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ORIGINAL PAPER

Encapsulated chitosan-moringa oil- based coating for preservation of rainbow trout (*Oncorhynchus mykiss*) fillets

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Abstract
Nanotechnology provides effective strategies in food preservation, especially through nanoemulsion-based systems that enhance stability and the controlled delivery of bioactive compounds.The aim of the present study was to investigate the bioactive effect of moringa oil (*Moringa oleifera*) (Mo) encapsulated in a nanoemulsion system containing chitosan (Ch) on the quality parameters of rainbow trout fillets. In line with this objective, characterization studies of Mo and Ch based nanoemulsion (Mo-N), which is known for its high antimicrobial and antioxidant content, were carried out by ultrasonic emulsification method. Than the *Oncorhynchus mykiss* fillets were coated with this nanoform and monitored for changes in microbiological, chemical and sensory parameters during 12 days of storage. TEM analysis confirmed the regular and spherical morphology of the nanoemulsion; the droplet size of 110.5 nm and PDI value of 0.32 indicated a relatively uniform distribution, suggesting favorable physical characteristics of the system. FT-IR analysis shows that the functional groups of the key components are retained after high-energy treatment. The findings revealed that Mo-N treatment effectively suppressed and delayed bacterial growth and had a positive effect on consumer acceptance. In addition, Mo-N minimized lipid and protein oxidation and discoloration of fillets and preserved the sensory parameters of fillets during storage compared to the control sample. According to the shelf life assessment, the developed Mo-N coating proved to be effective in preserving the quality characteristics of rainbow trout fillets, thus extending the shelf life by at least 3 days.

Keywords Trout · Shelf life · Nanoemulsion · Chitosan · *Moringa oleifera*

Introduction

In recent years, growing awareness of the relationship between diet and health, increasing concerns about food safety and synthetic additives, and the rising prevalence of chronic diseases have significantly influenced consumer behavior toward healthier dietary patterns. In addition, consumers increasingly perceive natural and minimally processed foods as safer and more beneficial for health, which further reinforces this shift. These factors have increased the demand for minimally processed and clean-label products. Especially with the recent increase in the trend towards healthy eating, the demand for natural preservatives and less processed products is increasing. In this context, the development of biocompatible and natural preservation systems, such as nanoemulsion-based coatings, has gained significant attention in the food industry [1–3]. Among these natural preservatives, plants and/or their oils are particularly popular. These products are advantageous because they have antimicrobial/antioxidant properties, have a positive effect on consumer health, are non-toxic, easily available and inexpensive. In a similar process, vegetable oils are natural agents that are frequently used and involved in nanotechnological applications. In particular, the most promising technology suitable for lipophilic actives with a controlled

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release rate and a good preference for topical application (improved bioavailability, enhanced solubility, stability and flexibility, and reduced side effects) is emulsions with droplet size in the nanometer range [4–6]. Nanotechnology is promising in areas such as the safety of agriculture and food production systems, increasing crop productivity, synthesizing new agrochemicals and developing new materials (Iavicoli et al., 2017 [4]).

Since food products deteriorate due to moisture, oxygen, light and heat factors, the importance of food packaging has increased day by day and innovative packaging materials are needed [7]. Food packaging materials that can release nanoscale antimicrobial compounds, antioxidants and/or flavors to improve the shelf life or sensory properties of a food are called "active". The incorporation of active compounds into food packaging materials is more common than packaging designed to release particulate nanomaterials into food. Nanostructured systems in foods; polymeric nanoparticles, liposomes, nanoemulsions and microemulsions [8].

Among emulsion applications, nanoemulsion applications draw attention in the preservation and functional properties as well as visual and physical qualities of foods. For the seafood processing sector, the main expectation from these applications is that they increase the shelf life with their antimicrobial/antioxidant properties as well as masking the characteristic odor of seafood products [9].

The constituents of *Moringa oleifera* (leaves, flowers, fruits and seeds) are important sources of nutrients and due to their therapeutic antibiotic and anti-inflammatory effects, *M. oleifera* is widely used in traditional medicine and has recently attracted the attention of researchers in the field of agriculture. Seed oil, which contains biologically active fatty acids and tocopherols with excellent oxidative stability, is utilized in different industries (food, illuminants, lubricants, biofuels and cosmetics). They have high oxidative stability and have physicochemical properties similar to olive oil [6, 10]. The oil from *M. oleifera* seeds is semi-solid, has a bitter almond smell and a yellowish-brown color. The mixed fatty acids in seed oil [palmitic acid − 1.04%, stearic acid − 3.58%, arachidic acid − 3.44%, behenic acid − 7.09%, palmitoleic acid − 2.38% and linoleic acid − 1.83%] play a role in protecting antioxidant molecules against free radicals [11]. Chitosan is a natural polymer frequently used for encapsulation in food applications, mainly for its controlled release of active ingredients, cross-linking thanks to free amine groups and increased stability [12].

Although nanoencapsulation of moringa oil has been previously reported, studies focusing on its incorporation into chitosan-based nanoemulsion systems remain limited. Existing research has mainly investigated moringa oil in its free form or within conventional delivery systems, with insufficient attention given to its interaction with chitosan and the resulting physicochemical and functional properties. Furthermore, the application of such systems in real food matrices, particularly in aquaculture products, has not been comprehensively evaluated. In this study, a chitosan-based moringa oil nanoemulsion was developed using ultrasonication and systematically characterized. In addition, its bioactivity potential was assessed on rainbow trout fillets. This study also aims to contribute to the development of nanoemulsion-based natural preservatives and to provide a basis for sustainable and cost-effective applications in the aquaculture processing sector. .

Material method

Nanoemulsion formulation and characterization studies

Moringa oil (Mo-N) chitosan (Ch) loaded nanoemulsion was prepared by modifying the method proposed by Kara et al., [13] (Table 1). Chitosan obtained from a commercial company (Sigma-Aldrich) was dissolved in acetic acid solution (7.5 g of chitosan in 500 mL of acetic acid solution (1.5%, v/v) to prepare a 1.5% (w/v) stock solution and to achieve complete dispersion of Ch, the stock solution was stirred overnight at room temperature (Alak et al., 2023). This stock solution was incorporated into the nanoemulsion formulation as a final ingredient at a concentration of 2% (v/v) [4]. Then, chitosan and Tween 80 (T80), a non-ionic, low molecular weight surfactant, and purified water were added to a beaker. The prepared solution was homogenized with a homogenizer (WiseTis) at 400 rpm for 3 min and moringa oil was added dropwise with a syringe simultaneously [13]. 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, moringa oil chitosan-loaded nanoemulsion (Mo-N /Ch) in oil-in-water (O/W) structure was obtained. Subsequently, nanoemulsion characterization, pH, viscosity, turbidity, conductivity, color and whiteness index, size

Table 1 Component content of nanoemulsion

Components	Percentage (%)
Chitosan (ChS)	2
Moringa oil (Mo-N oil)	6
Tween 80	24
Distilled water	68

distribution analysis were performed by Fourier Transform Infrared Spectroscopy (FT-IR) and morphological characterization by Transmission Electron Microscopy (TEM) [4, 14].

pH

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 [14].

Viscosity (mPa.S)

The changes in viscosity were determined with a viscosity device (SOIF NDJ-8 S (10 − 2*106 mPa.s, 30 rpm/3s–1 min - rotor number 3) according to [14] and measured with spindle number 3 at 30 rpm under a shear rate of 3 s-1 (10 − 2*106 mPa.s measurement interval).

Turbidity (NTU)

Turbidity was measured in a UV-spectrophotometer (WTW, Turb555) at room temperature (25 ± 2 °C) after 1:9 dilutions of the nanoemulsion with distilled water. Each result was then multiplied by the dilution coefficient in 3 replicates of this analysis [4, 14].

Conductivity (µS/cm)

Electrical conductivity was measured at 25 ± 2 °C by direct immersion of a HACH, HQ40d probe into the nanoemulsion [4, 14].

Color and whiteness index

*Lab** color values of the formulation were measured using a Konica Minolta (CR-200, Osaka, Japan) colorimeter. *Lab** values are given according to the International Commission on Illumination "CIELAB" (Commision Internationalele de I'E Clairage) standard. In this context, *Lab** showed that lightness or darkness, greenness (negative values) or redness (positive values), and blueness (negative values) or yellowness (positive values) of fish fillet (Sing et al., 2026). CIE *Lab** values were used to calculate the whiteness index (WI) [4]:

$$WI = 100 - \sqrt{\left[(100 - L^*)^2 + (a^{*2} + b^{*2})\right]}$$

Particle size distribution analysis

The Zeta potential, droplet size, and PDI of nanoemulsions were measured using the zetasizer device (Malvern Zetasizer Nano ZSP, United Kingdom). Nanoemulsions were diluted with ultrapure water at a ratio of 1 : 9. Zeta sizer measurements were collected in the capillary zeta cell DTS1070 at 25 °C, and 3 × 12 measurements were taken with a detector angle of 173° utilizing a laser of 633 nm [15]. This analysis was conducted at Atatürk University Eastern Anatolia High Technology Research and Application Center (DAYTAM).

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR analyses were determined by an FT-IR spectrophotometer (Perkin Elmer Spectrum 100) with a resolution of 4 cm^{-1} at a wavelength range of 4000–600 cm^{-1}. For the FT-IR analysis was used deuterated water for the preparation of nanoemulsions. This analysis was performed at the Recep Tayyip Erdogan University Central Research Laboratory.

Transmission electron microscope (TEM)

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.

Nanoemulsion coating of fillets and storage analysis

A total of 180 fillets of rainbow trout (*Oncorhynchus mykiss*) with an average weight of 280 ± 15 g obtained from Atatürk University Faculty of Fisheries, Inland Fish Research and Production Center were used. In the study, trout fillets were immersed in pure water as negative control (C), positive control (Ch) was composed of only chitosan solution and the treatment group was designed as nanoemulsion (Mo-N) coated fillets.

The prepared coating solutions were applied to the fillets by dipping method and the fillets were kept in the solution for at least 5 min under sterile conditions for an effective surface coating. They were then left to dry for 5 min in a sterile environment. After drying, the fillets were placed in plastic plates, wrapped in ordinary packaging with stretch film and subjected to cold storage for 12 days under refrigerator conditions. During this storage period, the following microbiological, chemical and sensory analyses were performed at certain intervals (on days 0, 3, 6, 9, and 12) to determine fillet quality and shelf life.

Microbiological analysis

Microbiological analyses were performed to determine fillet quality and shelf life at certain intervals (0., 3., 6., 9., and 12. days) during this storage period (Table 2).

Physico-chemical analysis

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.

Total volatile basic nitrogen (TVB-N) analysis 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 ∅125 mm, fast filtering). The resulting solution was distilled by adding 10% NaOH and TVB-N value was calculated as mg/100 g [16].

Determination of thiobarbituric acid reactive substance (TBARS) value Following the method given by Lemon (1975), 1 gram of tissue was taken, 6 mL of TCA solution was added and the mixture was homogenized for 30 s and then passed through Whatman 1 filter paper. The resulting filtrate was taken into a tube, 0.02 M TBA solution was added, the mixture was incubated (40 min at 100 °C) and the TBARS value was determined as µmol MA/kg by reading at 532 nm wavelength in spectrophotometer (Shimadzu, UV 160) [14, 17].

pH measurement After the mixture was homogenized in Ultra Turrax for 1 min, the pH value was determined with a pH meter [14].

Water holding capacity 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 [18].

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

Table 2 Bacteria and incubation references

Bacteria	Agar	Incubation
Total aerobic mesophilic bacteria	Plate Count Agar (PCA, Merck)	37 °C for 48 h aerobic
Psychrotrophic bacteria	Plate Count Agar (PCA, Merck)	10 °C for 7 days aerobic
Lactic acid bacteria	De Man Ragose Sharpe Agar (MRS, Merck)	30°C for 48 h anaerobic
Enterobacteriaceae	Violet Red Bile Dextrose Agar (VRBD. Merck)	30°C for 48 h anaerobic

Water activity analysis (a_w) Water activity measurements were performed with Aqualab (Decagon Devices, model series 3) water activity meter at 25 °C (±0.2 °C) [16].

Measurement of color intensity The color intensities of the samples were determined as *Lab** values using a colorimeter device [16, 19, 20].

Sensory analysis

The most preferred rating analysis was used, and the evaluations of general appearance, meat appearance, raw fish structure and raw fish odor, which are considered as reliable quality factors, were made according to the hedonic scale in the classification degrees specified in Alak et al., [1] and and Kumar et al., and [21]. These analyses were conducted by twenty-one panelists who had undergone prior training and they made evaluations of the treatment groups.

Statistical analysis The lactic acid number in the groups was accepted as 2 log CFU/g during storage. Data obtained at the end of the trial period were analyzed using GraphPad Prism 8 software. Results are expressed as mean ± standard deviation (mean ± SD), with statistical significance considered at the following levels: $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$, and not significant (ns) for $p>0.05$. Data were evaluated using two-way analysis of variance (ANOVA) for the evaluation of storage time and group effect. Tukey's multiple comparison test was applied for each group. Nanoemulsion characterization data are presented as mean ($n=3$) ± standard deviation (SD).

Results and Discussion

Characterization data

The pH, conductivity, turbidity viscosity, color (*Lab**), whiteness index (WI), Zeta potential value, particle size and polydispersity index results of the nanoemulsion are given in Table 3.

In this study, the pH value was determined as 3.93 ± 0.02 in the nanoemulsion system prepared using moringa oil and chitosan solution (Table 3). The results obtained were determined as acidic compared to the values determined in other studies in the literature (6.0 to 8.0) [6, 11, 22, 23]. This acidity is attributed to the preparation of chitosan in acetic acid, which is directly incorporated into the formulation as a carrier in the emulsion system; chitosan solutions prepared in this manner are typically reported to have a pH in the range of 3.5–4.5 [24, 25].

Table 3 Characterization data of nanoemulsion

pH	Conductivity (µS/cm)	Turbidity (NTU)	Viscosity (mPa.s)	Colour			Whiteness index (WI)
				L* (Lightness)	a* (Red/Green)	b* (Blue/Yellow)	
3.93 ± 0.02	204.67 ± 3.78	102.50 ± 0.71	237.70 + 2.50	82.54 ± 1.21	2.9 ± 1.30	15.96 ± 1.81	80.29 ± 1.28

Conductivity measurement in nanoemulsions is used as an important parameter to determine the emulsion type of the system. Generally, high conductivity values refer to O/W emulsions where the oil is dispersed in water; low conductivity values refer to W/O emulsions where water is dispersed in oil [13, 26]. The conductivity value determined for chitosan-based Mo-N was found to be 204.67 ± 3.78 µS/cm and appears to represent the O/W type nanoemulsion structure. It is well known that the homogeneous distribution of the nanoemulsion and the presence of a stable aqueous phase rich in ionic species contribute to the increase in the conductivity value [27]. In this context, the high conductivity of the Mo-N system is thought to be related to the ionic species originating from the chitosan solution. When chitosan dissolves in weak acids such as acetic acid, protonated amino groups ($-NH_3^+$) and acetate ions (CH_3COO^-) are formed. This directly contributes to increasing the overall conductivity of the system [28–30]. However, except for chitosan, emulsifiers (non-ionic surfactants such as Tween 80) in the formulation are not known to have a significant effect on conductivity. Similarly, the contribution of the oil phase (moringa oil) to conductivity is very limited, since the oil phase does not exhibit electrical conductivity nor does it release ions in aqueous media [31]. The total conductivity value of the system is therefore mainly due to the components of the aqueous phase.

Viscosity, which is commonly used as a preliminary indicator in determining emulsion type (O/W or W/O), is one of the main parameters affecting the physical stability of nanoemulsions [32]. Emulsion viscosity is known to vary depending on the chemical structure of the emulsifier used and the viscosity of the oil phase. In this research, increasing the emulsifier and oil ratio resulted in an increase in the viscosity of the nanoemulsion system (237.70 ± 2.50 mPa-s) (Table 3), which is consistent with previous findings [13, 33]. Although the relatively low viscosity observed in the nanoemulsion may suggest oil-in-water (O/W) characteristics, viscosity alone is not sufficient to definitively determine emulsion type [13]. Therefore, this parameter should be evaluated together with complementary analyses such as conductivity. In this study, the relatively high conductivity value supports the presence of a continuous aqueous phase, indicating that the nanoemulsion system exhibits O/W characteristics. Turbidity values of the Mo-N system were measured in NTU and found to be 102.50 ± 0.71 (Table 3). Surfactant concentration is positively correlated with viscosity and negatively correlated with turbidity. This decrease in turbidity for Mo-N can be attributed to the lower droplet diameter. The relatively low transparency of an emulsion system is due to reduced light scattering and high surfactant concentration [34, 35]. It is also known in the literature that the turbidity of nanoemulsions decreases with increasing number of passes and homogenization pressure, which is related to the decrease in droplet size [36].

For Mo-N system, L^* value was 82.54 ± 1.21, a^* value was 2.9 ± 1.30, b^* value was 15.96 ± 1.81 (Table 3). Nanoemulsions characteristically exhibit a hazy appearance due to their small droplet size, leading to intense scattering of light. This is recognized as one of the key elements shaping the optical properties of the systems. The L^* (gloss) values obtained in the study were above 80, indicating that the formulations have a high whiteness tendency and a balanced distribution of droplet sizes. This finding is directly related to the microstructural integrity of the nanoemulsions and supports the visual stability of the system. The nanoemulsion WI was determined as 80.29 ± 1.28. It is known that the appearance of the emulsion is mainly determined by the presence of oil droplets; therefore, parameters such as droplet size, concentration and refractive index directly affect the optical properties of the overall emulsion [37].

Analysis of size distribution

Nanoemulsions are isotropic colloidal dispersions consisting of droplets, usually below 100 nanometers, in which the oil and water phases are stabilized [38]. The average droplet size of 110.5 ± 2.25 nm determined in this study indicates that the system forms a stable structure with a high surface area (Fig. 1). In addition, the obtained value is in good agreement with the literature findings on nanoemulsions prepared with similar emulsifier ratios and reveals that the formulation successfully achieved the targeted microstructural properties ([13]; Mattar et al., 2024). There is an inverse relationship between viscosity and droplet size in nanoemulsion systems. It is known that an increase in emulsifier concentration increases the flow resistance of the system, preventing the droplets from coming together and thus reducing the average droplet size [39].

Zeta potential refers to the electrical potential at the interface separating the droplets that make up the inner phase from the outer phase in nanoemulsion systems [40]. The zeta potential value of the nanoemulsion obtained in this study was relatively low and was determined as -8.82 ± 0.19 (Table 4).

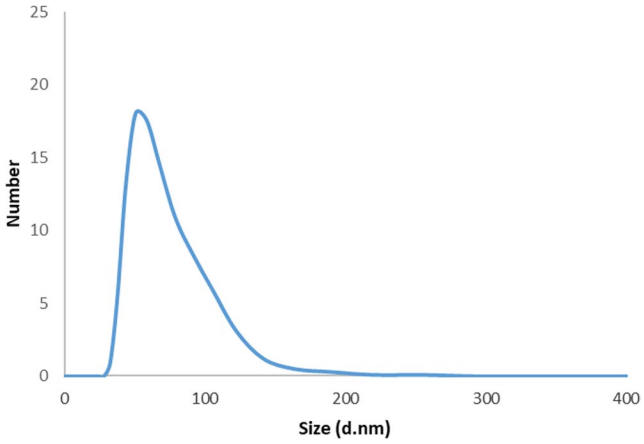

Fig. 1 Droplet size distribution of the nanoemulsion

Table 4 Nanoemulsion Zeta potential, droplet size, polydispersity index (PDI) values

Zeta (ζ) potential (mV)	Droplet size (nm)	Polydispersity index (PDI)
-8.82±0.19	110.5±2.25	0.32±0.06

Studies have reported that low zeta potential values are generally associated with the adsorption of cationic, anionic or non-ionic surfactants on the droplet surface [40, 41]. In the present study, the low zeta potential suggests that electrostatic stabilization is limited. However, the overall stability of the system can be attributed to steric stabilization provided by the non-ionic surfactant (Tween 80), which forms a protective layer around the droplets and prevents aggregation and coalescence.

PDI (polydispersity index), which is an important indicator for evaluating the homogeneity and stability of emulsion systems, also expresses the size distribution of droplets [42]. The obtained PDI value (0.32 ± 0.06) indicates a relatively narrow size distribution and supports the formation of a homogeneous nanoemulsion system. In general, a PDI below 0.3 reflects a narrow size distribution, whereas values above 0.5 indicate a broader distribution [43]. Although the PDI value obtained in this study is slightly above this threshold, it still suggests an acceptable level of uniformity for nanoemulsion systems. When compared with previous studies, Tahir et al., [44] reported a higher PDI value (0.446) for chitosan-based nanoemulsions prepared by ultrasonication, whereas Shao et al., [45] obtained significantly lower PDI values (< 0.3) for eugenol–chitosan nanoemulsions. These differences can be attributed to variations in formulation parameters such as the type of oil phase, surfactant composition, and preparation conditions. In particular, differences in emulsifier concentration and energy input during homogenization may significantly influence droplet size distribution. Therefore, the relatively low PDI value obtained in this study indicates that the developed nanoemulsion possesses a reasonably uniform droplet distribution and acceptable physical stability compared to similar systems reported in the literature [42].

Transmission electron microscopy (TEM)

The TEM image of Mo-N sample is exhibited in Fig. 2. The Mo-N nanoemulsion particles formed in this study have a spherical morphology and are in the size range of approximately 100 nm.

The ability to trace the particles with clear boundaries showed that the system was homogeneous and these findings were consistent with the analysis of Kumar and Mandal [46] on oil-in-water nanoemulsions. However, the denser regions observed in the center prove that the oil phase is concentrated in the core and stabilized by the chitosan coating on the surface. This morphological appearance of small, monodisperse and spherical particles is in agreement with Khan et al., [47] report with the same carrier.

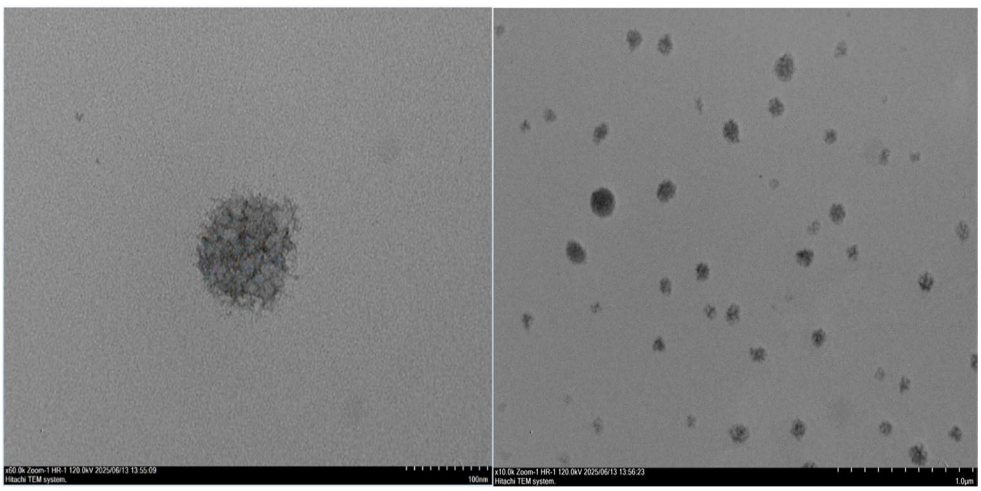

Fig. 2 TEM images of Mo-N nanoemulsion

Fourier transform infrared spectroscopy (FT-IR) analysis

When the FTIR spectra were examined to identify the functional groups of moringa oil and chitosan in the nanoemulsion (Fig. 3), the bands observed at 2922 and 2849 cm^{-1} in moringa oil (Mo-N oil) were attributed to aliphatic -CH_2 and -CH_3 stretching vibrations, while the prominent peak at 1744 cm^{-1} corresponded to ester carbonyl (C = O) vibrations of the triglyceride structure consistent with Shumi et al., [48] Similarly, the band at 1634 cm^{-1} in the chitosan solution (ChS) was assigned to the amide I group (C = O stretching and N-H bending) in agreement with Maciel et al., [49]. The shift of this band to approximately 1640 cm^{-1} in the nanoemulsion indicates the presence of hydrogen bonding and/or electrostatic interactions between the components. In addition, the characteristic bands of Tween 80 at 2857 cm^{-1} (–CH_2) and 1735 cm^{-1} (ester carbonyl) were found to be consistent with Kara et al., [13]. The preservation of the characteristic peaks of moringa oil (2922 and 2849 cm^{-1}) and Tween 80 (1735–1744 cm^{-1}) in the nanoemulsion spectrum confirms the successful incorporation of the components into the system. Slight shifts in peak position and changes in intensity suggest that intermolecular interactions are dominant rather than the formation of new covalent bonds. Furthermore, the absence of new peaks indicates that nanoemulsion formation is governed by physical interactions rather than chemical modifications.

Fillet storage data

In this study, moringa oil was encapsulated with chitosan, a polymeric wall material, and rainbow trout fillets were coated with this nanoemulsion and cold stored. When the data of the control and treatment groups (Ch and Mo-N) were evaluated, increases in pH, TBARS, TVB-N, bacterial loads, decreases in a_w and WHC, and increases/decreases in color data ($L^*a^*b^*$) were detected in the nanoemulsion (Mo-N) treated groups depending on the storage time. These changes were found to be statistically significant and are reflected in Figs. 4, 5 and 6.

While the difference in water holding capacity between Ch · Mo-N groups was insignificant during storage, the differences between C · Mo-N groups were statistically significant at $p<0.05$ and $p<0.01$ levels based on storage days. Similarly, while the changes in a_w activity between Ch x Mo-N in the first days of storage (0 and 3) were insignificant, this difference was determined at $p<0.001$ level in the last days of storage (6, 9, and 12), and the changes determined in terms of a_w in Mo-N group fillets were found to be significant at $p<0.05$ and $p<0.0001$ levels compared to C group fillets (Fig. 4). The similarity in a_w values suggests that the application of the coating did not affect the water content in the fish fillets.

Chemical parameters in fillet quality are reflected in Fig. 5. Increases in the determined parameters were recorded in all groups during storage. While these relative increases in TBARS levels were not significant until the last day of storage, on the last day of storage (day 12), the difference between the CH x Mo-N groups was again insignificant ($p>0.05$), while the changes between C x CH and C x Mo-N were significant at $p<0.05$ level. On the last day of storage, the difference in pH between C x Ch groups was insignificant, while the difference between C x Mo-N and Ch x Mo-N appeared at $p<0.0001$ level. In the TVB-N level, when the last day of storage was taken as a reference, the change

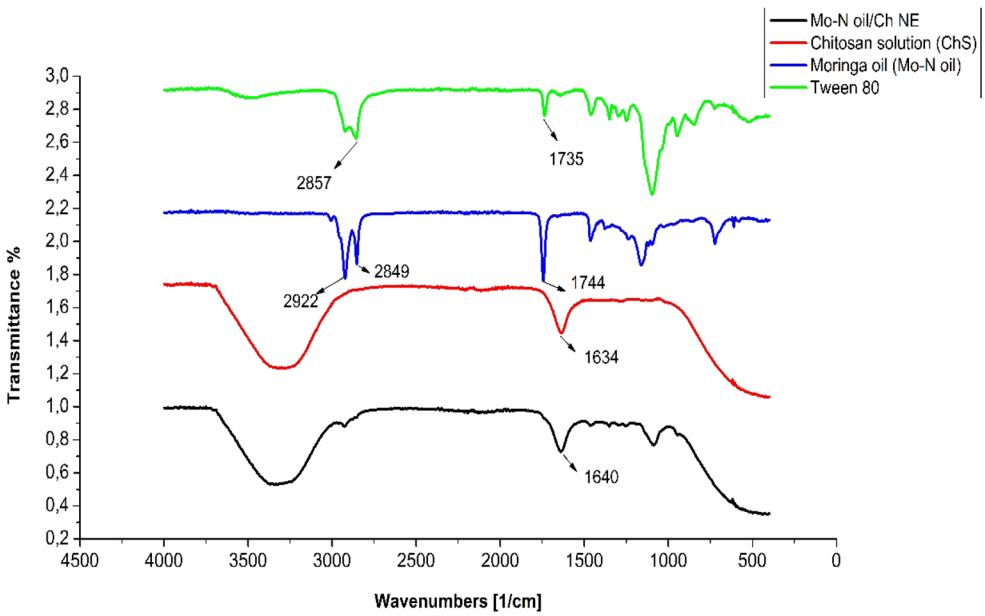

Fig. 3 FT-IR chart of nanoemulsion Mo-N oil/Ch NE, ChS, Mo-N oil, Tween 80

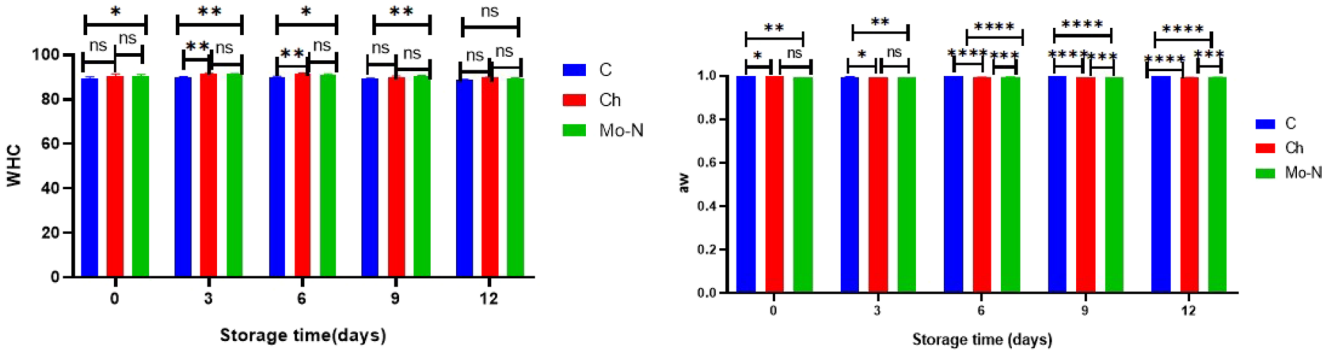

Fig. 4 Changes in water activity (a_w) and water-holding capacity (WHC) of fish fillets under refrigerated condition. Data are presented as mean ± SD. Statistical significance compared to the among treatment groups (C, Ch and Mo-N) is indicated as follows: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; ns not significant (ns) for p>0.05

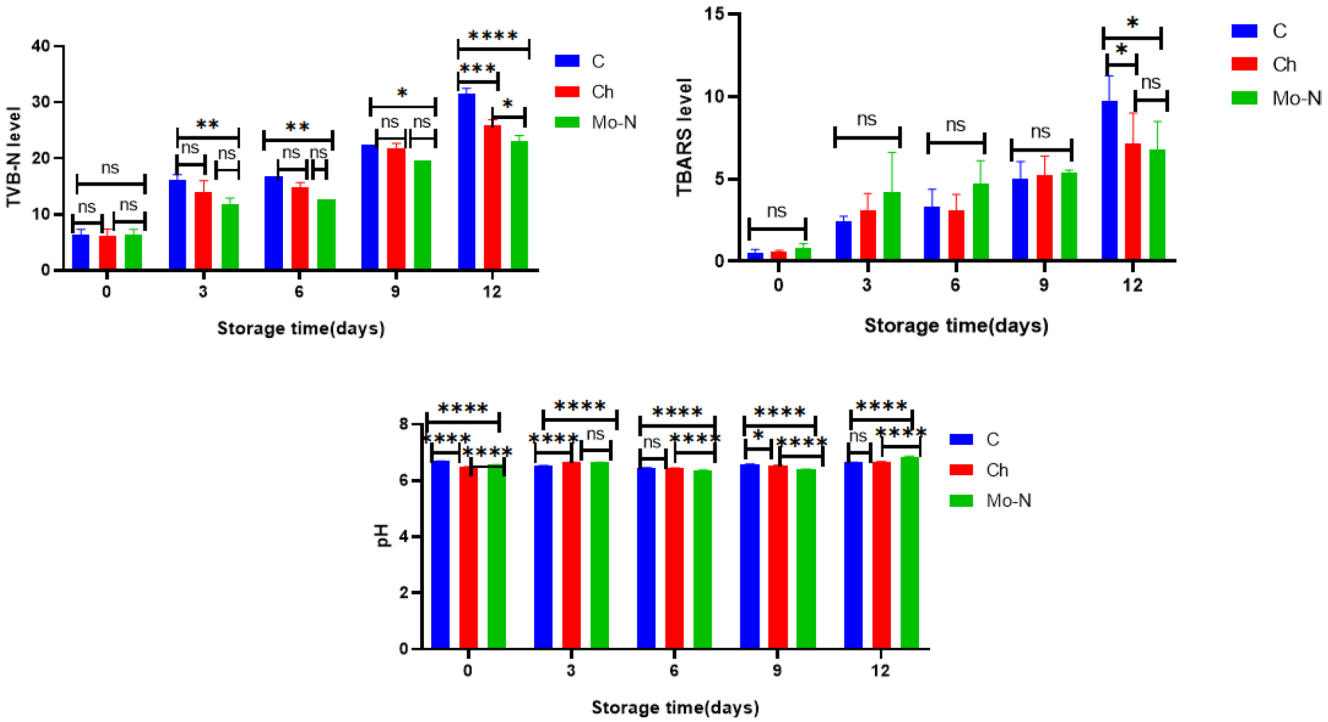

Fig. 5 Changes in chemical parameters (TVB-N, TBARS, and pH) of fish fillets under refrigerated condition (4 ±1°C). Data are presented as mean ± SD. Statistical significance compared to the among treatment groups (C, Ch and Mo-N) is indicated as follows: *p<0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; ns not significant (ns) for p>0.05

between C x Mo-N was found significant at p<0.0001, C x Ch at p<0.001 and Ch x Mo-N at p<0.05. When the Mo-N is compared with the control group (day 12 is taken as reference), the changes in these parameters were determined decrease as pH: 3%, TVB-N: 26.6% and TBARS: 29.8%. The control group (29.33 mg/100 g) exceeded the consumable limit value of TVB-N (25 mg/100 g) on day 12 of storage, followed by the Ch and Mo-N groups, respectively. The consumable limit value of Mo-N was not exceeded even at the end of day 12 among the treatment groups.

The lower pH values observed in Ch and Mo-N treatments compared to the control can primarily be attributed to the acidic nature of the chitosan/acetic acid-based coating, which directly affects the surface pH of the samples. In addition to this direct effect, biochemical processes such as ATP degradation leading to the release of inorganic phosphates and lactic acid accumulation due to anaerobic glycolysis may also contribute to the decrease in pH during storage. The subsequent increase in pH can be attributed to the accumulation of alkaline compounds such as biogenic amines and ammonia produced by microbial activity and endogenous enzymes [50].

The change in TVB-N and TBARS values of Mo-N treated fillets may be due to the content of *M. oleifera* (polyphenolic

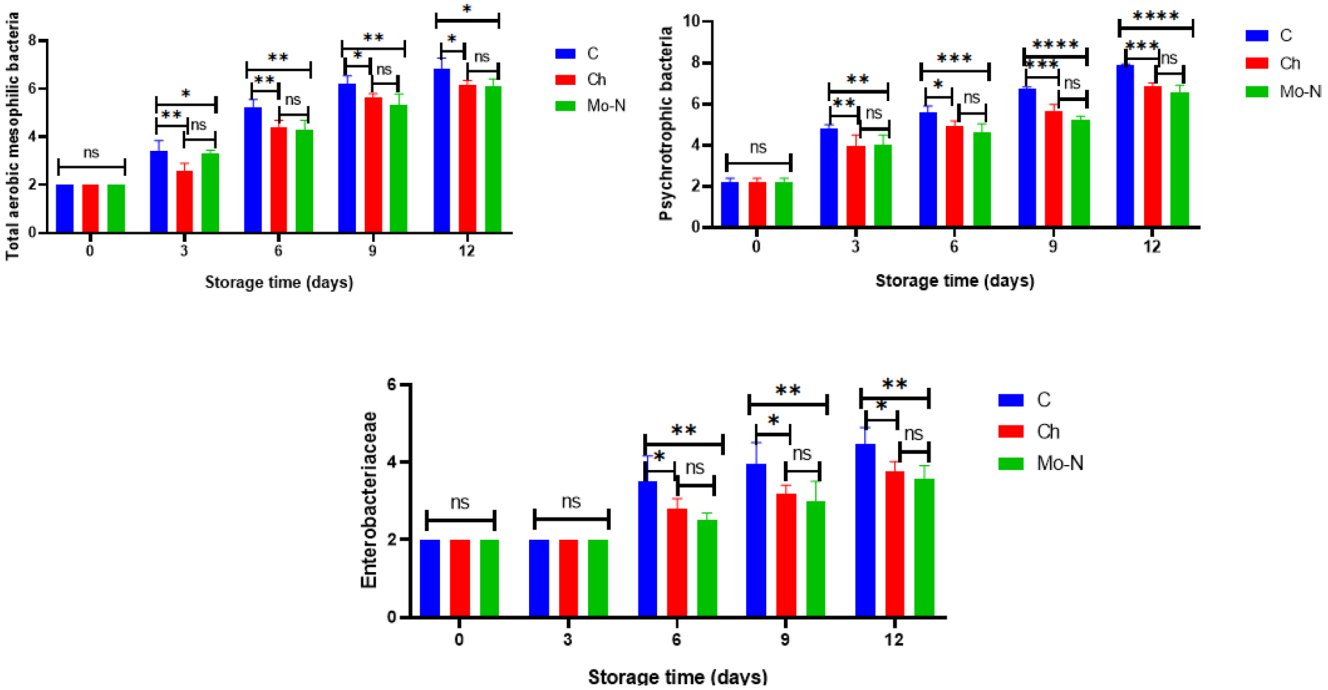

Fig. 6 Changes in microbiological parameters of fish fillets under refrigerated condition (4 ±1°C). Data are presented as mean ± SD. Statistical significance compared to the among treatment groups (C, Ch and Mo-N) is indicated as follows: *p<0.05, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns not significant (ns) for $p > 0.05$

compounds, proteins, antioxidants, oils and antioxidants, ascorbic acid, flavonoids, phenols and carotenoids) [14, 51]. The fact that the moringa oil in the nanoemulsion contains antioxidants that can fight oxidative damage by capturing free radicals and thus protect against the degradation of fat and proteins (Murillo and Fernandez, 2017). This reduction in lipid oxidation is further supported by the oxygen barrier and antioxidant properties of chitosan, as well as the presence of phenolic compounds in Mo-N, which inhibit oxidative reactions. They also quench singlet oxygen, absorb UV radiation, and chelate pro-oxidant metal ions such as Fe^{3+}, thereby limiting oxidative reactions. Through these mechanisms, oxidation is suppressed and TBARS formation is reduced [52]. This mechanism is consistent with the findings of Suwalsky et al., [53], who demonstrated that ellagic acid inhibits the penetration of oxidants into the lipid bilayer, thereby limiting their entry into cells and participation in oxidative reactions. In this context, the incorporation of antioxidants such as Mo into the coating formulation further enhances the effectiveness of chitosan, strengthening its ability to prevent lipid and protein oxidation. Acceptable TVB-N values for freshwater fish and seafood are limited to 25–35 mg N/100 g fish fillet [54, 55]. While the control group fillets exceeded the critical freshness limit on the 9th day of storage, Ch and Mo-N coated fillets maintained their quality on the ninth day. Interestingly, Mo-N coated fillets did not exceed the critical TVB-N limit during the storage period. The increase in TVB-N content can be attributed to many nitrogenous compounds such as ammonia, trimethylamine and dimethylamine produced by spoilage bacteria and endogenous digestive enzymes [54].

Microbiological findings also support the inhibition of chemical changes. Inhibition of microbial proliferation reduces the accumulation of proteases that cause protein degradation and subsequent accumulation of volatiles [56, 57]. Again, this mechanism is influenced by the fact that phytochemicals in moringa oil help prevent food spoilage by inhibiting the growth of microorganisms [50, 58] and extend the shelf life of foods [59]. Our microbial and chemical data are consistent with different research data [50, 58, 60–62] on the use of the same oil applications to prevent spoilage that causes quality loss in meat.

The lactic acid number was determined as 2 log CFU/g in the groups during the determined storage period. In other microbiological findings, microbial increases were determined in parallel with the storage time in all bacterial species and the changes between C x Mo-N were determined statistically insignificant in storage intervals, while the changes between C x CH and C x Mo-N showed changes at $p<0.05$, $p<0.001$, $p<0.0001$ and $p<0.00001$ levels (Fig. 6). The threshold of 6 log CFU/g, which is the consumption limit value when total aerobic mesophilic bacteria count is taken as a reference, was determined at the end of storage (12th day) in both treatment groups compared to the control, while

this value was exceeded on the 9th day in the control group fillets. Based on this table, it was observed that the treatments extended the shelf life by 3 days compared to the control, and this effect was also reflected on consumer tastes (Fig. 7).

The long shelf life of fillets treated with Mo-N nanoemulsion is in line with similar findings in other food preservation studies conducted by other researchers with the same oil emulsions [50, 58, 60, 61, 63, 64].

The inhibition observed in the treatment groups (Ch and Mo-N) compared to the control may be attributed to various interactions, including electrostatic interactions, van der Waals forces, Brownian motion, and collapse forces, as well as the disruption of adhesion forces required for biofilm formation [65]. Another possible mechanism for Mo-N is that the nanoemulsion reduces the availability of nutrients necessary for bacterial growth and adhesion [61]. The positive effect of MoSO nanoemulsion in extending fillet shelf-life is most likely due to its ability to penetrate microbial cell membranes [66], due to its large surface area relative to volume and stronger interfacial interactions, it facilitates more effective attachment to bacterial membranes [61].

In this study, the incomplete inhibition observed in Ch- and Mo-N-treated fillets could be attributed to the interaction of polyphenolic compounds with amine groups in Ch, reducing antimicrobial activity, or to the interaction between Mo and CH, but the characteristics of the specific microbial strains involved should also be taken into account. Because some strains may be more resistant to the antimicrobial properties of Mo and Ch or may adapt to the protective environment created by the coating [50].

Zhou et al. [67] reported that the antimicrobial properties of moringa nanoemulsions are closely related to their ability to disrupt biofilm integrity. Although biofilm can prevent the penetration of many antimicrobial agents, nanoemulsions can encapsulate antimicrobial agents and penetrate biofilms. In addition, essential oils can increase membrane permeability and alter the conformation of fatty acids, polysaccharides and phospholipid layers, leading to structural disruption of the cell wall and cytoplasmic membrane. This disruption leads to ion leakage, protein denaturation, a decrease in ATP levels and ultimately cell lysis [68].

In particular, it was found that panelist answers that referenced odor were given relatively high scores. While the color parameters of Mo-N coated fillets were determined better than the control, the acceptability level for this group was stated as “medium level” in line with the panelist opinions. The use of plant extracts in meat packaging has gained interest among researchers as it allows the inclusion of active compounds such as antioxidants and antimicrobials while minimizing undesirable sensory changes for consumers [69].

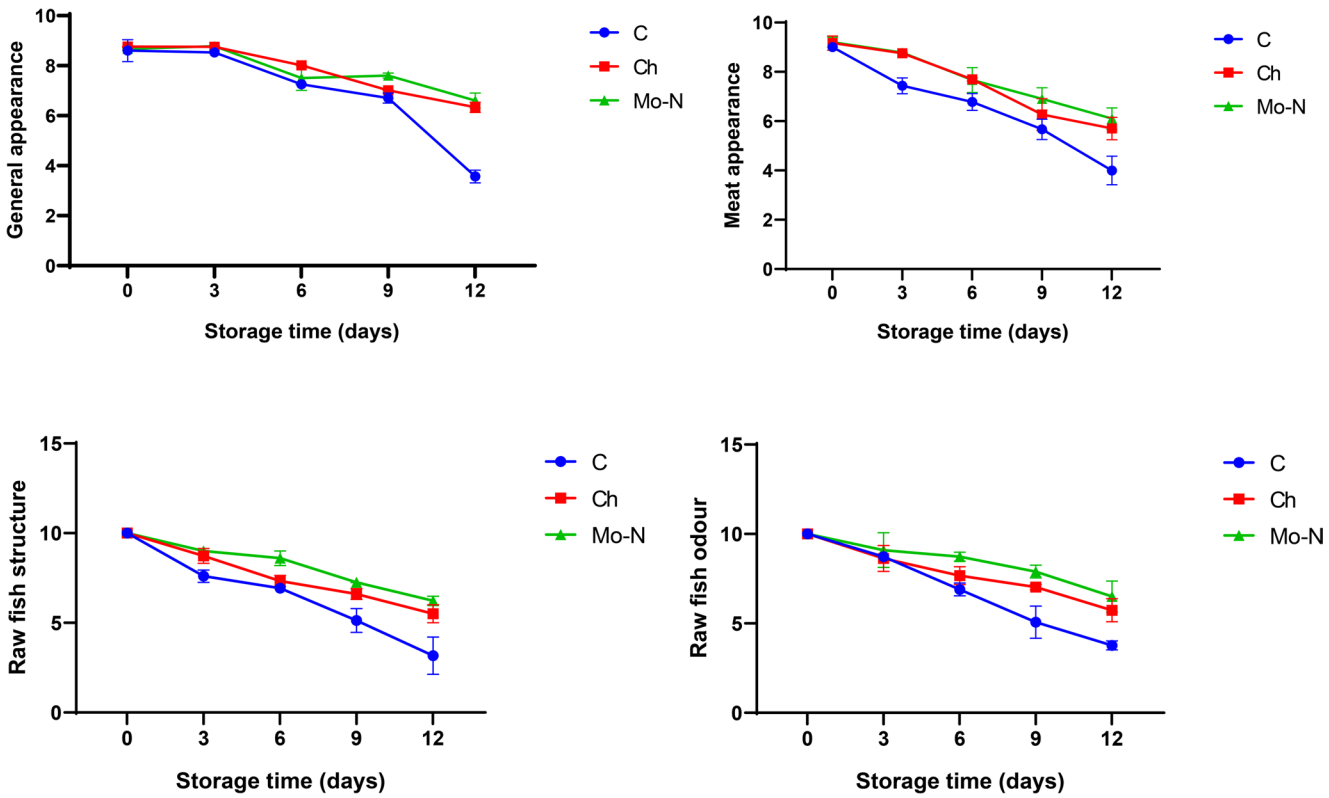

Fig. 7 Changes in sensory score of fish fillet under refrigerated condition (4 ±1°C) (days 0–12). Data are expressed as mean ± standard deviation (n = 3).

Contrary to the sensory parameters we obtained, in a study, it was reported that *M. oleifera* extract used at different concentrations in crustaceans changed the color and odor, making the treated samples unacceptable and there was no improvement in shelf life [70]. We think that this difference is due to the way moringa is used and the fact that the nanoemulsion form was included in our study. One of the major limitations in the application of plant extracts in food preservation is the impact on the natural taste, color and aroma of foods, especially when added in high concentrations [60].

Lower total color changes were measured in the coated samples compared to the uncoated sample. Discoloration of fish fillets during storage or processing is due to oxidation of myoglobin and its reaction with other muscle components [71]. Color changes are mainly seen in dark-fleshed fish species, and given that rainbow trout belong to the group of white-fleshed fish, keeping their color white may have contributed to their better appearance. Thus, the Mo-N has improved the preservation of the color of the fillet surface, limiting changes in texture and protecting it from environmental influences [54].

In this study, the differences between the groups in the L^* parameter are thought to be due to the changing moisture content of the samples with the storage period. In addition, changes in this parameter during storage can be explained by unstable pH values during storage, which cause brightness differences in meat products [72].

At the end of the storage period, a^* significant increase in b^* parameter was observed in the control sample, indicating an increase in yellow discoloration. In contrast, the coated sample presented significantly lower b^* values ($p<0.05$) compared to the control sample at the end of the storage period (Fig. 8). The high b^* value in the control sample can be attributed to lipid oxidation, since in fish products this reaction is usually associated with a shift towards a more yellowish color [73].

There are explanations in the literature that *M. oleifera* extracts significantly reduce the *Lab** values in meatballs made from mutton, and that this reduction is due to the green pigment (chlorophyll) of *M. oleifera* diluting hemoglobin, the meat pigment [60].

Conclusion

In this study, a nanoemulsion (Mo-N) was successfully formulated by ultrasonication using moringa oil (Mo) and chitosan (Ch). TEM analysis confirmed the regular and spherical morphology of the nanoemulsion; the droplet size of 110.5 nm and PDI value of 0.32 revealed the uniform distribution and high stability of the system. FT-IR analysis shows that the functional groups of the key components are retained after high-energy treatment. All characterization data indicated that the nanoemulsion exhibited adequate stability and effectiveness. Application of the nanoemulsion to fish fillets to evaluate its protective coating efficacy resulted in the suppression of microbial growth, reduction of oxidative changes, and preservation of sensory and quality parameters. These findings suggest that the moringa oil and chitosan-based nanoemulsion has the potential to serve as a promising delivery system for the efficient and controlled

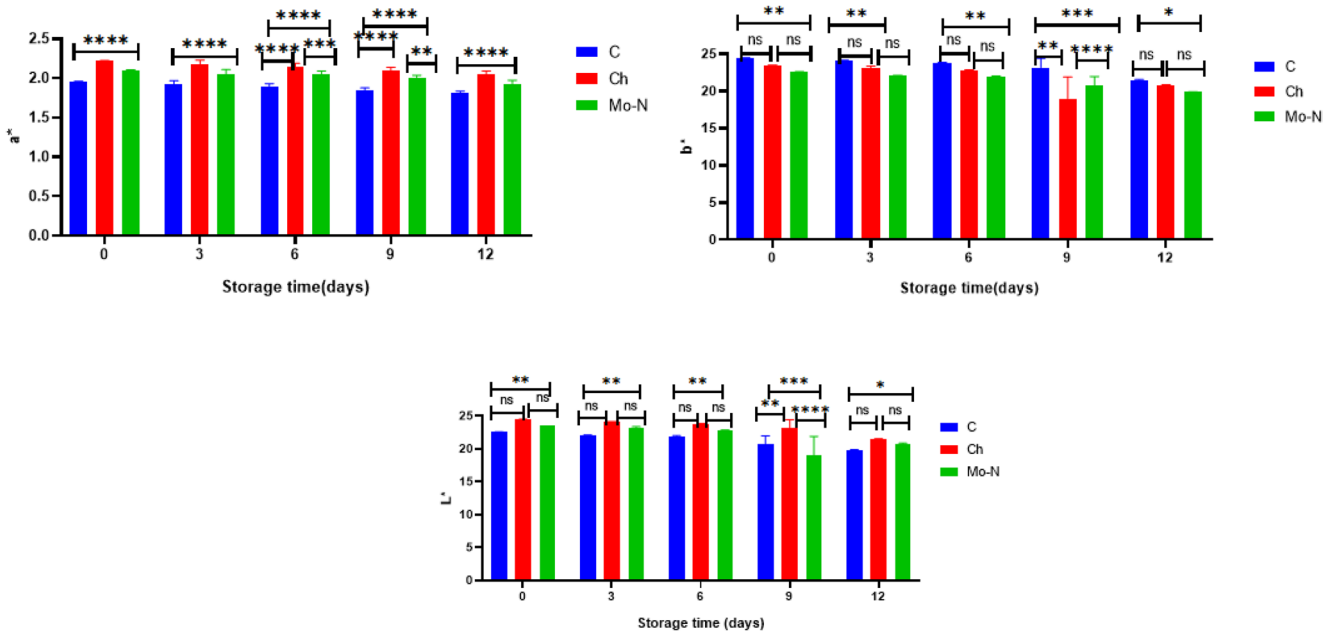

Fig. 8 Changes in color score of fish fillet under refrigerated condition (4 ±1°C) (days 0–12).

transport of bioactive compounds in the food industry. This innovative approach supports food sustainability and offers practical benefits for the seafood industry and consumers. Moreover, these findings highlight the importance of circular and blue economy principles in seafood preservation using innovative coatings. Investigating consumer preferences and acceptability will be crucial to ensure that Mo-N does not compromise the sensory qualities of fish fillets. Future studies should evaluate the effect of Mo-N on aroma and flavor profiles of different processed/semi-processed fish species.

Author contributions Gonca Alak: Conceptualization; Investigation; Writing - original draft; Validation; Methodology; Visualization; Writing - review & editing; Project administration; Supervision; Resources; Formal analysis. Ayşe Kara: Conceptualization; Investigation; Validation; Methodology; Formal analysis; Project administration; Resources. Muhammed Alaeddin Ekiz: Analyzed and interpreted the data, Investigation; Resources; Formal analysis. Evren Toraman, Onur Güçlüdağ, Elif Zeynep Özçelik, Koray Koşar, Tuba Akbey: Analyzed and interpreted the data. Muhammed Atamanalp: Writing - review & editing.

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Data Availability The datasets generated during the current study are available from the corresponding author on request.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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