https://doi.org/10.17221/116/2025-CJFS

The effect of chitosan enriched with different essential oils on the physicochemical and microbiological quality of trout burgers stored at 4 °C

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Citation: Elsheikh W., Ucak I. (2026): The effect of chitosan enriched with different essential oils on the physicochemical and microbiological quality of trout burgers stored at 4 °C. Czech J. Food Sci., 44: 62–70.

Abstract: This study examined the effects of chitosan films enriched with various essential oils on the quality characteristics of rainbow trout burgers stored at (4 ± 1 °C) for 21 days. Five groups were prepared: a control group (C), a chitosan film group (CF), and groups of chitosan films enriched with 1% oregano essential oil (OEO), citrus essential oil (CEO), and rosemary essential oil (REO). Microbiological counts and physicochemical factors were assessed. Significant differences ($P < 0.05$) in physicochemical properties were observed among the treatments, with OEO showing the lowest pH (6.67), the lowest peroxide value (*PV*; 12 meq $O_2 \cdot kg^{-1}$), and the lowest thiobarbituric acid reactive substances (TBARS) level [1.159 malondialdehyde (MDA)$\cdot kg^{-1}$]. Microbial results indicated that the shelf life of the treated groups was extended by up to 9 days compared to the control. The lowest counts of Enterobacteriaceae, yeast and mould, and lactic acid bacteria in OEO were 5.17, 4.87, and 5.10 log CFU$\cdot g^{-1}$, respectively, while the lowest counts of psychrophilic and mesophilic bacteria were observed in the REO group, at 6.34 and 6.29 log CFU$\cdot g^{-1}$, respectively. In conclusion, combining essential oils, particularly oregano and rosemary, with chitosan effectively enhances seafood freshness and extends its shelf life.

Keywords: edible films; natural preservatives; lipid oxidation; antioxidants; antimicrobial; shelf life; seafood

Extending the shelf life of perishable seafood while upholding safety and customer satisfaction is a key challenge for the food packaging industry (Priya et al. 2023). Fish provide essential nutrients, such as omega-3 and omega-6 fatty acids, high-quality proteins, and mineral salts (Breda et al. 2017). Due to their biological composition, seafood products spoil quickly without preservatives or refrigeration (Dehghani et al. 2018). While synthetic preservatives are effective at slowing spoilage, they raise concerns about potential long-term health effects (Pisoschi et al. 2018), and some microorganisms can still resist these chemical additives even at acceptable levels (Reygaert 2018).

Recent advances in packaging technologies focus on improving both safety and shelf life by incorporating oxygen scavengers, antioxidants, antibacterial agents, and sensors (Ahmed et al. 2022). There is growing interest in natural preservatives because they are considered safer and effective for extending shelf life (Mariod 2016). Multicomponent edible polymer coatings, which have shown promise in preserving taste and fragrance, are becoming a key topic of research (Al Mahmud et al. 2024). Edible packaging, including films and coatings, provides an appealing alternative to non-biodegradable options by addressing environmental and food safety concerns (Iversen et al. 2022). These biodegradable, non-toxic,

Supported by the Scientific Research Projects Unit/ Nigde Omer Halisdemir University, Turkey, Project No. 2022/12-LUTPEP.

https://doi.org/10.17221/116/2025-CJFS

and biocompatible materials protect both the environment and food products by reducing oxidation, moisture loss, enzymatic activity, and microbial spoilage (Ahmed et al. 2022).

Chitosan, a natural polymer derived from the deacetylation of chitin, is recognised for its antibacterial and antioxidant properties, which improve the quality of various food products (Dong et al. 2020). In edible films, active chemicals – antimicrobials, antioxidants, colourants, and flavours – are combined to enhance food quality, stability, and safety (Salgado et al. 2015). Chitosan can also function as a carrier for plant essential oils, supporting their preservation (Pabast et al. 2018). Regulations for essential oil use differ across regions: in the U.S., many are classified as Generally recognized as safe (GRAS); in the EU, similar rules apply; and in Japan, each oil is assessed individually (Jackson-Davis et al. 2023). This work investigates the effects of chitosan incorporated with oregano, citrus, and rosemary essential oils on the physicochemical and microbiological properties of trout fish burgers during refrigeration.

MATERIAL AND METHOD

Materials

Fresh rainbow trout (*Oncorhynchus mykiss*) were purchased from a farm in the Nigde region, Turkey. Styrofoam boxes filled with ice were used to transport the fish to the Department of Animal Production and Technologies at Nigde Omer Halisdemir University in Nigde, Turkey, where the experiment was conducted. The fish meat was deboned and minced to prepare fish burgers. Essential oils (citrus, rosemary, and oregano oil) were purchased from the Nigde market.

Preparation of fish burgers

Fish burgers were made according to the method described by Ucak et al. (2011) (Table 1). A manual hamburger press was used to make 50 g fish burgers with a diameter of 110 mm and a thickness of 8 mm.

Table 1. Ratios of raw material and additives utilised in the manufacture of fish burgers

Ingredients	Burger mixture ratio (%)
Minced trout flesh	87.8
Corn flour	6
Wheat flour	4
Salt	1.2
Sugar	0.6
Onion powder	0.2
Garlic powder	0.2

Preparation of edible chitosan films and their application to fish burgers

Chitosan films were produced using a modified casting technique based on the method by Moradi et al. (2012). A solution was prepared by mixing 2% (w/v) chitosan with 1% (v/v) glacial acetic acid, swirling the mixture on a hot plate at 50 °C to form a film-forming mixture. This mixture was filtered through Whatman filter paper to remove undissolved particles. After filtering, the solution was returned to the hot plate, and a plasticiser solution containing 0.5 mL of glycerol per gram of chitosan was added after 30 min. To improve the dispersion of essential oils in the film-forming solution, the oils were emulsified with 0.2% (v/v) Tween 80. Following 15 min of agitation, the emulsified essential oils were added to the chitosan solution to achieve a final concentration of 1% essential oils relative to chitosan, and the mixture was homogenised for 2 min. The resulting films were prepared in square polystyrene foam plates, and all film solutions were stored in a cupboard to cure at room temperature for 36 h at 51% relative humidity. Once dried, the films were removed from the foam dishes and disinfected under UV light for 10 min. After preparing the fish burger, it was encased between two layers of film. The study included the following groups: a control without chitosan film (C), a control with chitosan film (CF), and chitosan films infused with oregano oil (OEO), rosemary oil (REO), and citrus oil (CEO). The samples were stored at 4 ± 1 °C for 21 days. Quality analyses were conducted on samples from each treatment at intervals of 0, 3, 6, 9, 12, 15, 18, and 21 days.

Physicochemical characteristics analysis

The chemical composition of fish meat, including protein, fat, moisture, and ash, was determined utilising standard AOAC (2000) procedures method 950.46, 981.10, 960.39, and 920.153, respectively.

pH. For pH measurements, 10 g of the samples and 10 mL of distilled water were homogenised and combined for two minutes. A pH meter was used at a consistent temperature during the measurement (Manthey et al. 1988).

Peroxide value. The AOAC (1990) method 965.33 determines the peroxide value (PV). Two grams of the sample were mixed with 30 mL of a solution of 2 parts glacial acetic acid and three parts chloroform. After adding 1 mL of saturated potassium iodide solution, the mixture was kept in the dark for 5 min. The mixture was then titrated with 0.1 M sodium thiosulfate ($Na_2S_2O_3$), using starch as an indicator, after the introduction of 75 mL of distilled water. The results are reported in meq $O_2 \cdot kg^{-1}$.

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Thiobarbituric acid reactive substances (TBARS). The spectrophotometric measurements used for the TBAR analysis were based on the principle that the malondialdehyde in the samples imparts colour to the TBA reagent (Cd 19-90, AOCS 1998). The TBA reagent was added after dissolving a 5 mL sample of trout oil in *n*-butanol. For 120 min, the sample was maintained at 95 °C in a water bath to accelerate the reaction. After cooling, the samples were analysed by spectrophotometry at 530 nm. The findings were computed using the following formula and represented as mg malondialdehyde·kg^{-1} sample according to Equation 1:

$$TBA = 50 \times \left(absorbance\ of\ oil\ sample - blank\ absorbance\right) / sample\ weight\ (\mathrm{mg}) \quad (1)$$

Microbiological analysis

A 10 g sample of fish flesh was homogenised in 90 mL of Ringer solution for microbial analysis. To prepare the Ringer solution, one Ringer tablet was dissolved in 500 mL of distilled water and then autoclaved. Nine millilitres of this solution were placed in tubes to create serial dilutions. The prepared dilutions were plated on plate count agar (PCA) using the smear culture method for mesophilic bacteria, and the plates were incubated for 2 days at 30 °C. To count total aerobic psychrophilic bacteria, the dilutions were cultured on PCA for a week at 8–10 °C (Ogunkalu and Ucak 2024). The smear-sowing method was also used to transfer dilutions onto potato dextrose agar (PDA) medium, adjusted to pH 3.5, for counting moulds and yeasts. The Petri dishes were incubated for five days at 25 °C (Ogunkalu and Ucak 2024). To enumerate Enterobacteriaceae bacteria in the samples, dilutions were seeded into violet red bile agar (VRBA) using the bulk sowing method and incubated for 24 to 48 h at 37 °C (Ogunkalu and Ucak 2024). Lastly, de Man, Rogosa and Sharpe agar was used to quantify lactic acid bacteria via the spreading method, with plates incubated for 48 h at 30 °C in anaerobic jars (Ogunkalu and Ucak 2024). All culture media were obtained from Merck Millipore (Germany).

Statistical analysis

Statistical analysis and mean comparisons were performed using SPSS 19.0 (SPSS Inc., U.S.), with findings assessed using Duncan's multiple-range test and analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical characteristics. The chemical composition of fish meat was determined to be 16.77% crude protein, 6.97% fat, 72.51% moisture, and 1.13% ash. The initial pH in this study was 6.55 and increased significantly during storage in both untreated and treated samples ($P < 0.05$). At the end of the storage period, the OEO group had the lowest pH (6.68), and the control had the highest (7.05) (Figure 1). These findings are consistent with those of Volpe et al. (2015), whereas Ucak et al. (2020) reported slightly lower values. The pH rise during storage is likely due to rapid degradation and the formation of alkaline compounds during bacterial growth (Li et al. 2012), as well as the dissociation of carbonic acid (Ucak et al. 2020). There were significant differences among treatments throughout storage ($P < 0.05$).

PV was measured to assess fat oxidation and the production of primary oxidation products, which are key contributors to fish spoilage after microbial activity (Ucak et al. 2020). Oregano oil effectively prevented lipid oxidation in trout burgers, keeping peroxide values well within the fish oil safety limit of 20 meq O_2·kg^{-1} (Jairoun 2020). On day 21, peroxide values ranged from 12 meq O_2·kg^{-1} in the OEO group to 18.50 meq O_2·kg^{-1} in the C group (Figure 2). These findings are consistent with Ucak et al. (2021), who reported similar results. Furthermore, Ucak et al. (2020) showed that neutral additives and natural extracts can significantly reduce PV, enhancing the antioxidant properties of chitosan films. The incorporation of compounds, such as essential oils, further enhances the antioxidant, antibacterial, and antifungal activity of chitosan films (Sharma et al. 2021).

Numerous investigations have demonstrated the positive effects of chitosan on lipid oxidation in meat products, particularly when combined with certain natural antioxidants (Ucak et al. 2020). The TBAR test is commonly employed to evaluate rancidity in lipid-rich foods by detecting secondary oxidation products, including malondialdehyde (MDA) (Moselhy et al. 2013). The minimum value of thiobarbituric acid reactive substances was noted in the OEO group at 1.159 MDA·kg^{-1}. In contrast, the maximum was reported in the C group at 1.503 MDA·kg^{-1} on day 21 (Figure 3). Combining essential oils with a chitosan coating slows lipid oxidation in fish meatballs (Ucak and Afreen 2022). One important indicator of lipid oxidation is the TBARS value (Kuley et al. 2012), which reflects the formation of secondary lipid oxidation products. This is closely linked

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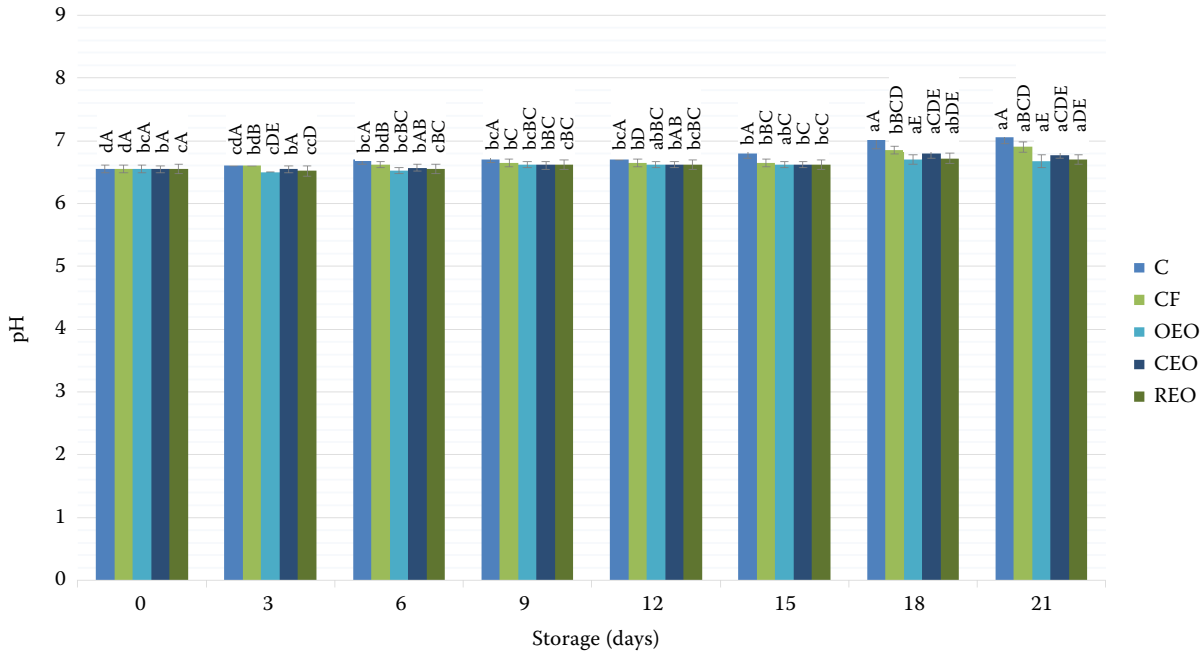

Figure 1. Changes in pH of trout fish burger treated with chitosan films enriched with different essential oils, stored at 4°C

$^{A–E}$different letters indicate treatment impact ($P < 0.05$); $^{a–d}$different letters indicate significant differences ($P < 0.05$) between means, indicating storage impact
C – control without chitosan film; CF – control with chitosan film; OEO, REO and CEO – chitosan films infused with oregano, rosemary and citrus oil, respectively

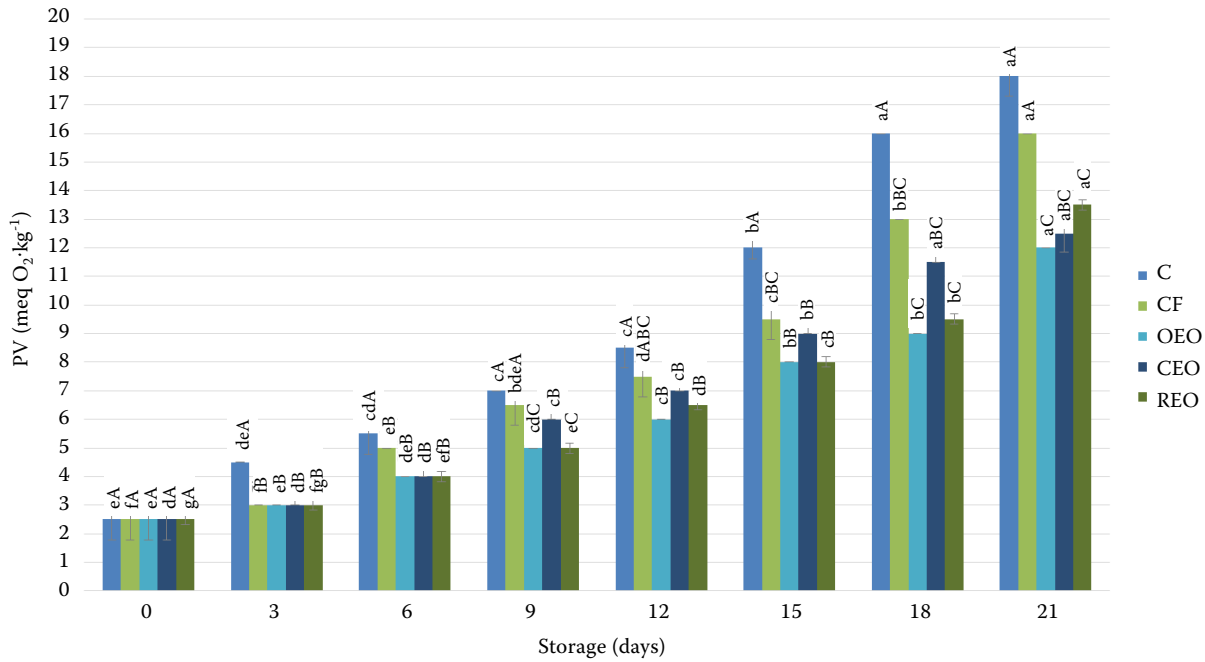

Figure 2. Changes in peroxide value (*PV*) of trout fish burger treated with chitosan films enriched with different essential oils, stored at 4 °C

$^{A–C}$different letters indicate treatment impact ($P < 0.05$); $^{a–g}$different letters indicate significant differences ($P < 0.05$) between means, indicating storage impact
C – control without chitosan film; CF – control with chitosan film; OEO, REO and CEO – chitosan films infused with oregano, rosemary and citrus oil, respectively

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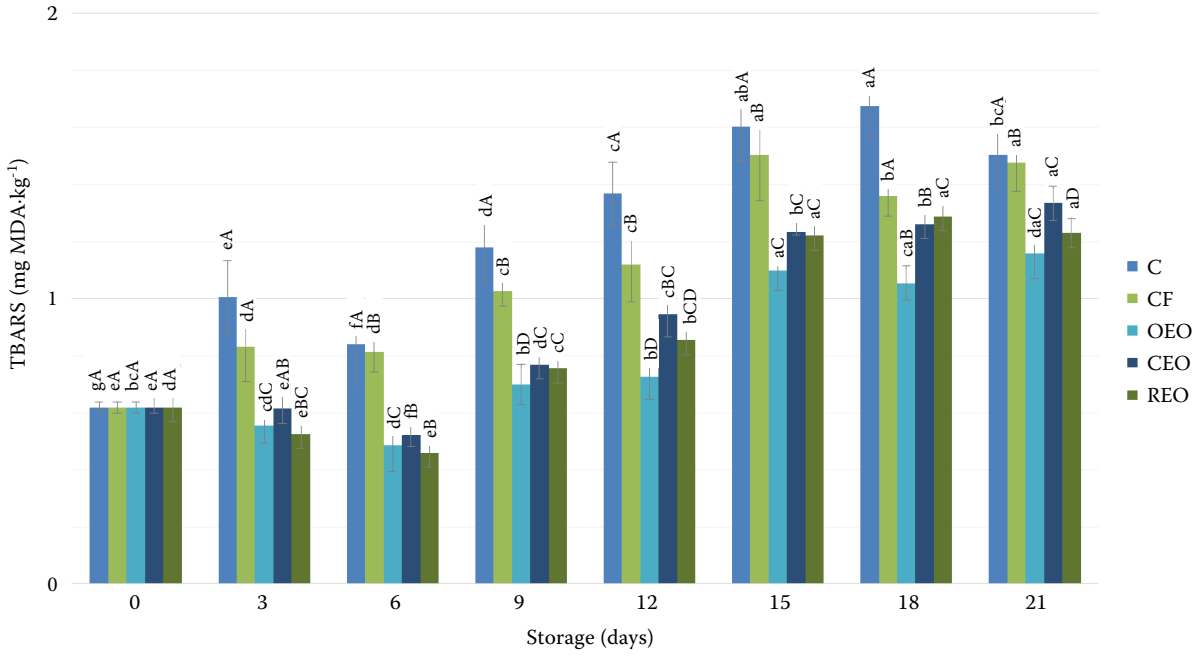

Figure 3. Changes in thiobarbituric acid reactive substances (TBARS) of trout fish burger treated with chitosan films enriched with different essential oils, stored at 4 °C

$^{A–D}$different letters indicate treatment impact ($P < 0.05$); $^{a–g}$different letters indicate significant differences ($P < 0.05$) between means, indicating storage impact
C – control without chitosan film; CF – control with chitosan film; OEO, REO and CEO – chitosan films infused with oregano, rosemary and citrus oil, respectively; MDA – malondialdehyde

to the unpleasant taste and odour of fish products. According to Gokoglu and Ucak (2020), seafood products should have a TBA value below 3 mg MDA·kg^{-1} and no more than 5 mg·kg^{-1}. All groups in our study showed TBARS values within these acceptable limits, indicating decreased oxidation. Oregano oil exhibits strong antioxidant properties, effectively delaying fat oxidation and neutralising reactive oxygen species (Rodriguez-Garcia et al. 2016). These results highlight the enhanced effectiveness of chitosan when enriched with essential oils, particularly oregano oil.

Microbiological analysis. Fish deterioration is primarily caused by microbial growth (Li et al. 2021). The differences in total Enterobacteriaceae counts in trout burgers stored at 4 ± 1 °C are shown in Table 2. The total Enterobacteriaceae count increased in all groups ($P < 0.05$), with the control group exhibiting the highest level at 8.31 log CFU·g^{-1} on day 21, which exceeds the acceptable limit of 7 log CFU·g^{-1} for fresh fish (ICMSF 1986). The lowest count was 5.17 log CFU·g^{-1} in the chitosan treated with oregano essential oil. Table 2 illustrates the variations in total psychrophilic bacterial counts in trout burgers stored at 4 ± 1 °C; on day 0, it measured 3.22 log CFU·g^{-1}, indicating acceptable microbial quality. On day 21 of storage, the total numbers of psychrophilic bacteria were 5.17, 5.53, 6.21, 6.26, and 8.31 in the OEO, CEO, REO, CF, and C groups, respectively, with only the C group exceeding the recommended limit. A statistically significant increase in microbial growth ($P < 0.05$) was observed during storage.

At day 0, the mesophilic bacteria count in trout fish burgers was 2.17 log CFU·g^{-1}. During storage, this count increased in all groups, but to a lesser extent in the treated groups than in the C and CF groups. By day 21, the chitosan group enriched with oregano and rosemary had the lowest mesophilic bacterial count (6.29 log CFU·g^{-1}), while the control group had the highest (8.38 log CFU·g^{-1}). For trout burgers stored at 4 ± 1 °C, the lactic acid bacteria count started at 1.79 log CFU·g^{-1} and increased throughout storage across all groups (Table 2). The lactic acid bacteria increased, mirroring the trend seen for mesophilic bacteria, and were significantly lower ($P < 0.05$) in the essential oil-treated group than in the C and CF groups. These findings indicate that combining essential oils with chitosan more effectively controls

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Table 2. Variations in microbiological of trout fish burgers treated with chitosan films enriched with different essential oils stored at 4 °C for 21 days (log CFU·g^{-1})

	Storage (days)	C	CF	OEO	CEO	REO
Enterobacteriaceae bacteria	0	2.12 ± 0.02^{hA}	2.12 ± 0.02^{fB}	2.12 ± 0.02^{gE}	2.12 ± 0.02^{fD}	2.12 ± 0.02^{fC}
	3	3.36 ± 0.01^{gA}	3.02 ± 0.00^{eB}	2.60 ± 0.02^{fD}	2.66 ± 0.01^{eC}	2.76 ± 0.00^{eC}
	6	4.24 ± 0.02^{fA}	3.84 ± 0.15^{dB}	2.85 ± 0.02^{eD}	3.23 ± 0.03^{dC}	3.09 ± 0.02^{dC}
	9	6.38 ± 0.01^{eA}	3.80 ± 0.01^{dB}	3.46 ± 0.03^{dC}	3.44 ± 0.01^{dB}	3.82 ± 0.03^{cC}
	12	6.93 ± 0.05^{dA}	3.99 ± 0.01^{dB}	3.63 ± 0.06^{dC}	3.96 ± 0.01^{cB}	3.67 ± 0.09^{cC}
	15	7.32 ± 0.02^{cA}	5.00 ± 0.02^{cB}	4.12 ± 0.03^{cC}	4.67 ± 0.05^{bB}	4.68 ± 0.08^{bB}
	18	8.02 ± 0.03^{bA}	6.02 ± 0.18^{bB}	4.52 ± 0.03^{bC}	4.87 ± 0.15^{bC}	4.79 ± 0.03^{bC}
	21	8.31 ± 0.11^{aA}	6.26 ± 0.07^{aB}	5.17 ± 0.08^{aD}	5.53 ± 0.05^{aC}	6.21 ± 0.02^{aB}
Total psychrophilic	0	3.22 ± 0.02^{eA}	3.22 ± 0.02^{fA}	3.22 ± 0.02^{eA}	3.22 ± 0.02^{fA}	3.22 ± 0.02^{eA}
	3	3.11 ± 0.01^{eA}	2.69 ± 0.08^{gB}	2.57 ± 0.09^{gBC}	2.53 ± 0.05^{hC}	2.56 ± 0.01^{fBC}
	6	4.46 ± 0.01^{dA}	3.99 ± 0.03^{eB}	2.78 ± 0.18^{fgC}	2.74 ± 0.06^{gC}	2.65 ± 0.01^{fC}
	9	6.47 ± 0.01^{cA}	3.92 ± 0.10^{eB}	3.00 ± 0.06^{efD}	3.68 ± 0.02^{eC}	3.68 ± 0.14^{efC}
	12	6.46 ± 0.01^{cA}	4.31 ± 0.04^{dB}	4.11 ± 0.05^{dC}	4.25 ± 0.00^{dB}	3.82 ± 0.01^{dD}
	15	7.28 ± 0.13^{bA}	5.40 ± 0.08^{cB}	4.66 ± 0.05^{cC}	5.31 ± 0.04^{cB}	5.23 ± 0.00^{cB}
	18	7.35 ± 0.09^{bA}	6.00 ± 0.02^{bB}	5.18 ± 0.10^{bD}	5.52 ± 0.07^{bC}	5.26 ± 0.03^{bD}
	21	8.25 ± 0.03^{aA}	7.07 ± 0.02^{aB}	6.47 ± 0.03^{aC}	6.44 ± 0.03^{aCD}	6.34 ± 0.08^{aD}
Mesophilic bacteria count	0	2.17 ± 0.03^{gA}	2.17 ± 0.03^{fA}	2.17 ± 0.03^{gA}	2.17 ± 0.03^{fA}	2.17 ± 0.03^{fA}
	3	3.26 ± 0.04^{fA}	2.81 ± 0.05^{eB}	2.66 ± 0.03^{fC}	2.61 ± 0.04^{efC}	2.67 ± 0.03^{eC}
	6	5.43 ± 0.02^{eA}	4.04 ± 0.08^{dB}	2.99 ± 0.12^{eC}	3.03 ± 0.03^{defC}	2.65 ± 0.09^{eC}
	9	6.38 ± 0.03^{dA}	4.11 ± 0.00^{dB}	3.72 ± 0.05^{dD}	3.84 ± 0.02^{cdeC}	3.41 ± 0.07^{dE}
	12	7.28 ± 0.01^{cA}	4.15 ± 0.02^{dB}	3.80 ± 0.01^{dE}	4.08 ± 0.02^{cdC}	3.88 ± 0.03^{cD}
	15	7.47 ± 0.12^{bA}	5.31 ± 0.07^{cB}	4.78 ± 0.01^{cC}	5.21 ± 0.02^{bcB}	5.07 ± 0.06^{bB}
	18	8.23 ± 0.04^{aA}	6.21 ± 0.01^{bB}	5.34 ± 0.03^{bD}	6.06 ± 0.02^{abC}	5.15 ± 0.04^{aE}
	21	8.38 ± 0.03^{aA}	8.28 ± 0.07^{aA}	6.29 ± 0.14^{aB}	7.19 ± 0.08^{aAB}	6.29 ± 0.05^{aB}
Lactic acid bacteria	0	1.79 ± 0.10^{hA}	1.79 ± 0.10^{gA}	1.79 ± 0.10^{eA}	1.79 ± 0.10^{gA}	1.79 ± 0.10^{gA}
	3	2.85 ± 0.02^{gA}	2.78 ± 0.06^{fA}	1.76 ± 0.09^{eD}	2.52 ± 0.06^{fB}	2.06 ± 0.17^{gC}
	6	3.11 ± 0.05^{fA}	2.98 ± 0.12^{bfA}	2.81 ± 0.02^{dB}	2.89 ± 0.00^{eAB}	2.65 ± 0.18^{fB}
	9	3.87 ± 0.05^{eA}	3.23 ± 0.07^{eB}	2.74 ± 0.02^{dD}	2.82 ± 0.10^{De}	3.04 ± 0.01^{eC}
	12	4.41 ± 0.08^{dA}	3.95 ± 0.02^{dB}	3.86 ± 0.02^{cB}	3.91 ± 0.01^{Bd}	3.65 ± 0.09^{dB}
	15	5.33 ± 0.01^{cA}	4.49 ± 0.08^{cB}	4.41 ± 0.02^{bB}	4.20 ± 0.04^{Cc}	4.04 ± 0.06^{cD}
	18	5.57 ± 0.15^{bA}	5.47 ± 0.07^{bA}	4.59 ± 0.16^{bB}	4.66 ± 0.18^{Bb}	4.94 ± 0.14^{bB}
	21	6.04 ± 0.08^{aA}	5.76 ± 0.10^{baA}	5.10 ± 0.12^{aA}	5.48 ± 0.02^{BCa}	5.29 ± 0.05^{aCD}
Yeast and mould	0	1.79 ± 0.10^{fA}	1.79 ± 0.10^{fA}	1.79 ± 0.10^{eA}	1.79 ± 0.10^{fA}	1.79 ± 0.10^{fA}
	3	2.74 ± 0.15^{eA}	2.55 ± 0.09^{beA}	1.87 ± 0.12^{eC}	2.38 ± 0.09^{eB}	2.38 ± 0.08^{eB}
	6	3.02 ± 0.18^{dA}	2.77 ± 0.14^{dB}	2.70 ± 0.12^{dC}	2.85 ± 0.14^{dC}	2.82 ± 0.01^{dC}
	9	4.19 ± 0.02^{cdA}	3.66 ± 0.01^{cB}	3.06 ± 0.08^{cC}	3.21 ± 0.19^{cC}	3.03 ± 0.11^{dC}
	12	4.34 ± 0.03^{cA}	4.14 ± 0.02^{cA}	3.52 ± 0.18^{cBC}	3.93 ± 0.02^{cAB}	3.13 ± 0.07^{cC}
	15	4.46 ± 0.01^{cA}	4.16 ± 0.01^{cB}	3.84 ± 0.08^{bcD}	4.02 ± 0.02^{cC}	3.95 ± 0.04^{cCD}
	18	4.79 ± 0.05^{bA}	4.41 ± 0.07^{bB}	3.98 ± 0.03^{bD}	4.31 ± 0.12^{bBC}	4.15 ± 0.04^{bCD}
	21	5.45 ± 0.02^{aA}	5.26 ± 0.01^{aB}	4.87 ± 0.02^{aC}	5.28 ± 0.03^{aB}	4.89 ± 0.03^{aC}

$^{A–E}$different letters indicate significant differences ($P < 0.05$) in the same row, indicating the impact of treatment; $^{a–h}$different letters indicate significant differences ($P < 0.05$) between the means in the same column, indicating the impact of storage
C – control without chitosan film; CF – control with chitosan film; OEO, REO and CEO – chitosan films infused with oregano, rosemary and citrus oil, respectively

https://doi.org/10.17221/116/2025-CJFS

bacterial growth. On day 21, the group with chitosan, oregano, and rosemary had a yeast and mould count of 4.87 log CFU·g^{-1}, compared to 5.45 log CFU·g^{-1} in the control (Table 2). Several studies support these results. Ucak et al. (2021) showed that gelatine membranes containing citrus seed extract inhibited Enterobacteriaceae growth in fish fillets compared with the control. Agrimonti et al. (2019) found that oregano oil inhibited Enterobacteriaceae in ground beef. Chen et al. (2021) demonstrated that combining oregano essential oil with chitosan reduced both quality deterioration and the growth of mesophilic bacteria and Enterobacteriaceae. At the start of storage, the psychrophilic bacterial count in trout fish burgers was about 3.22 log CFU·g^{-1}, consistent with findings by Jouki et al. (2014) and Ucak et al. (2018), who reported counts of 3.1 log CFU·g^{-1} and 2.47 log CFU·g^{-1}, respectively. In our study, treated samples showed lower psychrophilic bacterial counts than controls. Similarly, Yang et al. (2023) reported that encapsulated rosemary oil was more effective for preserving marine products than traditional methods.

The aerobic mesophilic bacteria count is a key indicator of the microbiological quality of fish products in food hygiene (Anihouvi et al. 2019). Initially, trout fish burgers had a mesophilic bacterial count of 2.17 log CFU·g^{-1}, which is lower than values reported by Ehsani et al. (2020) and Hashemi et al. (2023). Over the storage period, bacterial counts rose in all groups, but the treated groups showed reduced growth compared to the others. On day 21, the oregano and rosemary groups both had counts of 6.29 log CFU·g^{-1}. According to the threshold for fresh fish (ICMSF 1986), the control group surpassed the acceptable limit within 9 days. Meanwhile, the CF and CEO groups stayed below the threshold for 18 days. Notably, the OEO and REO groups maintained levels below the limit for more than 18 days, demonstrating extended microbiological stability. Encapsulated essential oils enhance antibacterial activity by disrupting bacterial membranes (Jafarinia et al. 2022). Samples with chitosan and essential oils had significantly reduced microbial growth. Essential oils include compounds that interfere with enzyme systems, increase membrane permeability, disrupt cell structure, and impair cell and conidia development (Jayasena and Jo 2013). The combination of chitosan and essential oils, specifically oregano and rosemary, demonstrated a synergistic antibacterial effect, effectively reducing microbial loads.

Citrus essential oil slows the proliferation of mould and harmful bacteria in sea bass fillets (Boulares et al. 2018). Rosemary and oregano essential oils enhance the physical and antimicrobial properties of chitosan-based films for fish (Yang et al. 2023). Essential oils are also used for antifungal coatings that improve food quality and shelf life (Sharma et al. 2021). Citrus seed extracts reduced mould and yeast in fish fillets (Ucak et al. 2021), and chitosan coatings with lemon and thyme oils extended the shelf life of fish fillets to 16 days (Cai et al. 2018). Similarly, lemon verbena essential oil in sodium alginate coatings controlled bacterial growth (Li et al. 2021). Quince seed mucilage films with 2% thyme oil significantly extended the microbial shelf life of rainbow trout fillets by up to 11 days (Jouki et al. 2014).

CONCLUSION

Combining chitosan with essential oils – namely, oregano, citrus, and rosemary – improved the physicochemical characteristics of trout burgers during refrigerated storage at 4 ± 1 °C. Additionally, combining essential oils with chitosan films enhanced the films' antioxidants and antibacterial abilities, as the treated groups showed the lowest microbiological counts in this work. The findings provide practical insights into seafood quality, helping control microbial spoilage and improve stability for economic gains. Therefore, we recommend combining essential oils with chitosan films to enhance the quality and shelf life of trout burgers.

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Received: July 23, 2025
Accepted: December 5, 2025
Published online: February 23, 2026