. Journal of Food Quality 2020(1): 1540925.

© Akdeniz University Faculty of Agriculture