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Enhancing Shelf Life in Rainbow Trout Fillets Using Zinc Oxide Nanoparticles-Infused Edible Coatings from *Vicia Villosa*

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A B S T R A C T

Background: Rainbow trout (*Oncorhynchus mykiss*) is widely consumed due to its nutritional value; however, it remains highly perishable. In this study, we investigated the effects of an edible coating derived from *Vicia villosa* protein isolate (VVPI) combined with zinc oxide nanoparticles (ZnO NPs) to prolong the shelf life of rainbow trout fillets under refrigerated conditions (4 ± 1 °C).

Methods: VVPI was extracted from defatted *Vicia villosa* seeds, and three coating formulations were prepared: VVPI alone, VVPI with10 mg ZnO/100 mL, and VVPI with 20 mg ZnO/100 mL. Rainbow trout fillets were coated and monitored for 12 days. Various chemical indices including pH, free fatty acids, peroxide value, thiobarbituric acid-reactive substances, and total volatile nitrogen along with microbial loads, and sensory attributes were evaluated on Days 0, 4, 8, and 12.

Results: The results showed that fillets coated with VVPI-containing ZnO NPs had significantly lower levels of lipid oxidation and microbial growth than the control and VVPI-only samples. Higher concentrations of ZnO NPs (20 mg/100 mL) exhibited the strongest protective effect, delaying the onset of rancidity and maintaining sensory quality. The shelf life of the fillets was effectively extended by four days, highlighting the potential of this approach to preserve fish fillets.

Conclusion: Incorporating ZnO NPs into VVPI-based coatings presents a promising strategy for enhancing the freshness and acceptability of rainbow trout fillets.

1. Introduction

Rainbow trout (*Oncorhynchus mykiss*), belonging to the Salmonidae family, has been in increasing demand due to its favorable attributes, high nutritional content, and comparatively low production costs relative to red meats such as lamb or beef. Nonetheless, fresh fish products typically exhibit shorter shelf life than many other food items (Saei et al., 2021). The primary factors contributing to their rapid spoilage include oxidative reactions and microbial activity (Shahbazi & Shavisi, 2018). Consequently, there is a pressing need to develop and optimize methods that can extend the shelf life of rainbow trout meat during harvesting, processing, and refrigerated storage (Hashemi et al., 2022). Among the preservation techniques now commonly employed, edible films and coatings have gained attention for their ability to curb oxidative spoilage, mitigate microbial growth, and retain the nutritional quality of various types of meat (Mirbagheri et al., 2023; Shahbazi & Shavisi, 2018). Acting as effective barriers to moisture, carbon dioxide, and oxygen, biopolymer-based films often rely on polysaccharides, proteins, or lipids (Chen et al., 2019). *Vicia villosa* (*V. villosa*) seeds are an appealing protein source for such films thanks to their abundance of fiber, protein, and minerals (Shokrollahi Yancheshmeh et al., 2022). The protein isolate (VVPI) derived from these seeds exhibits promising

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functional properties, is low in cost, and features a high extraction yield (Javan et al., 2024). Its largely hydrophilic character, however, can result in limited water-barrier capabilities, highlighting the need to incorporate additional active agents to effectively hinder lipid oxidation and inhibit microbial proliferation. Recently, inorganic nano-scale additives have been explored for enhancing coatings' preservation effects. Among these, zinc oxide (ZnO) nanoparticles stand out for their antimicrobial properties and stability under high pressures and temperatures (Mizielińska et al., 2018). Classified as "generally recognized as safe" (GRAS) by the United States Food and Drug Administration (21CFR182.8991), ZnO nanoparticles disrupt bacterial cell walls, interfere with DNA replication, and generate reactive oxygen species owing to their large surface area. Such mechanisms can significantly delay microbial spoilage and extend the shelf life of food products (Mizielińska et al., 2017; Javan et al., 2024). Numerous investigations corroborate the efficacy of coatings enriched with various additives in enhancing rainbow trout quality during storage. For instance, chitosan coatings with *Mentha spicata* essential oil and zinc oxide nanoparticles reduced both microbial proliferation and chemical degradation in refrigerated fillets (Shahbazi & Shavisi, 2018), while fish protein-based coatings improved preservation indicators under both cold and frozen conditions (Özyurt et al., 2015). Fish protein isolate/fish skin gelatin-ZnO nanocomposite film incorporated with basil leaf essential oil has also shown encouraging results in maintaining fish fillet quality (Arfat et al., 2015). Additionally, whey protein coatings supplemented with ascorbic acid retarded bacterial growth and lipid oxidation, resulting in a four-day shelflife extension (Ahmadabad et al., 2012). Together, these studies highlight that protein-based coatings, fortified with diverse antimicrobial and antioxidant agents, are highly effective in preserving the overall quality of rainbow trout fillets. Despite these advances, no published research has yet examined VVPI as a new and widely available plant-based protein for developing edible coatings to extend the shelf life of fish products. Thus, the present study aimed to investigate the effectiveness of an active VVPI-based coating containing ZnO nanoparticles on the storage life of rainbow trout fillets, with an emphasis on chemical, microbial, and sensory attributes.

2. Materials and Methods

2.1 Materials

Fresh rainbow trout fillets (approximately 550-600 g each, skinless and boneless) were purchased from a local market in Semnan, Iran. The fillets were transferred to the laboratory in insulated polystyrene boxes containing ice packs. *Vicia villosa* seeds were obtained from an agricultural research center in Shahrekord, Iran. Zinc oxide nanopowder (ZnO NPs, 10-30 nm particle size, 81.38 g/mol, 20-60 g/m^2) was supplied by Fine Nano (Tehran, Iran). All chemicals and reagents-including potassium hydroxide (KOH), glycerol, magnesium hydroxide (MgO), hydrochloric acid (HCl), and boric acid were of analytical grade and purchased from Sigma-Aldrich Co. LLC. (St Louis, Mo, USA). Plate count agar (PCA) and de Man, Rogosa, and Sharpe (MRS) agar were obtained from Merck Co. (Darmstadt, Germany).

2.2 Protein Extraction

The *Vicia villosa* seeds were manually cleaned to remove foreign materials and then ground using a grinder (IKA M20, IKA, Staufen, Germany). The resulting powder was defatted by mixing with hexane at a 1:5 (w/v) solid-to-solvent ratio under continuous stirring (300 rpm) at room temperature (25°C) for 6 h. Following extraction, the hexane was removed using a Buchner vacuum funnel, and the powder was air-dried in the shade at ambient temperature. The defatted powder was then passed through a 40-mesh sieve and stored at 4 °C. Subsequently, VVPI was extracted from the defatted protein powder following the method described by Shokrollahi Yancheshmeh et al. (2022). In brief, 50 g of VVPI powder was mixed with distilled water (1:15, w/v) at pH 9.5 for 45 min, followed by centrifugation at 5,000 × g for 20 min. The resulting supernatant was collected, and its pH was adjusted to 4.5 to precipitate the protein. After a second centrifugation step at 5,000 × g for 20 min, the protein precipitate was washed twice with deionized water; then its pH was raised to 7 using 1 M NaOH. Protein extraction was carried out at 25 °C (room temperature). Finally, the obtained VVPI was freeze-dried using Operon FDB-550 freeze-drier (South Korea) and stored at 4 °C for further analyses.

2.3 Preparation of the Active Coating

Freeze-dried VVPI powder (5% w/v) was dissolved in deionized water at 55 °C under continuous stirring (Pole Ideal Tajhiz Co., Tehran, Iran) until fully dissolved. Glycerol (1 g) was then added as a plasticizer, and stirring continued for an additional 1 h. ZnO nanoparticles were incorporated into the VVPI solution at concentrations of 0, 10, and 20 mg per 100 mL (Javan et al., 2024). The coating solutions were subsequently homogenized at 12,000 rpm for 10 min using an Ultra-Turrax homogenizer (D125, Janke & Kunkel, Germany).

2.4 Coating the Fish Fillets

Boneless, skinless trout fillets were aseptically cut into 1×1×1 cm^3 pieces and randomly divided into four groups including Control (NC): Immersed in sterile distilled water for 30 s, drained on a sterile steel sieve for 2 min.C1: Immersed in VVPI solution (0% ZnO NPs) for 30 s, then drained for 2 min.C2: Immersed in VVPI solution containing 10 mg ZnO NPs for 30 s, then drained for 2 min.C3: Immersed in VVPI solution containing 20 mg ZnO NPs for 30 s, then drained for 2 min. Each coating step was repeated once more for all treatments except the control. The coated and control fillet samples were then aseptically packaged in polyethylene bags and stored at 4 ± 1 °C. Chemical, microbial, and sensory evaluations were carried out on Days 0, 4, 8, and 12.

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2.5 Chemical Analysis

2.5.1 Determination of pH

The pH of each fish sample was measured according to the procedure described by Javan et al. (2024). Briefly, 10 g of each sample was dispersed in distilled water (10 mg w/v) at ambient temperature using a blender. The pH of the resultant suspension was then recorded with a Sartorius laboratory pH meter (Sartorius, USA). The pH meter was calibrated with a three-point calibration mode using the standard buffer solutions.

2.5.2 Total Volatile Basic Nitrogen (TVB-N) Assay

The Total Volatile Basic Nitrogen (TVB-N) content in fish fillets was measured using a Kjeldahl-type apparatus, combining methods described by Goulas and Kontominas (2005) and Urmila et al. (2015) with slight modifications. A sample of 10 g of homogenized fish fillet was blended with 100 mL of distilled water and centrifuged at 3,000 rpm for 10 min. The supernatant was filtered using Whatman No. 1 filter paper. 5 mL of the filtrate was alkalinated by adding 5 mL of MgO solution (10 g/L). Steam distillation was carried out using a Kjeldahl distillation unit for 5 min. The distillate was collected in a receiving flask that contained 10 mL of 20 g/L boric acid and a few drops of 0.1% methyl red and bromocresol green indicators. The distillate solution was finally titrated with 0.1 mol/L HCl and the TVB-N content was calculated using the following equation:

$$TVB\text{-}N\ (mg\ N/100\ g) = ((V \times C \times 14)/W) \times 100$$

Where W is the sample weight (g), V is the volume of titrant (mL), and C is the normality of the acid (mol/L). Results were expressed as mg of nitrogen per 100 g of fish fillet.

2.5.3 Quantification of Free Fatty Acid (FFA) Content

Free fatty acids (FFAs) were determined following the method outlined by Hashemi et al. (2022). In this assay, 5 g of each fillet sample was pressed in 25 mL of ethanol containing 1% phenolphthalein. The extracted lipid fraction was then titrated with 0.1 N NaOH until a stable pink color persisted. Each test was performed in triplicate, and the results were reported as the mean percentage of oleic acid.

2.5.4 Peroxide Value (PV)

Peroxide value (PV) was measured according to Hashemi et al. (2022) with minor modifications. After lipid extraction by the method of Bligh and Dyer (1959), 1 g of extracted lipid and 30 mL of the acetic acid: chloroform (3:2) solution were transferred to a 250 mL Erlenmeyer and lipids were dissolved by gentle shaking. Distilled water (30 mL) and saturated potassium iodide solution (0.5 mL, 1% w/v) were added to the mixture and kept in the dark for 1 min. Then, the released iodine was titrated with 0.01 N sodium thiosulfate. PV value was calculated using the below equation and the results were expressed as milliequivalents peroxide oxygen/kg lipid.

$$PV = (V \times N)/W) \times 1000$$

where W is the sample weight (g), V is the volume of sodium thiosulfate (mL), and N is the normality of the sodium thiosulfate.

2.5.5 Thiobarbituric Acid Reactive Substances (TBARS) Assay

TBARS, which are secondary products of lipid peroxidation, were measured following Hashemi et al. (2022). First, 5 g of each fish fillet sample was homogenized with 15 mL of deionized water. Subsequently, 2 mL of the homogenate was mixed with 2 mL of a 0.67% thiobarbituric acid (TBA) solution prepared in distilled water. The mixture was transferred to a glass test tube, sealed, and heated in a water bath at 90°C for 1 h to facilitate the reaction between malondialdehyde (MDA) and TBA. After cooling to room temperature, the tubes were centrifuged at 2,000×g for 15 min, and the absorbance values of both the blank (AB) and the samples (AS) were measured at 532 nm using a UV-Vis spectrophotometer (Cecil, Cambridge, England). A standard calibration curve was prepared using 1, 1, 3, 3-tetramethoxypropane (TMP) as the MDA standard. A series of standard solutions with known concentrations of MDA equivalents (ranging from 0.5 to 10 µM) were processed in the same manner as the samples. The TBARS value was expressed as mg of malondialdehyde (MDA) per kg of fish fillet, calculated based on the calibration curve. All measurements were performed in triplicate to ensure reproducibility. Blank samples, prepared without fish tissue, were included in each batch to account for any reagent-specific absorbance.

2.6 Microbial Analysis

The microbial examination was carried out by mixing 25 g of each fish sample with 225 mL of 0.1% peptone water for 3 min in a laboratory blender (Model: Waring CB15, Torrington, Connecticut, USA). Subsequent serial dilutions were prepared, and aliquots were plated on Plate Count Agar (PCA) for total psychrotrophic count (TPC) using the pour-plate method. Plates were incubated at 4 °C for 12 days. The results were expressed as $\log_{10}$ colony-forming unit/g (log CFU/g) of the meat samples.

2.7 Sensory Analysis

Sensory evaluation was conducted following Javan et al. (2024). A 12-member trained panel assessed the odor, texture, and color of the samples using a 5-point hedonic scale (Scale: 1-dislike extremely; 2-dislike slightly; 3-neither like nor dislike; 4-like slightly; 5-like extremely). Panelists were instructed on the sensory evaluation procedure and relevant organoleptic properties before testing.

2.8 Statistical Analysis

All experiments were conducted in triplicate, and each set of experiments was repeated twice. The resulting data were analyzed using SAS software (Version 9.1; SAS Institute Inc., Cary, NC, USA) at a significance level of 0.05. One-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test, was employed to compare mean values at the 95% confidence level.

3. Results and Discussion

3.1 pH

pH is a well-established indicator of microbial spoilage in marine products (Kazemi et al., 2021). In this study, the control sample showed a steady increase in pH, exceeding the maximum permissible level by Day 8 (Figure 1. A). In contrast, the pH of the VVPI-coated samples dropped significantly on Day 4 relative to Day 0, indicating that the coating initially mitigates spoilage-related changes. However, pH subsequently increased, returning to near the initial level on Day 8 and reaching the maximum acceptable limit which is 7 by Day 12 (Gonçalves, 2017). Notably, samples treated with VVPI coatings supplemented with ZnO nanoparticles first showed a pH increase on Day 4, followed by a decrease close to the initial level on Day 8-an effect suggesting that VVPI coatings can delay spoilage. By the end of the storage period, these samples also climbed back to the maximum acceptable pH threshold, though more slowly than the control treatment. Ahmadabad et al. (2012) reported that glycogen breakdown and lactic acid accumulation in fish fillets lower pH, as does the dissolution of CO_2 into the muscle′s aqueous phase or the packaging environment–producing carbonic acid. Eventually, as bacterial and endogenous enzymes release nitrogenous bases, pH rises (Salehi & Sahari, 2021). The pH drop at the outset of storage may be related to the production of acids through CO_2 involvement, glycogen breakdown, and VVPI fermentation. In fillets coated with VVPI containing ZnO nanoparticles, the antimicrobial properties of ZnO NPs likely suppressed bacterial growth and slowed enzymatic activity, preventing a rapid increase in pH. Nonetheless, endogenous fish muscle enzymes (lipase, phospholipase, and protease) gradually drove pH higher toward the end of storage by breaking down lipid and protein compounds (Sadeghi et al., 2020).

3.2 TVN-B

Volatile nitrogen bases (VNB) constitute a key indicator for assessing the freshness of fishery products (Zhou et al., 2024). In this study, at Day 0, no significant differences in TVB-N levels were observed between the treatments ($p > 0.05$). By Day 4, the No-Coat treatment exhibited significantly higher TVB-N levels compared to the other treatments ($p < 0.05$), with the Coat 0 mg ZnO, Coat 10 mg ZnO, and Coat 20 mg ZnO treatments showing lower values. On Day 8, No-Coat continued to show the highest TVB-N levels, followed by Coat 10 mg ZnO and Coat 0 mg ZnO, while Coat 20 mg ZnO had the lowest levels, with significant differences between groups ($p < 0.05$). On Day 12, No-Coat showed the highest TVB-N values, significantly different from both Coat 10 mg ZnO and Coat 0 mg ZnO treatments ($p < 0.05$), while Coat 20 mg ZnO exhibited the lowest TVB-N levels, with statistically significant differences across treatments. These bases include trimethylamine (originating from spoilage bacteria), dimethylamine (generated by autolytic enzymes during freezing), ammonia (derived from amino acid deamination), nucleotide catabolites, and other essential nitrogenous compounds (Javan et al., 2024). Their levels rise in direct proportion to storage time. Since 25-26 mg of TVNB/100 g fish meat is considered the maximum acceptable limit, the control samples in this study surpassed that threshold on Day 8 (Figure 1. B) and were thus classified as spoiled (Sadeghi et al., 2020). Meanwhile, fillets treated with VVPI slightly exceeded the maximum permissible level on Day 12 (Figure 1. B). In contrast, the two treatments coated with VVPI formulations containing ZnO nanoparticles experienced a gradual increase in VNB and remained below the critical threshold until the end of the storage period. This observation was most pronounced in samples coated with 20 mg ZnO NPs, underscoring the potential of such coatings for meat preservation. Ahmadabad et al. (2015) reported that the application of edible coatings can curb total volatile nitrogen (TVN) levels by restricting bacterial infiltration at the tissue surface. In a similar vein, Haghighi and Yazdanpanah (2023) demonstrated that edible coatings with antimicrobial properties effectively retard the rise in volatile nitrogen bases. Consequently, the introduction of ZnO nanoparticles into such coatings exerts an antimicrobial effect by disrupting bacterial cell walls. This action inhibits microbial growth and thereby delays the accumulation of volatile nitrogen bases.

3.3 Free Fatty Acids

The results of FFA percentage analysis during storage at 0, 4, 8, and 12 days are shown in Figure 1. C. The FFA percentage in all treatments increased over time. At Day 0, there were no significant differences among the treatments ($p > 0.05$). However, on Day 4, the No coat group exhibited significantly higher FFA levels compared to the other treatments ($p < 0.05$), while Coat 0 mg ZnO, Coat 10 mg ZnO, and Coat 20 mg ZnO showed significantly lower values. By Day 8, the No coat treatment continued to show the highest FFA levels, with Coat 10 mg ZnO and Coat 0 mg ZnO showing intermediate values and Coat 20 mg ZnO exhibiting the lowest FFA levels. These differences were statistically significant ($p < 0.05$). On Day 12, No-Coat showed the highest FFA percentage, significantly differing from the other treatments ($p < 0.05$), while Coat 20 mg ZnO consistently exhibited the lowest FFA values. These results suggest that ZnO coatings effectively reduce FFA formation, thus potentially inhibiting lipid oxidation and improving the quality of the samples during storage. FFA formation signals the progression of lipid oxidation (Salehi & Sahari, 2021) and serves as an important

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indicator of food spoilage (Paktarmani et al., 2017). In line with Sadeghi et al. (2020), our findings also revealed an increase in FFAs over time. The antimicrobial activity of the VVPI-based coating moderated this rise by inhibiting bacterial growth. Psychrotrophic bacteria, which proliferate under cold conditions, secrete enzymes such as lipase and phospholipase, thereby generating compounds (including ketones, aldehydes, and volatile sulfides) that further elevate FFA levels (Rashidi et al., 2022). Javadian and Ebrahimian (2017) observed that phenolic compounds in a plant extract-based edible coating reduced microbial counts and consequently slowed FFA release. Consistent with these results, VVPI's phenolic constituents hinder bacterial activity. Moreover, the addition of ZnO nanoparticles confers additive antimicrobial effects, with higher ZnO NP concentrations notably enhancing bacterial inhibition.

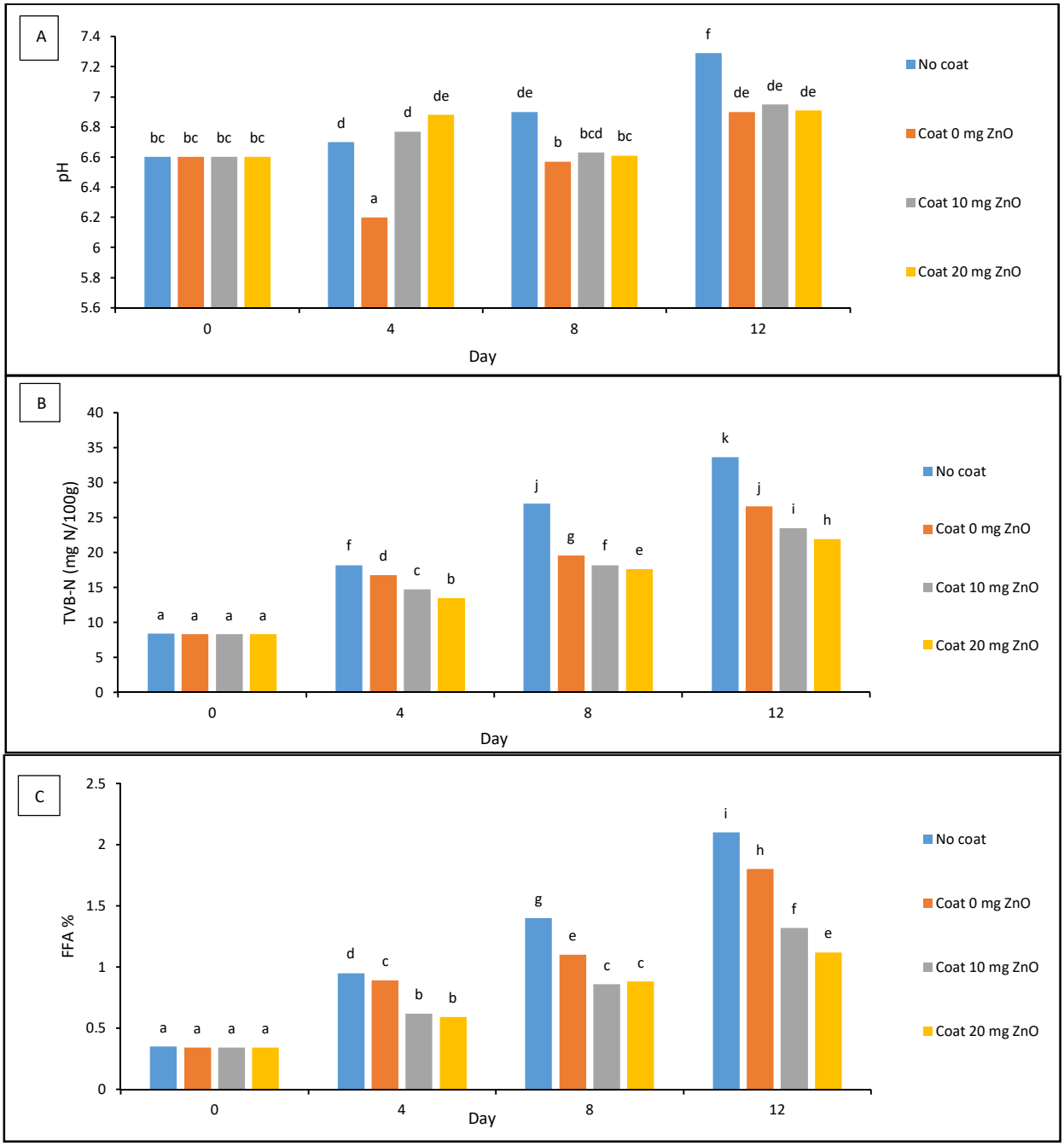


Figure 1. Changes in (A) pH, (B) TVB-N, (C) Free Fatty acid of rainbow trout fillets coated with different coatings during cold storage at 4 °C. Different letters indicate significant differences ($p < 0.05$)

3.4 Peroxide value (PV)

The peroxide value (PV), an indicator of lipid oxidation, was measured over 12 days under different coating treatments (No coat, Coat 0 mg ZnO, Coat 10 mg ZnO, and Coat 20 mg ZnO). At the start (Day 0), all treatments displayed comparable PV levels (~1 meq/kg fish meat), with no statistically significant differences among the treatments ($p > 0.05$) (Figure 2. A). This indicates that the initial oxidative state of the samples was uniform across treatments. By Day 4, significant differences emerged among the treatments. The treatment without coating exhibited the highest PV ($p < 0.05$), suggesting rapid lipid oxidation in the absence of a protective barrier. The Coat 20 mg ZnO treatment displayed the lowest PV ($p < 0.05$), while Coat 10 mg ZnO and Coat 0 mg ZnO exhibited intermediate PV levels ($p < 0.05$, labeled "c" and "d," respectively). These findings suggest that ZnO coatings, particularly at higher concentrations, effectively mitigate lipid oxidation. Lipid oxidation intensified in all treatments by Day 8, with the No-Coat treatment reaching significantly higher PV levels ($p < 0.05$) compared to the coated treatments. The Coat 20 mg ZnO treatment maintained the lowest PV ($p < 0.05$), followed by Coat 10 mg ZnO and Coat 0 mg ZnO ($p < 0.05$, labeled "f" and "g," respectively). The ability of ZnO to inhibit oxidation is likely due to its known antioxidant properties, such as free radical scavenging. By the end of the study, PV levels peaked in all treatments. The No-Coat treatment exhibited the highest PV ($p < 0.05$), indicating extensive lipid oxidation. The Coat 0 mg ZnO treatment also showed a high PV ($p < 0.05$), albeit lower than the uncoated treatment. In contrast, the Coat 20 mg ZnO treatment exhibited the lowest PV ($p < 0.05$), followed by Coat 10 mg ZnO ($p < 0.05$). These results reinforce the protective effect of ZnO coatings against lipid oxidation, with higher ZnO concentrations providing better stability. Kazemi et al. (2021) observed that protein-based coatings, analogous to VVPI, effectively limit both oxygen infiltration and microbial growth in fish tissue. Rezaei and Hamzeh (2011) further demonstrated that incorporating antioxidant agents-such as those present in VVPI enriched with ZnO nanoparticles-can suppress the activity of reactive oxygen species and microbial populations. Additionally, Barzegari Firouzabadi et al. (2016) highlighted that ZnO nanoparticles, owing to their antimicrobial properties, inhibit microbial growth and the secretion of enzymes like lipase and phospholipase, thereby slowing peroxide formation and bolstering product safety during storage.

3.5 Thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid reactive substances (TBARS) test was used to measure secondary lipid oxidation products, particularly malondialdehyde (MDA) (Kazemi et al., 2021). The trends in TBARS values (mg MDA/kg fish meat) are illustrated in Figure 2. B. At the start of the study, all treatments showed similar TBARS values (~0.5 mg MDA/kg fish meat) with no statistically significant differences ($p > 0.05$). By Day 4, significant differences in TBARS values were observed among treatments. The No-Coat treatment exhibited the highest TBARS value ($p < 0.05$), indicating accelerated secondary lipid oxidation. In contrast, the Coat 20 mg ZnO treatment displayed the lowest TBARS value ($p < 0.05$), followed by Coat 10 mg ZnO and Coat 0 mg ZnO ($p < 0.05$, labeled "c" and "e," respectively). These results suggest that ZnO coatings effectively inhibit secondary oxidation, with higher ZnO concentrations providing superior protection. At the end of the study, TBARS values peaked across all treatments. The No-Coat treatment exhibited the highest TBARS value ($p < 0.05$), signifying extensive lipid oxidation. The Coat 0 mg ZnO treatment showed elevated TBARS levels as well ($p < 0.05$), though still lower than the uncoated treatment. The Coat containing 10 mg ZnO and 20 mg ZnO treatments exhibited significantly lower TBARS values ($p < 0.05$), demonstrating their protective effects against lipid degradation, with Coat 20 mg ZnO being the most effective. The results demonstrate that ZnO coatings are effective in reducing secondary lipid oxidation, as evidenced by the lower TBARS values in coated samples compared to the uncoated control. Lipid oxidation is a significant concern in food storage (Javan et al., 2024), as it generates volatile compounds that compromise sensory qualities and shelf life. ZnO, particularly at higher concentrations, acts as a potent antioxidant, reducing the formation of malondialdehyde and other secondary oxidation products. These findings are consistent with previous studies highlighting ZnO's antioxidant properties, which are attributed to its ability to scavenge free radicals and prevent the propagation of oxidative reactions (reference relevant studies). The No-Coat treatment exhibited a rapid and significant increase in TBARS values throughout the study, underscoring the vulnerability of unprotected samples to oxidative stress. In contrast, the Coat 20 mg ZnO treatment maintained the lowest TBARS levels across all time points, highlighting its superior ability to retard secondary lipid oxidation. The Coat 10 mg ZnO treatment also demonstrated a protective effect, albeit less pronounced than the Coat 20 mg ZnO treatment. The Coat 0 mg ZnO treatment, while providing some level of protection, was significantly less effective, suggesting that the presence of ZnO is critical for effective oxidative control. Lipase and phospholipase, released by both fish tissue and microbial activity, catalyze fat breakdown (Kazemi et al., 2021). When oxygen reacts with these liberated fatty acids or their oxidative byproducts, spoilage ensues. Thus, moderating bacterial growth and reactive oxygen species is essential for keeping TBARS levels in check. By functioning as an oxygen barrier, the protein-based edible coating in this study helped maintain lower TBARS values (Khan et al., 2015). These findings closely align with those of 24. Raeisi et al. (2014), who used carboxymethyl cellulose (CMC) coatings enriched with *Zataria multiflora* and grape seed extract on rainbow trout fillets, highlighting the reliability of our approach. Salehi and Sahari (2021) similarly demonstrated that coatings with both antioxidant and antimicrobial properties reduce bacterial populations and curb active oxygen. Our results replicate this trend: VVPI′s antioxidant capacity and ZnO NPs′ antimicrobial effects together deliver outcomes consistent with those reported by Jamali et al. (2023).

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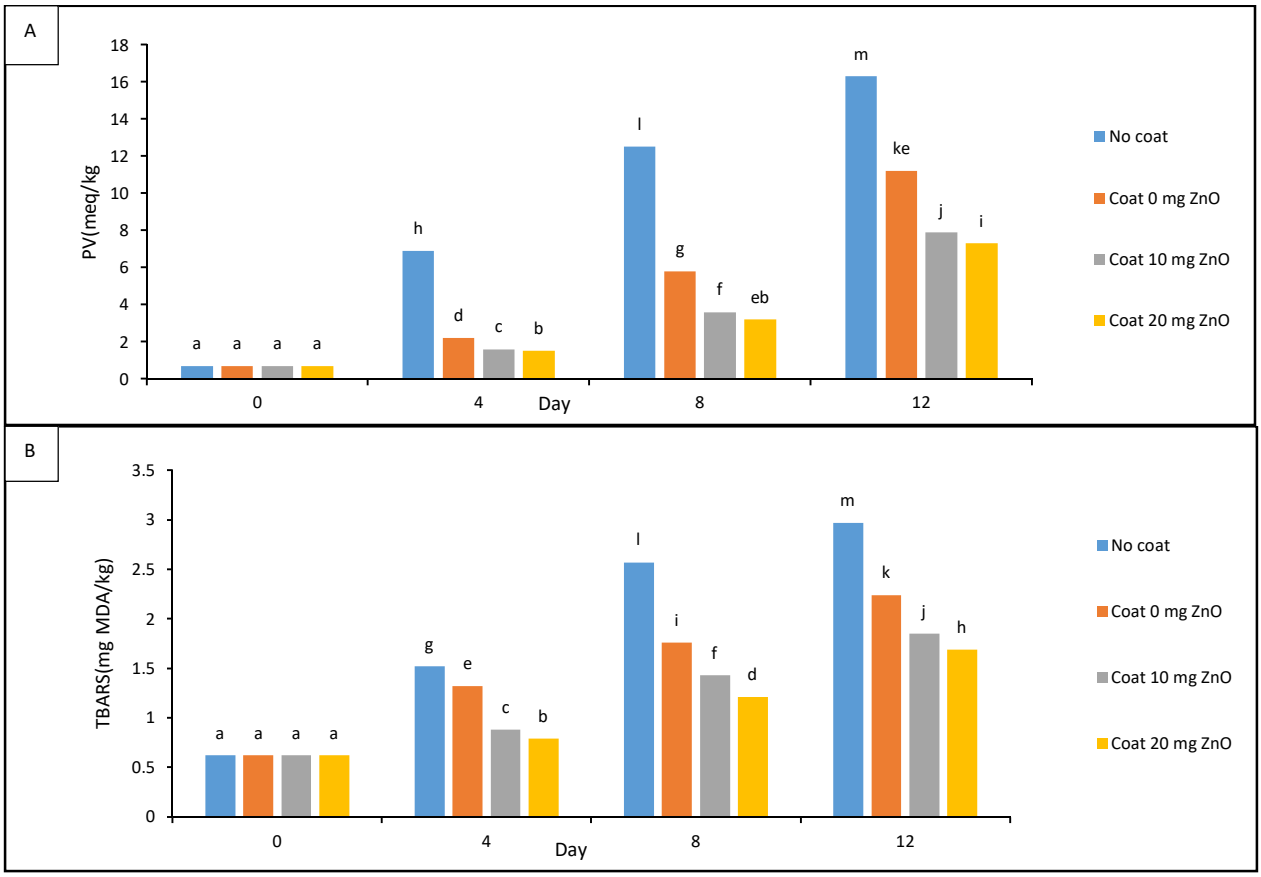


Figure 2. Changes in (A) PV, (B) TBARS of rainbow trout fillets coated with different coatings during cold storage at 4 °C. Different letters indicate significant differences ($p < 0.05$)

3.6 Evaluation of microbial properties

The impact of different coatings with varying zinc oxide (ZnO) concentrations on the growth of psychrotrophic bacteria (log CFU/g) was analyzed over 12 days (Figure 3). At Day 0, all treatments displayed comparable bacterial counts (~3 log CFU/g), with no statistically significant differences ($p > 0.05$). By Day 4, a substantial increase in bacterial growth was observed across all treatments, with significant differences becoming evident. The No-Coat treatment demonstrated the highest bacterial count ($p < 0.05$), while the Coat 20 mg ZnO treatment exhibited the lowest bacterial count ($p < 0.05$). The Coat 10 mg ZnO and Coat 0 mg ZnO treatments showed intermediate levels of bacterial growth ($p < 0.05$). This trend suggests that the presence of ZnO in the coating, particularly at higher concentrations, effectively inhibits psychrotrophic bacterial growth during this period. On Day 8, bacterial counts continued to rise, with the No coat treatment reaching the highest levels ($p < 0.05$). Among the coated treatments, bacterial growth remained significantly lower, with Coat 20 mg ZnO displaying the least growth ($p < 0.05$). This result highlights the sustained antimicrobial activity of ZnO at higher concentrations, which effectively suppresses bacterial proliferation. By Day 12, psychrotrophic bacterial counts peaked in all treatments. The No-Coat treatment reached the highest bacterial load ($p < 0.05$), followed by Coat 0 mg ZnO ($p < 0.05$) and Coat 10 mg ZnO ($p < 0.05$). In contrast, the Coat 20 mg ZnO treatment exhibited the lowest bacterial count ($p < 0.05$), demonstrating its superior antimicrobial effect over the study period. The findings indicate that ZnO coatings significantly inhibit the growth of psychrotrophic bacteria, with higher ZnO concentrations (20 mg ZnO) proving most effective. The antimicrobial properties of ZnO are well-documented, attributed to mechanisms such as the generation of reactive oxygen species (ROS) and disruption of bacterial membranes, which ultimately limit bacterial proliferation (Li et al., 2020). The Coat 0 mg ZnO treatment exhibited a limited inhibitory effect compared to the uncoated treatment, suggesting that even the physical presence of a coating may marginally reduce bacterial growth. However, the addition of ZnO, especially at 20 mg concentrations, provides a substantial enhancement in antimicrobial activity. These results align with previous studies demonstrating the dose-dependent efficacy of ZnO in controlling bacterial populations in food systems or storage environments (Javan et al., 2024; Gao et al., 2024). Interestingly, the NoCoat treatment consistently showed the highest bacterial counts across all time points. This observation highlights the importance of coatings as a protective barrier against microbial contamination and proliferation. By Day 12, the Coat 20 mg ZnO treatment maintained significantly lower bacterial loads compared to

all other treatments, underlining its potential as an effective antimicrobial strategy in extended storage applications. Consistent findings have been reported in other studies. Similar findings were reported on using Chitosan coatings containing *Mentha spicata* essential oil and zinc oxide nanoparticles, which significantly reduced microbial growth and maintained chemical quality during refrigerated storage (Shahbazi & Shavisi, 2018). Similarly, fish protein-based coatings extracted from Klunzinger's ponyfish improved microbial and chemical parameters in cold and frozen storage (Özyurt et al., 2015). Whey protein coatings combined with vitamin C demonstrated antibacterial activity and extended fillet shelf life by 4 days (Ahmadabad et al., 2012). Chitosan nano-gel/emulsion coatings incorporating *Bunium persicum* essential oil and nisin effectively inhibited E. coli O157:H7 growth in rainbow trout fillets (Kazemeini et al., 2019). These studies consistently show that protein-based coatings with various antimicrobial additives can significantly reduce bacterial counts and maintain the quality of rainbow trout fillets during storage.

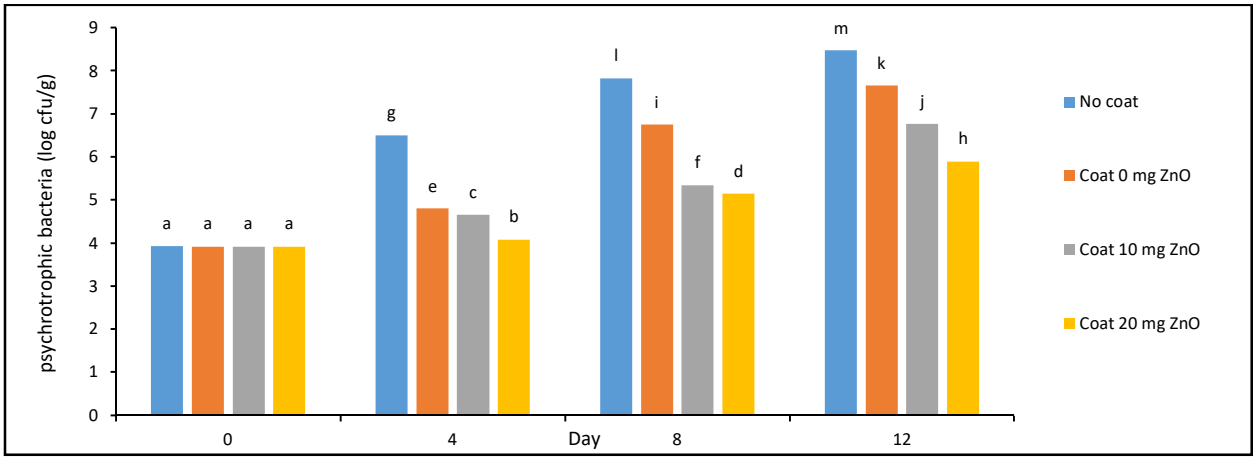


Figure 3. Psychrotrophic bacteria count in rainbow trout fillets coated with different coatings during cold storage at 4 °C. Different letters indicate significant differences ($p < 0.05$)

Microbial spoilage results from the utilization of free amino acids by spoilage bacteria, which subsequently produce various byproducts, including trimethylamine and ammonia (Sadeghi et al., 2020). This contamination often arises due to improper storage conditions as well as secondary contamination (Kazemi et al., 2021). The International Committee for Microbiological Specification of Foods defines the upper limit for psychrotrophic bacteria at 7 log CFU/g (Sadeghi et al., 2020). Recent studies have demonstrated the effectiveness of edible coatings in controlling bacterial growth: Javan et al. (2024) showed that ZnO nanoparticle-infused VVPI-based coatings effectively inhibited bacterial growth in chicken meat, in agreement with earlier findings by Rashidi et al. (2022). The antibacterial properties of ZnO nanoparticles are believed to be due to the generation of reactive oxygen species (ROS) through zinc oxide photocatalysis, as well as the release of zinc cations from the nanoparticle surface. Upon contact with bacterial cells, ZnO nanoparticles are absorbed on the cell surface, disrupting the cell wall. In parallel, ROS penetrates the cell membrane and induces oxidative stress, leading to damage to essential cellular components such as DNA, lipids, and proteins (Rashidi et al., 2022).

3.7 Sensory evaluation

The sensory evaluation charts demonstrate that the application of ZnO coatings (especially at 20 mg) significantly enhances the texture, color, and odor acceptability of rainbow trout fillets during cold storage at 4°C ($p < 0.05$) (Figure 4). No significant difference was found between 10 and 20 mg ZnO coatings except odor ($p > 0.05$). The no-coat fillets show a significant decline in all sensory attributes over time, while the ZnO-coated fillets, particularly those with 20 mg ZnO, maintain superior sensory quality throughout the storage period. The letters above the bars highlight the statistical significance of differences between the various treatments, with 20 mg ZnO consistently outperforming the other treatments, including the no-coat control ($p < 0.05$). This suggests that ZnO coatings, especially at higher concentrations, are effective in preserving the overall sensory quality of rainbow trout fillets during cold storage. The sensory attributes of fish are highly influenced by microbial activity and muscle autolysis (Rashidi et al., 2022). As shown in Figure 4, the results of the present study align with those reported by Rashidi et al. (2022). Specifically, the VVPI-based coating enriched with ZnO nanoparticles, known for its antioxidant and antibacterial properties, yielded superior sensory scores by mitigating oxidative spoilage and inhibiting microbial growth. Elevated levels of free fatty acids can lead to unfavorable tastes and odors, as these acids interact with proteins and denature their structure, ultimately altering the product's texture (Salehi & Sahari, 2021). The earliest sensory change following lipid oxidation is the perception of a bitter flavor, followed by a yellow discoloration of the tissue. Furthermore, microbial activity during storage or processing degrades proteins, resulting in reduced tissue

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firmness toward the end of storage (Kazemi et al., 2021). Psychrotrophic bacteria are especially significant in fish spoilage; they deaminate free amino acids and produce volatile nitrogenous compounds that not only compromise nutritional value but also generate off-flavors and undesirable odors (Sadeghi et al., 2020). Recognizing these mechanisms enables the development of better preservation strategies to curb spoilage factors. Protein-based coatings have shown considerable promise in maintaining the quality of fish and poultry products during storage (Javan et al., 2024). Among these, coatings derived from fish protein, whey protein, or gelatin have been reported to enhance sensory attributes and overall acceptability compared to uncoated controls (Özyurt et al., 2015; Sabzipour-Hafshejani et al., 2022; Rodriguez-Turienzo et al., 2011). By minimizing microbial proliferation, lipid oxidation, and chemical degradation, these coatings can extend shelf life. However, their effectiveness often depends on formulation and application methods. For instance, although gelatin coatings alone demonstrate limited antimicrobial capacity (Andevari & Rezaei, 2011), whey protein coatings formulated with glycerol effectively safeguard frozen salmon (Rodriguez-Turienzo et al., 2011). Moreover, the timing of the coating application can influence its efficacy, with post-freezing application sometimes yielding superior results (Rodriguez-Turienzo et al., 2011). Overall, protein-based coatings offer a promising pathway to enhance product quality and extend shelf life, forecasting a bright outlook for fish preservation.

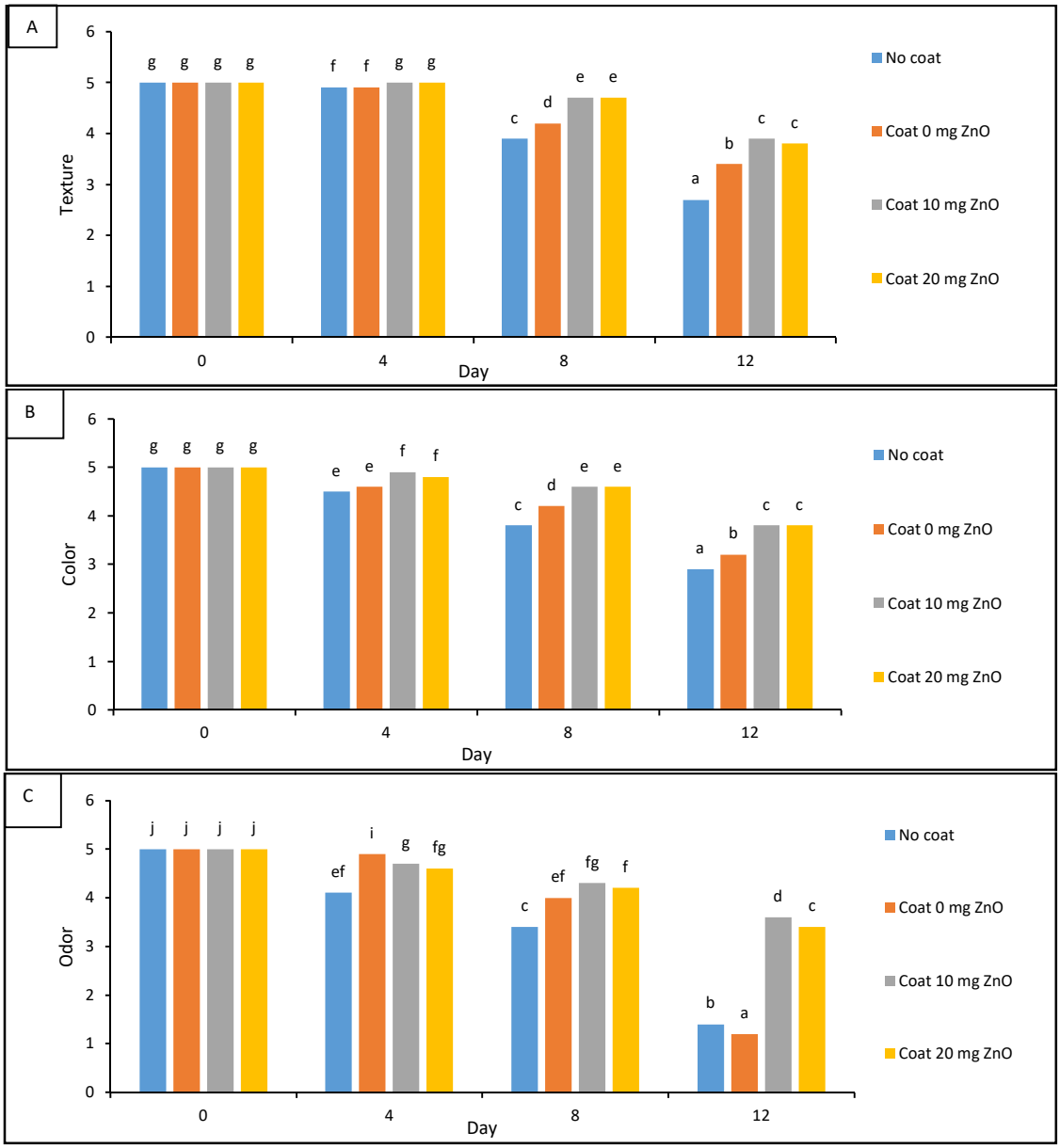


Figure 4. Sensory Evaluation of rainbow trout fillets coated with different coatings during cold storage at 4 °C. scores of (A) texture, (B) Color, (C) Odor acceptability. *Different letters indicate significant differences ($p < 0.05$)

4. Conclusion

A VVPI-based edible coating containing ZnO nanoparticles was evaluated for its preservative efficacy on rainbow trout fillets stored under refrigeration. Results demonstrated that coated samples exhibited significantly lower microbial counts, thereby delaying the onset of microbial spoilage. In addition, all measured chemical spoilage indicators including thiobarbituric acid reactive substances (TBARS), pH, and total volatile nitrogen (TVB-N) were more favorable in the coated fillets than in non-coated controls over the storage period. Sensory assessments likewise indicated good overall acceptability of the coated samples, offering reassurance about product quality. Although direct visualization techniques (e.g., SEM) were not employed in this study to confirm nanoparticle distribution, prior evidence suggests that ZnO nanoparticles can be uniformly dispersed within edible coatings. The antibacterial and antioxidant attributes observed here are consistent with effective nanoparticle release, thereby contributing to shelf-life extension. Taken together, our findings highlight the potential of this bio-based coating to mitigate both microbiological and chemical deterioration, prolonging the shelf-life of fresh rainbow trout fillets by up to four days. These results underscore the promise of natural, nanotechnology-enhanced solutions for fish preservation and broader food packaging applications.

Authors' Contributions

Bahar Lotfi: Investigation; Writing-original draft. **Sara Mehdizadeh Mood:** Conceptualization; Project administration; Supervision; Writing-review & editing. **Ashkan Jebeli Javan:** Conceptualization; Project administration; Validation. **Behdad Shokrollahi Yancheshmeh:** Data curation; Formal analysis.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethical considerations

The present study was approved by the Vice-Chancellor for Education and Research of Semnan University. (Registration Code IR.SU.80).

Using artificial intelligence

No artificial intelligence was used in the writing of this article.

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