IJVR
ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol.25 No.4 Ser. No.89 2024

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Enhancing the shelf life of rainbow trout fillets using edible sodium caseinate coating with *Oliveria decumbens* essential oil nanoemulsion, vitamin E and sodium dodecyl sulfate

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10.22099/ijvr.2024.48884.7185

(Received 31 Dec 2023; revised version 10 Dec 2024; accepted 28 Dec 2024)

Abstract

Background: The use of essential oil nanoemulsions has emerged as a promising strategy for extending the shelf life of highly perishable foods, like fish, by leveraging their natural antimicrobial and antioxidant properties. **Aims:** This study investigated the effects of an edible sodium caseinate coating incorporating *Oliveria decumbens* essential oil (*Od*-EO) and its nanoemulsion (*Od*-NEO), along with vitamin E and sodium dodecyl sulfate (SDS), on the shelf life of rainbow trout fillets during refrigeration. **Methods:** Fish samples were coated by immersion in pure sodium caseinate (SC), SC+*Od*-EO, and SC+*Od*-NEO. Sensory (odor, color, texture, overall acceptance), chemical (pH, total volatile basic nitrogen [TVB-N], thiobarbituric acid [TBA], and peroxide value [PV]), and microbiological analyses (total mesophilic aerobic bacteria, psychrophilic anaerobic bacteria, and *Pseudomonas* spp. counts) were conducted over 15 days of storage at 4°C. **Results:** The study results indicated that fillets treated with SC+*Od*-NEO exhibited significantly higher quality compared with other treatments. Additionally, the combination of nanoemulsion, vitamin E, and SDS showed synergistic effects in reducing microbial growth, delaying lipid oxidation, and improving organoleptic quality, extending the shelf life of rainbow trout fillets up to 15 days. Overall, the correlation analysis highlights the interdependence of microbial, chemical, and sensory parameters and reinforces the role of SC-NEO-based treatments in mitigating spoilage and maintaining the sensory quality of fish fillets during storage. **Conclusion:** The results highlight the potential of nanoemulsion-based coatings as an innovative and effective strategy for extending the shelf life of highly perishable fish products. Coting with SC+*Od*-NEO+Vit E+SDS can be recommended as an effective food preservative.

Key words: Coating, Nanoemulsion, *Oliveria decumbens*, SDS, Shelf life extension

Introduction

Essential oils (EOs) are natural bioactive compounds extracted from various plant parts, including flowers, buds, seeds, leaves, bark, wood, fruits, and roots (Jayasena and Jo, 2013). The application of EOs in the food industry has gained significant attention due to their natural bioactive properties, including antioxidant, antimicrobial, antiviral, and antifungal activities. These attributes make EOs valuable not only in food preservation but also in enhancing the overall quality of food products. However, their direct use is often limited by challenges such as poor water solubility, chemical instability under environmental stress (e.g., heat, light, and oxygen), intense aroma, high volatility, and potential toxicity at high concentrations (Burt, 2004; Shahrampour and Razavi, 2023). Furthermore, the unpleasant organoleptic effects of free EOs can significantly affect consumer acceptance of the final product (Li *et al*., 2015).

To address these limitations, nanotechnology has emerged as a transformative solution. Nanoencapsulation techniques, such as nanoemulsions, micelles, and liposomes, have shown great potential in improving the functional properties of EOs by enhancing their solubility, stability, and controlled release. Nanoemulsions, in particular, have gained attention as efficient delivery systems due to their unique physicochemical properties. These systems, characterized by droplet sizes ranging from 10-200 nm, offer enhanced transparency, physical stability, and reduced impact on food taste and aroma (Rao and McClements, 2011).

In addition to addressing stability and sensory

challenges, nanoemulsions significantly boost the antimicrobial efficacy of EOs. This enhancement is attributed to the increased surface area of the nano-sized droplets and their ability to penetrate microbial phospholipid membranes more effectively. The antimicrobial activity of these nanoemulsions is highly dependent on their method of preparation, which affects factors such as droplet size, charge, and overall distribution (Shahbazi *et al*., 2017). Moreover, nanocarriers can facilitate targeted delivery, minimize toxicity, and prolong the release of EOs, thereby reducing the concentration required for effective preservation.

Oliveria decumbens, a medicinal plant from the Umbelliferae family, is native to the warm regions of Southern and Western Iran. Renowned for its traditional applications in treating abdominal pain, dyspepsia, diarrhea, and fever, it is also valued for its anti-inflammatory, antioxidant, and antimicrobial properties (Amin *et al*., 2005). The essential oil derived from *O. decumbens* (*Od*-EO) has been applied in the food industry as a natural flavoring and preservative (Esmaeili *et al*., 2018). While studies have demonstrated the antimicrobial (Behbahani *et al*., 2018), antioxidant (Karami *et al*., 2020), and wound-healing properties (Amin *et al*., 2022) of *Od*-EO, research on its nanoemulsion form (*Od*-NEO) remains scarce.

Bioactive edible coatings, formulated from natural biopolymers such as proteins, polysaccharides, and lipids, serve as protective barriers against external factors like oxygen, moisture, and aroma, thereby enhancing food quality and extending shelf life (Homayonpour *et al*., 2021). Sodium caseinate (NaCaS), a widely available, water-soluble biopolymer, is particularly effective in preserving food quality due to its transparency, protective properties, and high nutritional value. It is frequently employed in extending the shelf life of various meat products by reducing spoilage during storage (Umaraw and Verma, 2017).

Sodium dodecyl sulfate (SDS), an anionic surfactant with potent antimicrobial activity, has gained prominence as a safe and effective agent for disrupting bacterial cell membranes (Maktabi *et al*., 2018). Research highlights its applicability for surface disinfection of diverse food products, with notable efficacy against various foodborne pathogens (Rigotti *et al*., 2017; Maktabi *et al*., 2024; Olivera and Gomes, 2024). SDS is considered non-hazardous and safe for human consumption (Morales-delaNuez *et al*., 2011).

Vitamin E (Vit. E), a fat-soluble antioxidant, plays a critical role in delaying lipid oxidation and preventing chemical spoilage during storage, making it a valuable additive in food preservation (Avila-Ramos *et al*., 2012).

Rainbow trout (*Oncorhynchus mykiss*), a member of the Salmonidae family, holds significant economic and nutritional importance as a cold-water aquaculture species. In Iran, rainbow trout production and consumption have risen sharply in recent years (Kalbassi *et al*., 2013). With increasing demand for innovative and sustainable preservation methods, the use of biodegradable films and coatings enriched with natural antimicrobials and antioxidants, such as EOs, has gained traction (Ozogul *et al*., 2020).

The use of essential oil nanoemulsions has emerged as a promising strategy for extending the shelf life of highly perishable foods, such as fish, by leveraging their natural antimicrobial and antioxidant properties. Fresh fish is prone to rapid spoilage due to microbial growth, lipid oxidation, and enzymatic activities, which not only reduce its quality but also pose health risks. Incorporating EOs into nanoemulsion systems enhances their efficacy by increasing their water solubility, stability, and bioavailability, while minimizing their strong aroma and potential toxicity at high concentrations. The nano-sized droplets provide a larger surface area for interaction with microbial cells, allowing for better penetration and disruption of their membranes, thereby inhibiting spoilage microorganisms. Furthermore, the antioxidant properties of nanoemulsions help prevent lipid oxidation, maintaining the sensory qualities and nutritional value of fish during storage. Unlike traditional preservation methods, EO nanoemulsions are a natural and sustainable alternative that meets consumer demands for clean-label products. Their ability to provide controlled and prolonged release of bioactive compounds ensures effective preservation over extended periods, making them an innovative and practical solution for maintaining the freshness and safety of fish and other perishable foods. Several studies have demonstrated the efficacy of incorporating EO nanoemulsions into edible coatings to enhance the shelf life of seafood products (Shokri *et al*., 2020; Homayonpour *et al*., 2021; Jahangiri *et al*., 2022).

This study aims to: (a) evaluate the effects of sodium caseinate-based edible coatings containing *Od*-EO or *Od*-NEO on the quality of rainbow trout fillets during refrigerated storage, and (b) assess the synergistic impact of SDS and Vit. E in combination with *Od*-NEO on extending the shelf life of trout fillets.

Materials and Methods

Preparation and characterization of *Od*-EO and *Od*-NEO

The essential oil of *O. decumbens* was extracted using a Clevenger-type apparatus following the European Pharmacopoeia guidelines (Singh *et al*., 2008). The composition of the essential oil was analyzed using Gas Chromatography/Mass Spectrometry (GC/MS) (Agilent 5977B, USA) as described by Adams (2017). *Od*-NEO was prepared by emulsifying *Od*-EO (2% v/v) with Tween 80 (30% EO weight) in distilled water using an ultrasonic device (200 W, 20 kHz; Hielscher, Germany) according to Noori *et al*. (2018). The physicochemical properties of *Od*-NEO, including nano droplet size, zeta potential, and polydispersity index (PDI), were determined using dynamic light scattering (DLS) with a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) as described by Shafiq *et al*. (2007).

Preparation of coating solutions

Sodium caseinate (4 g) was dissolved in 100 ml of sterile distilled water and stirred at 1100 rpm at 30°C for 3 h. Glycerol monostearate (1.2 g) was added as a plasticizer to enhance the strength and flexibility of the solution (Noori *et al*., 2018). *Od*-EO or *Od*-NEO was then added separately with continuous stirring to obtain a final concentration of 2% (v/v). In the second phase, for the combination treatments, vitamin E (1%) and SDS (1%) were added to the SC-*Od*-NEO coating solution, which had shown the best results in preliminary tests.

Preparation and coating of fish fillets

Fresh rainbow trout samples were purchased from a local market in Ahvaz, Khuzestan Province, and immediately transported to the laboratory under refrigeration. The fish were gutted, filleted, washed with tap water, and randomly divided into four groups for the first phase of the experiment. The fillets were coated by immersion in drinking water as a control (C), pure sodium caseinate (SC), sodium caseinate + 2% *Od*-EO (SC-EO 2%), and sodium caseinate + 2% *Od*-NEO (SC-NEO 2%) for 2 min at 25°C. The coated fillets were drained, individually packaged in lidded polyester (PS) trays, and stored at 4°C for 15 days. Analyses were conducted at 0, 3, 6, 9, 12, and 15 days of storage. In the second phase, fillets were treated with either control (C), sodium caseinate + 2% *Od*-NEO (SC-NEO 2%), or SC-NEO + SDS + Vit. E coating solution, and stored and sampled as described above. All experiments were performed in triplicate for greater accuracy.

Microbiological analysis

Ten g of fish fillets were aseptically transferred to sterile bags containing 90 ml of 0.1% peptone water, and homogenized in a stomacher (Lab Blender 400, Interscience, France) for 60 s. Serial dilutions (1:10) were prepared, and 0.1 ml of each dilution was cultured on appropriate media. Mesophilic and psychrotrophic bacteria were enumerated after incubation on plate count agar (Merck, Germany) for 48 h at 37°C and 7 days at 7°C, respectively. *Pseudomonas* spp. were enumerated using pseudomonas CFC selective agar (Merck, Darmstadt, Germany) after 48 h of incubation at 30°C. Microbial counts were expressed as log CFU/ml (Ojagh *et al*., 2010).

Chemical analysis

pH measurement

Fish samples (10 g) were homogenized in 90 ml of distilled water. The pH of the filtered homogenate was measured using a pH meter (AZ-86555, Taiwan) (Fan *et al*., 2009).

Total volatile base nitrogen (TVB-N) determination

The TVB-N content was determined using the microdiffusion assay as described by Raeisi *et al*. (2016). In brief, 2 g of MgO and 2-3 drops of silicone were added to a mixture of 5 g of fish sample and 60 ml of distilled water, then transferred to tubes for distillation using a Kjeldahl apparatus. The distillate was absorbed in 40 ml of boric acid solution containing a mixed indicator (0.1 g methyl red and 0.1 g methyl blue dissolved in 100 ml absolute ethanol). The boric acid solution was titrated with 0.1 N hydrochloric acid (HCl). The TVB-N content was calculated using the following equation:

%mg TVB-N = (V*C*14*100)/5

Where,
V: The volume of HCl used
C: Its concentration

Peroxide value (PV) determination

The peroxide value (PV) was determined according to the AOCS method Cd 8b-90, as meq of peroxide oxygen per kg of fat (Brühl, 1997). Lipids were extracted from fish fillets using the Bligh and Dyer (1959) method. One g of extracted fish oil was mixed with 25 ml of chloroform-acetic acid solution (2:3 v/v), followed by the addition of 1 ml of saturated potassium iodide solution. The mixture was kept in the dark for 5 min, and then 30 ml of distilled water was added and shaken. The liberated iodine was titrated with 0.01 N sodium thiosulfate using starch as an indicator. PV was calculated using the equation:

PV (meq/kg) = [(a-b)*t/w]*1000

Where,
a: The volumes (ml) of sodium thiosulfate used for the sample
b: The volumes (ml) of sodium thiosulfate used for the blank
t: The concentration (M) of sodium thiosulfate
w: The weight (g) of the sample

Thiobarbituric acid reactive substances (TBARS) determination

The TBARS value was measured as an indicator of lipid oxidation using the colorimetric assay described by Cheng *et al*. (2014). Five g of each sample were homogenized with 25 ml of 20% trichloroacetic acid and 20 ml of distilled water, then centrifuged at 8,000 rpm for 10 min. Three ml of the supernatant were mixed with 3 ml of thiobarbituric acid reagent (0.02 M TBA in 90% acetic acid) in a glass tube. The tube was heated in a water bath at 90°C for 1 h, and then cooled. The absorbance (As) of the sample and the absorbance (Ab) of the reagent were measured at 532 nm using a spectrophotometer, with distilled water as the blank. The TBARS value was expressed as mg of malondialdehyde (MDA) per kg of fish sample, using the following equation:

TBA= [(As-Ab)*50]/200

Sensory analysis

Sensory evaluation was conducted by a panel of 10 trained sensory assessors (6 females and 4 males), aged 25-35, all of whom were postgraduate students in food science with prior experience in sensory analysis. Before the evaluation, the panelists underwent a preliminary training session to familiarize themselves with the

evaluation criteria and scale interpretation. They evaluated attributes such as odor, color, texture, and overall acceptability using a 5-point hedonic scale. The scale rated:
Color: 5 = no discoloration, 1 = extreme discoloration
Odor: 5 = extremely desirable, 1 = extremely unacceptable/off-odors
Texture: 5 = firm, 1 = very soft
Overall acceptability: Calculated as the average of color, odor, and texture scores, with equal weighting

Assessments were conducted under controlled environmental conditions (20 ± 2°C, 60% relative humidity) in individual sensory booths to minimize bias. Samples scoring below 3 on the hedonic scale were considered unacceptable for consumption. The sensory analysis methodology followed the recommendations of Lawless and Heymann (2010).

Statistical analysis

All experiments, including sensory evaluations, were conducted in triplicate. Data were analyzed using SPSS version 19 software, with statistical significance set at P<0.05. Quantitative data were analyzed using analysis of variance (ANOVA) with repeated measurements, followed by the LSD post-hoc test. Sensory evaluation data, being non-parametric, were analyzed using the Kruskal-Wallis test for comparison across groups and the Friedman test for evaluating the changes in sensory attributes over time.

Results

Characterization of *Od*-EO and *Od*-NEO

The results obtained from GC-MS analysis showed that the main components of *Od*-EO were thymol (53.4%), γ-terpinene (20.48%), p-cymene (18.02%), and myristicin (2.7%). After the formulation of *Od*-NEO, the average size of the nanoemulsion, its zeta potential, and PDI were 45.71 nm, -36.3 mV, and 0.42, respectively. The nanoemulsion also exhibited good stability for at least 15 days. The full characterization of *Od*-EO and *Od*-NEO has been published by our team (Nikravan *et al*., 2020).

Fish fillet quality

Microbial analysis

Table 1 presents the total viable counts (TVC), psychrotrophic counts (PTC), and *Pseudomonas* spp. viable count changes in the different groups during storage at the first stage. Overall, all groups showed a rising trend in TVC, PTC, and PsC during the storage period, with the control group showing a quicker pattern compared with other groups, and the lowest counts were observed for the *Od*-NEO group (P<0.05). The results of the second stage are presented in Figs. 1a-c. The SC-NEO + SDS + Vit. E treatment had a significant effect on the reduction of bacterial count during storage compared with the *Od*-NEO group (P<0.05).

Chemical analysis

***Measurement of pH*:** Table 2 and Fig. 2a show the pH changes in the first and second stages of trout fillet during 15 days of storage. On day 0, pH values were not significantly different among the experimental groups (P>0.05). On the 15th day of storage, the lowest pH was observed in the SC-NEO group (6.56 ± 0.1) (P<0.05), and the highest pH was observed in the control group (7.33 ± 0.11) (P<0.05). In the second stage, no significant difference was observed between the SC-NEO and SC-NEO + SDS + Vit. E treatments (P>0.05).

***TVB-N*:** The changes in TVB-N in the first and second stages are outlined in Table 2 and Fig. 2b. TVB-N content increased over the storage period in all groups, with the greatest increase observed in the control group (P<0.05). The initial amount of TVB-N in the control was 9.89 ± 1.15 mg N/100 g, with no significant difference (P>0.05) among the different groups at the beginning of storage. For the SC, SC-EO, and SC-NEO samples, a slower increase was observed. In this regard, there was no significant difference between the SC-NEO and SC-NEO + SDS + Vit. E-treated samples throughout the entire storage period (P>0.05) (Fig. 2b).

Table 1: Changes in TVC, PTC, and PsC of rainbow trout stored for 15 days

Parameter	Groups	Storage time (days)					
		0	3	6	9	12	15
TVC	C	3.97 ± 0.69^{aC}	5.66 ± 0.58^{aBC}	7.21 ± 0.42^{aAB}	8.03 ± 0.69^{aAB}	8.89 ± 0.61^{aA}	9.82 ± 0.71^{aA}
	SC	3.64 ± 0.40^{aBC}	5.13 ± 0.30^{aB}	7.12 ± 0.50^{aAB}	7.84 ± 0.57^{aA}	8.62 ± 0.51^{abA}	9.32 ± 0.38^{abA}
	SC-EO	3.63 ± 0.47^{aB}	4.6 ± 0.55^{aB}	6.5 ± 0.51^{abA}	6.63 ± 0.62^{abA}	7.35 ± 0.6^{bcA}	8.06 ± 0.56^{bcA}
	SC-NEO	3.48 ± 0.52^{aA}	3.94 ± 0.80^{aA}	5.61 ± 0.44^{bA}	5.99 ± 0.75^{bA}	6.84 ± 0.69^{cA}	7.48 ± 0.61^{cA}
PTC	C	4.35 ± 0.37^{aD}	5.57 ± 0.45^{aD}	7.26 ± 0.46^{aC}	7.84 ± 0.77^{aBC}	9.28 ± 0.46^{aAB}	10.14 ± 0.34^{aA}
	SC	4.27 ± 0.34^{aD}	5.28 ± 0.39^{aC}	7.22 ± 0.81^{aB}	7.77 ± 0.67^{aB}	9.22 ± 0.68^{aAB}	9.88 ± 0.25^{abA}
	SC-EO	4.02 ± 0.13^{aD}	4.9 ± 0.15^{aD}	6.52 ± 0.44^{aC}	6.46 ± 0.41^{aBC}	7.86 ± 0.23^{bAB}	8.92 ± 0.76^{bA}
	SC-NEO	4.15 ± 0.42^{aC}	4.06 ± 0.26^{bBC}	5.77 ± 0.75^{aAB}	6.26 ± 0.43^{aA}	6.97 ± 0.04^{bA}	7.62 ± 0.36^{cA}
PsC	C	3.49 ± 0.62^{aD}	4.94 ± 0.55^{aC}	6.54 ± 0.42^{aB}	7.37 ± 0.34^{aAB}	8.55 ± 0.44^{aA}	9.33 ± 0.44^{aA}
	SC	3.39 ± 0.28^{aE}	4.87 ± 0.14^{abD}	6.55 ± 0.43^{aC}	6.96 ± 0.12^{aBC}	8.32 ± 0.58^{aAB}	9.11 ± 0.54^{aA}
	SC-EO	2.94 ± 0.23^{aD}	3.73 ± 0.76^{bcCD}	5.85 ± 0.77^{aBC}	6.1 ± 0.48^{aABC}	7.05 ± 0.18^{bAB}	7.82 ± 0.66^{bA}
	SC-NEO	3.02 ± 0.17^{aC}	3.43 ± 0.39^{cBC}	5.19 ± 0.48^{aAB}	5.96 ± 0.75^{aAB}	6.55 ± 0.47^{bA}	7.36 ± 0.50^{bA}

Mean values of three replicate ± the standard deviation of the mean. This means that sharing the same letter in the same row (A-E) and column (a-d) are not significantly different (P>0.05). TVC: Total viable counts, PTC: Psychrotrophic counts, PsC: *Pseudomonas* spp. viable count, C: Control, SC: Sodium caseinate, EO: Essential oil, and NEO: Nanoemulsion essential oil

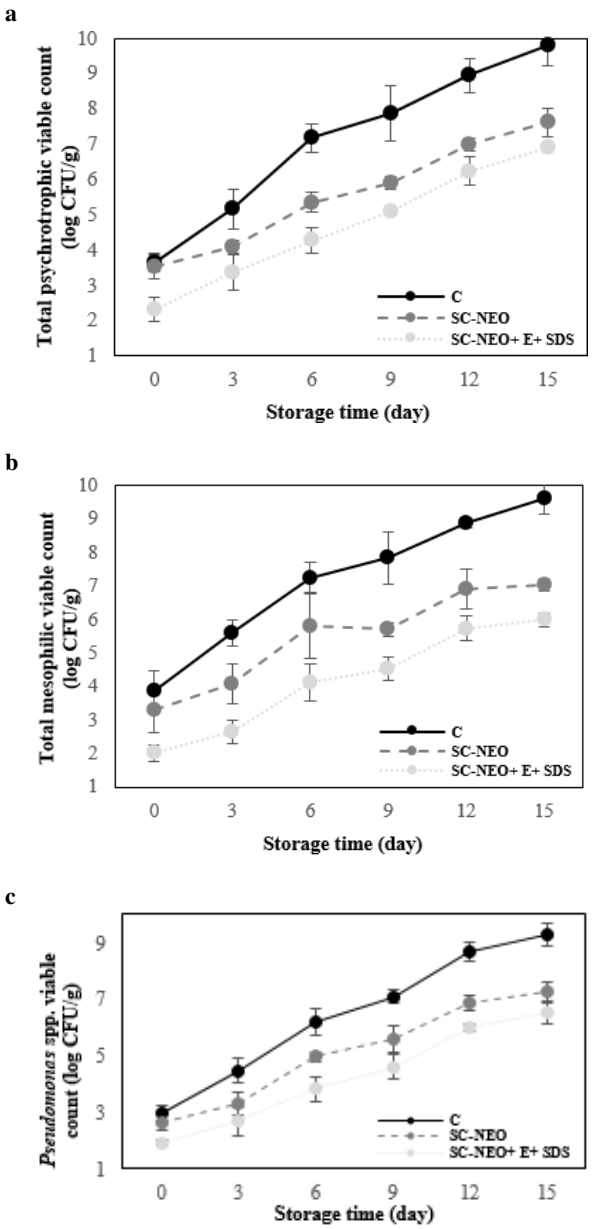
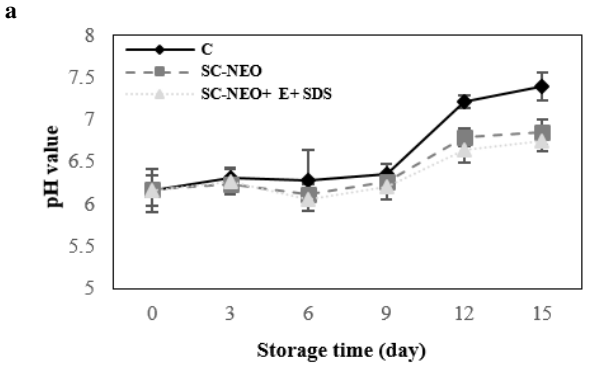


Fig. 1: The effect of different coating treatments on total psychotropic viable counts (**a**), total mesophilic counts (**b**), and *Pseudomonas* spp. counts (**c**) of rainbow trout fillets during storage at 4°C. C: Control, SC: Sodium caseinate, NEO: Nanoemulsion essential oil, E: Vitamin E, and SDS: Sodium dodecyl sulfate



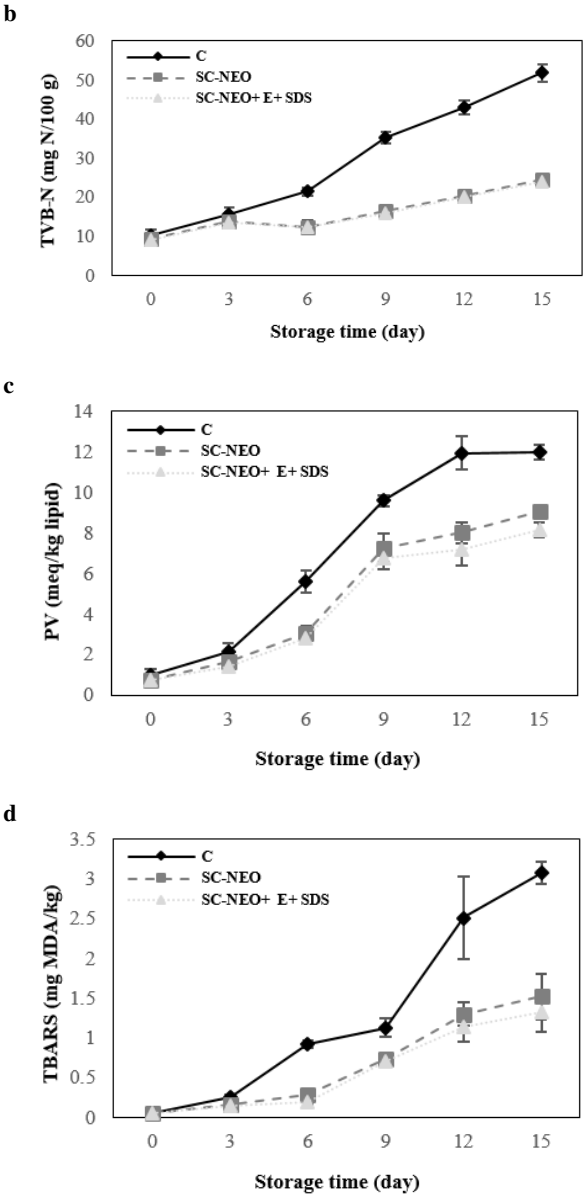


Fig. 2: The effect of different coating treatments on value changes of pH (**a**), TVB-N (**b**), PV (**c**), and TBARS (**d**) in rainbow trout fillets during storage at 4°C. C: Control, SC: Sodium caseinate, NEO: Nanoemulsion essential oil, E: Vitamin E, and SDS: Sodium dodecyl sulfate

PV: Table 2 and Fig. 2c show the PV levels during the storage of fish fillets over a 15-day period. The initial PV level in the fresh fillets was 1.03 ± 0.2 meq/kg. PV measurement results indicated that the coating had no significant effect on PV reduction during storage, except on days 6 and 9. As shown in Table 2, in the control and SC samples, PV showed an increasing trend from the beginning of the storage period to the 12th day of the experiment, followed by a decrease on the 15th day. In contrast, the SC-EO and SC-NEO treatments showed an increasing trend in PV until the end of the storage period ($P<0.05$). Although the difference between SC-NEO + SDS + Vit. E and SC-NEO samples was not significant, this trend held except for the last day of storage (Fig. 2c).
***TBARS*:** Changes in TBARS values are shown in Table

2 and Fig. 2d. At the beginning of the experiment, the initial TBARS level in the fresh fillets was 0.06 ± 0.01 mg MDA/kg, which increased to a maximum level of 3.56 ± 0.13 mg MDA/kg for the control group. Significant differences (P<0.05) were observed in TBARS values between the control and treatment groups during the storage period, as shown in Table 2. The TBARS value of SC-NEO + SDS + Vit. E was almost equal to that of the SC-NEO sample, with no significant difference between them (P>0.05) (Fig. 2d).

Sensory analysis

The results of the sensory analysis of rainbow trout treated throughout the storage period at stages 1 and 2 are shown in Table 3 and Figs. 3a-d, respectively. As can be seen, the control group scored lower and was less acceptable, but there was no significant difference (P>0.05) between the other samples until day 6. The best score during this period at the first stage was received by the SC-NEO, which remained above the acceptable limit (a score of 3) until the 12th day. Additionally, when SDS + Vit. E was used in the formulation of the SC-NEO coating in the second stage, the scores were higher than those of the other groups, and it was able to receive an acceptable sensory score by the 15th day of the experiment.

Table 2: Changes in pH, TVB-N (mg TVB-N 100 g-1), PV (meq O2 kg-1), and TBA (mg MA kg-1) content of rainbow trout stored for 15 days

Parameter	Groups	Storage time (days)					
		0	3	6	9	12	15
pH	C	6.40 ± 0.10^{aC}	6.31 ± 0.10^{aC}	6.49 ± 0.09^{aC}	6.71 ± 0.14^{aB}	7.18 ± 0.10^{aAB}	7.33 ± 0.11^{aA}
	SC	6.42 ± 0.12^{aB}	6.28 ± 0.09^{aB}	6.41 ± 0.02^{aB}	6.52 ± 0.10^{abB}	6.69 ± 0.01^{bB}	7.02 ± 0.02^{bA}
	SC-EO	6.37 ± 0.09^{aA}	6.24 ± 0.08^{aA}	6.31 ± 0.09^{abA}	6.44 ± 0.08^{bA}	6.60 ± 0.05^{bA}	6.79 ± 0.10^{cA}
	SC-NEO	6.36 ± 0.14^{aB}	6.13 ± 0.13^{aB}	6.23 ± 0.07^{bB}	6.31 ± 0.09^{bB}	6.46 ± 0.10^{bAB}	6.56 ± 0.10^{dA}
TVB-N	C	9.89 ± 1.15^{aF}	17.08 ± 0.68^{aE}	22.68 ± 0.68^{aD}	34.53 ± 2.67^{aC}	43.30 ± 3.21^{aB}	53.01 ± 2.34^{aA}
	SC	9.80 ± 0.68^{aD}	14.93 ± 1.08^{aD}	18.57 ± 1.39^{bC}	25.20 ± 0.45^{bB}	37.70 ± 4.57^{bA}	44.33 ± 0.80^{bA}
	SC-EO	9.14 ± 0.57^{aD}	11.38 ± 1.39^{bD}	14.84 ± 1.27^{cC}	19.22 ± 0.57^{cB}	24.26 ± 0.34^{cAB}	33.88 ± 5.14^{cA}
	SC-NEO	9.86 ± 1.03^{aE}	10.64 ± 0.60^{bDE}	12.13 ± 0.80^{dD}	17.82 ± 1.17^{cC}	20.90 ± 0.80^{cB}	24.73 ± 0.69^{dA}
PV	C	1.03 ± 0.20^{aD}	2.33 ± 0.32^{aD}	5.83 ± 0.53^{aC}	10.3 ± 0.86^{aB}	12.00 ± 1.68^{aAB}	11.53 ± 0.91^{aA}
	SC	1.00 ± 0.35^{aC}	2.00 ± 0.61^{aC}	3.93 ± 0.65^{bB}	9.73 ± 0.57^{aA}	11.10 ± 1.23^{aA}	10.93 ± 0.83^{aA}
	SC-EO	0.86 ± 0.24^{aD}	1.80 ± 0.53^{aCD}	3.43 ± 0.57^{bC}	9.03 ± 0.94^{abB}	10.20 ± 0.96^{aA}	10.26 ± 0.71^{aA}
	SC-NEO	0.83 ± 0.33^{aE}	1.46 ± 0.59^{aDE}	3.36 ± 0.49^{bD}	7.33 ± 0.93^{bC}	7.96 ± 0.09^{aB}	8.93 ± 0.09^{aA}
TBA	C	0.06 ± 0.01^{bE}	0.28 ± 0.03^{aD}	0.92 ± 0.06^{aC}	1.19 ± 0.08^{aB}	2.97 ± 0.27^{aA}	3.56 ± 0.13^{aA}
	SC	0.08 ± 0.01^{aE}	0.23 ± 0.02^{abD}	0.69 ± 0.13^{bC}	1.05 ± 0.13^{abB}	2.85 ± 0.24^{aA}	3.05 ± 0.09^{bA}
	SC-EO	0.05 ± 0.00^{bF}	0.20 ± 0.02^{bE}	0.35 ± 0.04^{cD}	0.84 ± 0.05^{bcC}	2.14 ± 0.27^{bB}	2.68 ± 0.13^{cA}
	SC-NEO	0.05 ± 0.00^{bD}	0.18 ± 0.01^{bC}	0.24 ± 0.05^{cC}	0.72 ± 0.06^{cB}	1.35 ± 0.18^{cA}	1.24 ± 0.19^{dA}

Mean values of three replicate ± the standard deviation of the mean. This means that sharing the same letter in the same row (A-E) and column (a-d) are not significantly different (P>0.05). C: Control, SC: Sodium caseinate, EO: Essential oil, and NEO: Nanoemulsion essential oil

Table 3: Sensory analyses of rainbow trout stored for 15 days

Parameter	Groups	Storage time (days)					
		0	3	6	9	12	15
Odor	C	4.86 ± 0.18^{aA}	3.66 ± 0.24^{aB}	2.93 ± 0.24^{bBC}	2.33 ± 0.24^{bCD}	1.80 ± 0.32^{cDE}	1.26 ± 0.24^{bE}
	SC	4.73 ± 0.09^{aA}	4.00 ± 0.16^{aAB}	3.73 ± 0.24^{abB}	3.33 ± 0.24^{aB}	2.33 ± 0.24^{bcC}	1.80 ± 0.16^{abC}
	SC-EO	4.6 ± 0.16^{aA}	4.46 ± 0.33^{aA}	3.93 ± 0.33^{aAB}	3.46 ± 0.09^{aBC}	2.80 ± 0.43^{abCD}	2.06 ± 0.33^{abD}
	SC-NEO	4.73 ± 0.09^{aA}	4.40 ± 0.16^{aAB}	4.13 ± 0.24^{aABC}	3.80 ± 0.16^{aBC}	3.53 ± 0.09^{aC}	2.40 ± 0.43^{aD}
Color	C	4.93 ± 0.09^{aA}	3.86 ± 0.24^{aB}	3.4 ± 0.32^{bBC}	2.53 ± 0.24^{cC}	1.86 ± 0.09^{bCD}	1.20 ± 0.16^{cD}
	SC	4.53 ± 0.09^{aA}	4.40 ± 0.16^{aA}	3.66 ± 0.33^{abAB}	3.06 ± 0.33^{bBC}	2.33 ± 0.41^{abC}	2.00 ± 0.48^{bcD}
	SC-EO	4.80 ± 0.16^{aA}	4.46 ± 0.33^{aAB}	3.86 ± 0.49^{abBC}	3.46 ± 0.18^{abCD}	2.60 ± 0.32^{abDE}	2.46 ± 0.49^{abE}
	SC-NEO	4.86 ± 0.09^{aA}	4.53 ± 0.24^{aA}	4.33 ± 0.24^{aA}	4.00 ± 0.16^{aAB}	3.13 ± 0.24^{aBC}	3.00 ± 0.28^{aC}
Texture	C	4.80 ± 0.16^{aA}	4.00 ± 0.16^{aA}	3.00 ± 0.43^{bB}	2.20 ± 0.32^{bBC}	1.66 ± 0.41^{bC}	1.00 ± 0.00^{bD}
	SC	4.73 ± 0.09^{aA}	4.26 ± 0.33^{aAB}	3.6 ± 0.58^{abB}	2.80 ± 0.28^{abBC}	2.13 ± 0.24^{abC}	1.06 ± 0.09^{bD}
	SC-EO	4.53 ± 0.18^{aA}	4.06 ± 0.24^{aA}	3.73 ± 0.41^{abAB}	3.00 ± 0.43^{abB}	2.46 ± 0.09^{abC}	1.60 ± 0.16^{abC}
	SC-NEO	4.66 ± 0.24^{aA}	4.13 ± 0.49^{aAB}	4.00 ± 0.32^{aAB}	3.33 ± 0.24^{aB}	3.00 ± 0.16^{aC}	2.00 ± 0.16^{aD}
Overall conception	C	5.00 ± 0.00^{aA}	3.20 ± 0.43^{cB}	3.06 ± 0.09^{bB}	1.46 ± 0.24^{cC}	1.00 ± 0.00^{cC}	1.00 ± 0.00^{bC}
	SC	5.00 ± 0.00^{aA}	4.00 ± 0.32^{bB}	3.53 ± 0.09^{abB}	2.46 ± 0.41^{bC}	1.20 ± 0.16^{bcD}	1.06 ± 0.09^{bD}
	SC-EO	5.00 ± 0.00^{aA}	4.53 ± 0.24^{abA}	3.73 ± 0.49^{aB}	3.00 ± 0.16^{abC}	1.53 ± 0.24^{bcD}	1.26 ± 0.24^{abD}
	SC-NEO	5.00 ± 0.00^{aA}	4.73 ± 0.09^{aA}	3.86 ± 0.61^{aB}	3.20 ± 0.43^{aC}	3.00 ± 0.16^{aC}	1.73 ± 0.24^{aD}

Mean values of three replicate ± the standard deviation of the mean. This means that sharing the same letter in the same row (A-E) and column (a-d) are not significantly different (P>0.05). C: Control, SC: Sodium caseinate, EO: Essential oil, and NEO: Nanoemulsion essential oil

Table 4: Correlation coefficients between quality parameters

Parameter	TVC	PTC	PsC	pH	TVB-N	PV	TBARS	Odor	Color	Texture	Overall conception
TVC	1	0.991	0.980	0.957	0.993	0.869	0.990	-0.975	-0.978	-0.971	-0.988
PTC	0.991	1	0.989	0.952	0.990	0.860	0.988	-0.969	-0.970	-0.967	-0.984
PsC	0.980	0.989	1	0.947	0.982	0.850	0.982	-0.960	-0.963	-0.961	-0.977
pH	0.957	0.952	0.947	1	0.945	0.819	0.945	-0.910	-0.921	-0.919	-0.936
TVB-N	0.993	0.990	0.982	0.945	1	0.867	0.996	-0.980	-0.982	-0.974	-0.992
PV	0.869	0.860	0.850	0.819	0.867	1	0.865	-0.854	-0.853	-0.850	-0.863
TBARS	0.990	0.988	0.982	0.945	0.996	0.865	1	-0.979	-0.980	-0.972	-0.991
Odor	-0.975	-0.969	-0.960	-0.910	-0.980	-0.854	-0.979	1	0.983	0.984	0.984
Color	-0.978	0.970-	0.963-	-0.921	-0.982	-0.853	-0.980	0.983	1	0.988	0.987
Texture	-0.971	-0.967	-0.961	-0.919	-0.974	-0.850	-0.972	0.984	0.988	1	0.985
Overall conception	-0.988	-0.984	-0.977	-0.936	-0.992	-0.863	-0.991	0.984	0.987	0.985	1

Values close to 1 or -1 indicate a strong correlation (positive or negative, respectively). High positive correlations exist among microbial counts (TVC, PTC, PsC), pH, TVB-N, PV, and TBARS. Sensory parameters (odor, color, texture, and overall conception) negatively correlate with microbial growth and spoilage markers, showing quality degradation over time

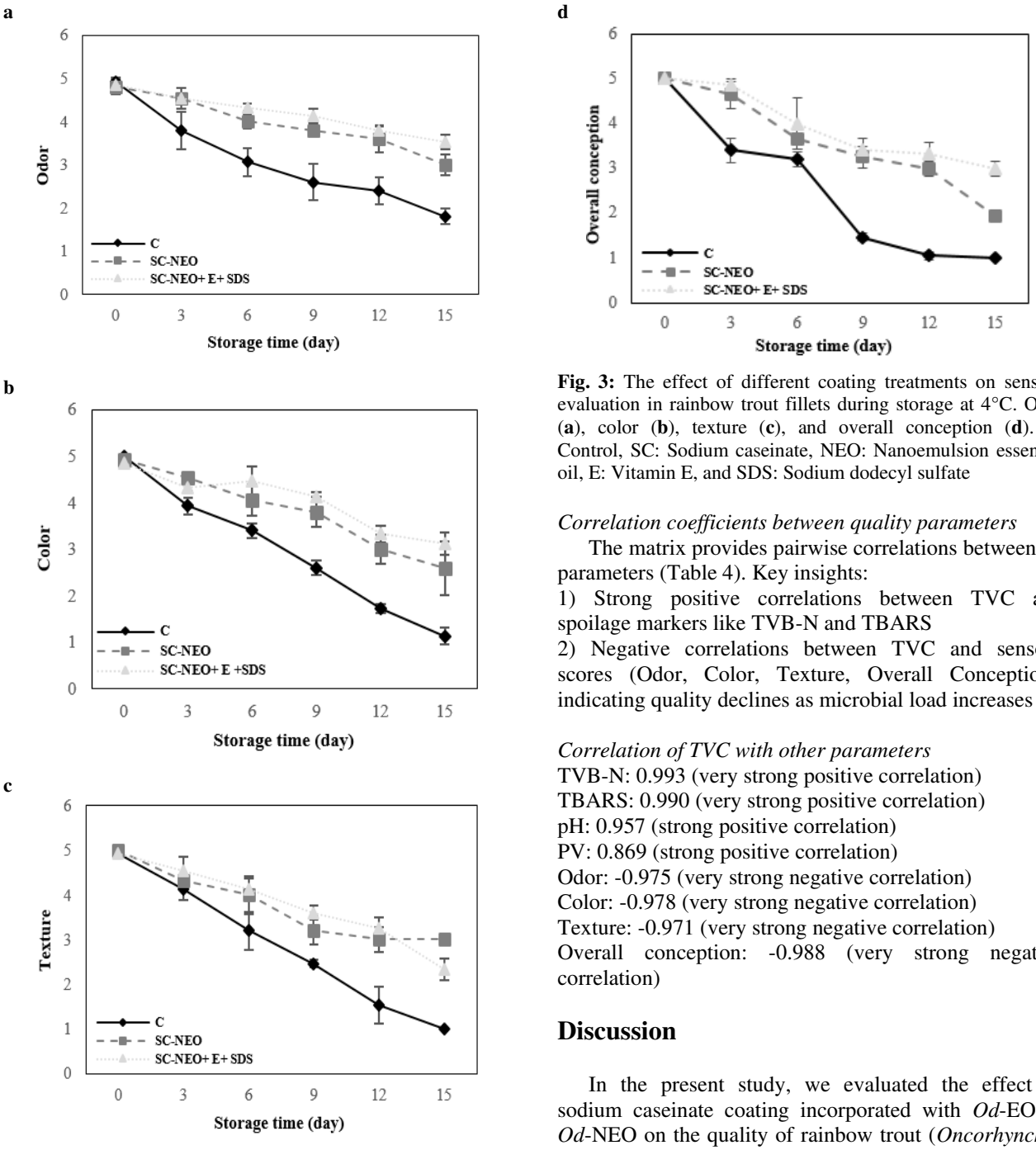


Fig. 3: The effect of different coating treatments on sensory evaluation in rainbow trout fillets during storage at 4°C. Odor (**a**), color (**b**), texture (**c**), and overall conception (**d**). C: Control, SC: Sodium caseinate, NEO: Nanoemulsion essential oil, E: Vitamin E, and SDS: Sodium dodecyl sulfate

Correlation coefficients between quality parameters

The matrix provides pairwise correlations between all parameters (Table 4). Key insights:

1) Strong positive correlations between TVC and spoilage markers like TVB-N and TBARS

2) Negative correlations between TVC and sensory scores (Odor, Color, Texture, Overall Conception), indicating quality declines as microbial load increases

Correlation of TVC with other parameters

TVB-N: 0.993 (very strong positive correlation)
TBARS: 0.990 (very strong positive correlation)
pH: 0.957 (strong positive correlation)
PV: 0.869 (strong positive correlation)
Odor: -0.975 (very strong negative correlation)
Color: -0.978 (very strong negative correlation)
Texture: -0.971 (very strong negative correlation)
Overall conception: -0.988 (very strong negative correlation)

Discussion

In the present study, we evaluated the effect of sodium caseinate coating incorporated with *Od*-EO or *Od*-NEO on the quality of rainbow trout (*Oncorhynchus*

mykiss) during refrigerated storage. The results obtained in the present study agree with the findings of Noori *et al*. (2018), who calculated the mean droplet size of ginger nanoemulsion and the polydispersity index, which were 57.4 nm and 0.22, respectively. According to other studies, differences in the mean droplet sizes of NEOs are due to the types of EOs and surfactants used to fabricate NEOs, and various techniques used to formulate NEOs (Özogul *et al*., 2022). In the present investigation, the PDI value was reported to be less than 0.5. A PDI with uniform size distributions close to zero indicates a higher level of homogeneity, while values close to one indicate greater heterogeneity (Acevedo-Fani *et al*., 2015). Zeta potential is another significant parameter that defines the electrical charge on the surface of NEO droplets (McClements and Rao, 2011). The negative charge could be attributed to the hydroxyl and carboxyl groups of fatty acids, which are released from the chemical compositions of EOs after ultrasonic emulsification and play a role in stability (Chen *et al*., 2013). *Od*-NEO was adequately stable during the storage time, and no phase separation (creaming) was observed after 15 days.

According to ICMSF, when the total bacterial count in fish fillets reaches 7-8 log CFU/g, the fillets are considered spoiled and are not recommended for consumption (Chotimarkorn, 2014). In this study, the C and SC groups exceeded the acceptable microbial level by the sixth day of storage, whereas the SC-EO group passed the threshold on day 12 (7.35 log CFU/g). The SC-NEO group exceeded the acceptable threshold (7.48 log CFU/g) on the last day of the period, but the SC-NEO + SDS + Vit. E treatment did not reach this limit over the storage time. The growth trends of psychrotrophic and *Pseudomonas* spp. bacteria were similar to those of TVC. Microbial growth was lower in the treated groups compared with the control group ($P<0.05$). Meral *et al*. (2019) demonstrated that thyme oil nanoemulsion reduced TPB growth in trout fillets from 7.93 log CFU/g to 6.42 log CFU/g, and TVC growth was successfully reduced by 28% compared with the control group sample. These results are in agreement with Shokri *et al*. (2020), who showed that an effective chitosan coating enriched with *Ferulago angulata* nanoemulsion (3%) increased the shelf life of trout samples during 16 storage days. In summary, the microbial growth of trout fillets at 4°C was decreased by the sodium caseinate coating, which indicate the effect of casein coating on contamination; however, the difference was not statistically significant ($P>0.05$). When the essential oil was added to the coating, the inhibitory effect increased, and by converting EO into NEO, the antimicrobial property increased significantly ($P<0.05$). Finally, by adding Vit. E and SDS to SC-NEO, a microbiological shelf life extension of 6 days was achieved compared with the control treatment. Ameur *et al*. (2022) showed that nanoemulsions prepared with cinnamon and grape seed essential oils could prolong the shelf life of flathead mullet (*Mugil cephalus*) fillets by at least 2 days. Zibaee and Shamekhi (2023) reported a significant antimicrobial potential of Kakol (*Suaeda aegyptiaca*) essential oil and its nanoemulsion in lowering TVC and TPB during storage at 4 ± 1°C.

The initial pH value for the control group was 6.4 on day 0, indicating freshness and good quality fillets according to the most acceptable value for rainbow trout (Angiş and Oğuzhan, 2013). The initial decrease in pH values of all groups resulted from the breakdown of glycogen and the formation of lactic acid following death (Chuesiang *et al*., 2020). This value subsequently increased significantly during storage. The pH rise of samples is mostly associated with the accumulation of alkaline compounds such as ammonia and TMA, which are produced due to bacterial activity and endogenous enzymes (Özogul *et al*., 2016). Our results revealed that the coated treatment groups had a significant effect on pH reduction compared with the control group ($P<0.05$) after the 6th day, but there was no marked difference between treatment groups except on day 15. Ameur *et al*. (2022) also reported a similar effect of cinnamon and grape seed nanoemulsion on reducing pH values compared with the control group, with no significant differences shown between the treated groups ($P>0.05$).

TVB-N is a parameter for determining the spoilage levels of muscle tissues based on protein degradation and the formation of volatile nitrogen compounds due to microbial activity (Orban *et al*., 2011). With increases in bacterial counts in all groups, the TVB-N gradually increased during storage. However, the TVB-N in treated samples was significantly lower than in the control group at all sampling times. The quantity of 25 mg N per 100 g is the most acceptable rate for rainbow trout (Gimenez *et al*., 2002). The TVB-N limit was achieved by the control and SC groups on the 9th day, and this limit was reached by the SC-EO group on the 12th day. However, the SC-NEO and SC-NEO + SDS + Vit. E groups remained below this limit for at least 15 days. Using chitosan - *Ferulago angulata* NEO to extend the shelf life of rainbow trout fillets stored at 4°C yielded similar results (Shokri *et al*., 2020). Zibaee and Shamekhi (2023) also reported that Kakol (*Suaeda aegyptiaca*) essential oil nanoemulsion significantly delayed the increasing trend of TVB-N value in rainbow trout.

PV is a parameter of lipid oxidation that measures preliminary oxidation products like peroxides and hydroperoxides. Pradhan *et al*. (2018) reported that a PV of 10-20 meq O2/kg in fat fish is the acceptable limit. As shown in Table 2, the PV levels were significantly affected ($P<0.05$) throughout storage for up to 6 days, but there were no significant differences in treatment, except on days 6 and 9. It was generally observed that the treatment group with NEO yielded lower PV levels than the other groups over the storage period. In addition, according to Fig. 2c, the addition of Vit. E to SC-NEO caused a significant reduction in PV. The control group reached 12 ± 1.68 on day 12 with the highest PV, which then declined due to the decomposition of hydroperoxide into secondary oxidation compounds. These results were similar to those reported by Shadman *et al*. (2017) and Ameur *et al*. (2022).

The TBARS index is used to assess the degree of secondary lipid oxidation by evaluating aldehydes such as malondialdehyde (Jouki *et al*., 2014). A TBARS value of 1-2 mg MDA/kg is generally considered the limit for natural odor or flavor (Dehghani *et al*., 2018). As shown in Table 2, the lower TBARS value in the SC treatment compared with the C group was due to the oxygen barrier properties of the sodium caseinate coating in preventing lipid oxidation, which is in agreement with Zargar *et al*. (2016). Further reduction of TBARS in SC-EO and SC-NEO may be associated with the antioxidant properties of essential oil. Generally, groups treated with NEO showed lower TBARS levels than the aforementioned limits throughout the entire storage period, as reported by Özogul *et al*. (2016) and Khedri and Roomiani (2019). As shown in Fig. 2d, the lower amount of MDA in SC-NEO + SDS + Vit. E compared with SC-NEO could be due to the presence of Vit. E, a strong antioxidant.

Sensory panelists were able to distinguish between the control and the treatment groups based on the odor, color, texture, and overall perception of the rainbow trout fillets. In our study, the sensory scores followed the results of microbial and chemical analyses. The results of chemical and microbial analysis and sensory evaluations indicate that a coating based on sodium caseinate - *Oliveria decumbens* essential oil nanoemulsion can lead to the retention of good quality characteristics and the extension of the shelf life of rainbow trout fillets during storage. This finding is supported by other studies that have reported that plant essential oil nanoemulsions reduce the odor of fish, improve sensory properties, and extend the shelf life of fish (Özogul *et al*., 2016; Shadman *et al*., 2017; Shokri *et al*., 2020; Ameur *et al*., 2022).

The cumulative chemical and microbial analyses suggest that under the environmental conditions of the study, the storage duration of fish fillets was 6 days for the control group, 12 days for the SC-NEO group, and 15 days for the SC-NEO + SDS + Vit. E group. These findings indicate that coating with SC-NEO extended the storage time by 6 days, and the inclusion of sodium dodecyl sulfate (SDS) and Vit. E in the coating extended it by an additional 3 days, without significant alterations in microbial, chemical, or sensory properties. The observed effects of the coatings, as well as the addition of SDS and Vit. E on microbial growth, lipid oxidation, pH dynamics, and sensory attributes, result from their distinct yet complementary mechanisms of action. This synergistic interplay not only inhibits microbial growth but also reduces lipid oxidation, regulates pH levels, and preserves sensory attributes, ultimately extending the shelf life and maintaining the quality of coated fillets during storage.

Correlation results align with expected spoilage trends in fish, where microbial growth is closely tied to biochemical changes and sensory degradation. These findings demonstrate the interconnected nature of microbial, chemical, and sensory changes during storage and reinforce the effectiveness of the SC-NEO and SC-NEO + SDS + Vit. E treatments in preserving trout fillet quality. Further studies could explore the mechanistic pathways of these interactions to optimize formulations for extended shelf life.

This study investigated the effects of sodium caseinate coatings enriched with *Od*-EO and *Od*-NEO, along with SDS and Vit. E, on the quality and shelf life of rainbow trout fillets during refrigerated storage. The study results indicate that the incorporation of *Od*-NEO significantly reduced microbial growth, lipid oxidation, and TVB-N formation while maintaining desirable sensory attributes. The addition of SDS and Vit. E further enhanced the stability and effectiveness of the coating, extending the shelf life by up to 15 days compared with 6 days in the control group.

The results of correlation analysis revealed strong interdependencies between microbial, chemical, and sensory parameters, emphasizing the holistic impact of spoilage factors on product quality. Notably, microbial spoilage (TVC and PTC) exhibited a strong negative correlation with sensory acceptability, while chemical spoilage markers such as TBARS and TVB-N were positively correlated with microbial counts. These findings provide valuable insights into the mechanisms by which nanoemulsion-based coatings can improve food preservation.

The results highlight the potential of nanoemulsion-based coatings as an innovative and effective strategy for extending the shelf life of highly perishable fish products. Future research should focus on optimizing formulations, scaling up production, and evaluating the economic feasibility of such coatings for commercial applications.

Acknowledgements

We would like to express our appreciation to the Research Council of Shahid Chamran University of Ahvaz, Iran, for their financial support (grant No. SCU.VF 99.534).

Conflict of interest

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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