

Effect of a developed bilayer film of acorn starch and zein electrospun enriched with cinnamon oil on microbiological, chemical, and sensory characteristics of rainbow trout fillet

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Abstract

This study aimed to investigate the effect of a bilayer active packaging film composed of Persian acorn starch and zein electrospun nanofibers incorporated with cinnamon essential oil (CEO) on the microbiological, chemical, and sensory attributes of fresh rainbow trout fillets stored at 4°C. Six treatments were prepared: control (C), acorn starch film (AS), electropun zein (Z), electropun zein contain CEO (ZCEO), bilayer acorn starch/zein nanofiber film (AS/Z), and bilayer acorn starch/zein nanofiber containing CEO (AS/ZCEO). The total psychrotrophic count (TPC) of control samples surpassed the acceptable limit of 7 log CFU/g by day 6 (7.1 log CFU/g), whereas AS/ZCEO delayed this threshold to day 12 (7.28 log CFU/g). AS/ZCEO treatment also significantly suppressed the growth of lactic acid bacteria (1.88 log CFU/g), Enterobacteriaceae (1.85 log CFU/g), yeast and molds (2 log CFU/g) throughout storage. A gradual increase in pH and total volatile basic nitrogen (TVB-N) was found in all treatments; however, these increases were markedly attenuated in CEO-loaded films. After 12 days, AS/ZCEO samples exhibited a pH of 6.84 and a TVB-N value of 25.2 ± 1.4 mg N/100 g, while the control exceeded 7.3 and 45.5 mg N/100 g, respectively. Sensory evaluation further confirmed superior color, odor, texture, and overall acceptability in CEO-treated samples, extending sensory shelf life at least 3 days. Overall, AS/ZCEO demonstrated strong potential as an active biodegradable packaging material for enhancing the microbial and chemical stability, and sensory quality of refrigerated rainbow trout fillets.

Introduction

Recently, consumers have increasingly preferred fish because of its rich nutritional composition, including proteins, omega-3 polyunsaturated fatty acids (PUFA), and vitamins as well as its health benefits, palatable flavor, and convenience of preparation ¹. The rainbow trout (*Oncorhynchus mykiss*), a member of the *Salmonidae* family, is among the most widely cultivated and consumed fish species globally. Because of its rapid growth rate, adaptability to a broad range of water temperatures and qualities, and resistance to environmental fluctuations, it has become one of the dominant aquacultures in many countries ². Moreover, the increasing demand for processed fish products, such as trout fillets, highlighting its economic and dietary value ³. On the other hand, fish meat is a perishable food due to its highly water content, weak connective tissues, and high pH ⁴. Moreover, maintaining the freshness of fish remains a major challenge since it deteriorates quickly through a series of biochemical and microbial processes. These include microbial decomposition, lipid oxidation, and breakdown of proteins by enzymatic (autolytic) reactions, which generate volatile nitrogen compounds, free fatty and amino acids, and ultimately lead to the formation of secondary metabolites such as aldehydes and ketones ⁵. One way to increase the shelf life of food is packaging.

Packaging serves as a crucial factor in preserving food quality, extending shelf life, and facilitating both product transport and marketing. Although plastic materials have long dominated the packaging industry because of their durability and low cost, growing awareness of its environmental impact has spurred a widespread search for biodegradable packaging alternatives in research and industry ⁶.

Biodegradable packaging films can control key factors that cause food to spoil, such as oxygen, moisture, and carbon dioxide levels. Furthermore, their compatibility with antimicrobial agents and other functional biomolecules enhances their potential as active packaging materials for food preservation ⁷. Overall, these attributes present promising directions for the development of innovative film technologies in the food industry.

A recent development in this field is the shift from petroleum-derived plastics to environmentally friendly biopolymers, including starch, chitosan, gelatin, polylactic acid, whey protein isolate, and zein, as alternative packaging materials ^{5,8}. Zein is a water-insoluble and nonpolar protein with excellent film-forming, biodegradable, and biocompatible properties, making it suitable for electrospinning applications⁹. Zein-based electrospun nanofibers have been explored for encapsulating bioactive compounds and active food packaging. However, pure zein nanofibers often exhibit weak tensile strength and brittleness, requiring modification or blending to improve performance so that each layer helps improve the mechanical properties and permeability of the film ¹⁰. Based on this, the bilayer or laminated structures combining hydrophilic starch with hydrophobic polymers(zein) have been developed. Starch-based films are transparent biodegradable materials with excellent oxygen barrier properties but limited mechanical strength and high water sensitivity ¹¹. But when used in a bilayer film structure, they enhance barrier performance and strength, making them more suitable for food packaging applications ¹².

Globally, most commercial starches come from cereals and tubers ¹³, but rising demand in food and non-food industry caused risks compromising food security. In addition, native starches characterized by limited mechanical resistance, pronounced sensitivity to water, and great oxygen barrier, driving research toward modified forms or non-traditional sources ¹⁴. Studies have shown that non-conventional source such as banana, barley, black bean seed, mango and acorn could provide sustainable options with comparable properties ¹⁵. Acorns have long served as both food and traditional medicine in Iran ¹⁶. Acorns are rich in starch (50–60% dry weight), along with proteins, lipids, and tannins ¹⁷. Acorn starch exhibits higher gelatinization temperature, stronger gel strength, and greater resistant starch content than cereal starches ¹⁸. These properties make it a promising material for value-added products in food active packaging applications.

Active packaging involves incorporating bioactive agents such as bacteriocins, enzymes, organic acids, and essential oils (EOs) directly or onto packaging materials to improve food safety and shelf life ⁴. EOs have attracted significant attention in the food industry for their natural origin, low toxicity, and cost-effectiveness ¹⁹. Due to their strong antimicrobial, antifungal, and antioxidant activities, EOs are widely incorporated into active packaging films to enhance food preservation²⁰. Among them, cinnamon essential oil (CEO), is notable for its antibacterial, antioxidant, and anticarcinogenic properties, and has been effectively applied in biodegradable packaging systems such as starch/poly(butylene adipate-co-terephthalate) ²¹, sodium carboxymethyl cellulose (CMC)/cellulose nanofiber ²², and corn starch/gelatin

bilayer films¹² to inhibit microbial growth and protein degradation in foods. However, the strong aroma, volatility, and instability of EOs limit their direct use, necessitating encapsulation techniques such as electrospinning, which enables controlled release and protection of volatile compounds²³. Electrospun nanofiber films incorporating EOs (e.g., thyme, clove, rosemary, and pepper oils) have shown efficacy in extending the shelf life of fruits and other perishable products²⁴. Zein as a biopolymers have been increasingly employed as electrospinning substrates due to their biodegradability, biocompatibility, and safety, making them promising candidates for developing sustainable food active packaging materials²⁵. In our previous study, we developed and characterized the acorn starch/zein bilayer film and investigated its effect on the total mesophilic bacterial count and lipid oxidation of rainbow trout fillets during refrigerated storage²⁶. Building upon those findings, the present study provides a more comprehensive evaluation of the bilayer packaging film composed of Persian acorn starch and electrospun zein incorporating CEO in controlling microbial growth and chemical changes in rainbow trout fillets stored at refrigerated condition for 12 days.

Materials and methods

Materials

Persian acorns (*Q. brantii*) were collected from the Khorramabad area in Lorestan Province, Iran, during the ripening season and kept in a cool, dark location until the experiments were conducted. Rose Bengal Chloramphenicol selective agar was sourced from Liofilchem (Roseto degli Abruzzi, Italy). De Man, Rogosa, and Sharpe broth (MRS) from Ibresco (Ibresco Co., Tehran, Iran), nutrient broth, nutrient agar, peptone water (PW), plate count agar (PCA), and violet red bile glucose agar (VRBGA) were purchased from Quelab Laboratories (Quebec, Canada). Sulfuric acid, 2-thiobarbituric acid, boric acid, magnesium oxide, and hydrochloric acid were obtained from Merck (Darmstadt, Germany). All other reagents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Preparation and coating of rainbow trout fillets

Fresh rainbow trout (*Oncorhynchus mykiss*) with an average weight of 500 ± 50 g and a length of approximately 29 ± 2 cm were obtained from a local fish farm and transported to the laboratory in insulated containers with crushed ice within 0.5 hours after fishing. Then, fish were decapitated, eviscerated, and filleted manually under hygienic conditions to obtain two fillets per fish. The fillets were divided into six groups and covered with the films: control (C, without film), acorn starch film (AS), electropun zein (Z), electropun zein contain 10% (v/w) CEO (ZCEO), bilayer acorn starch/zein nanofiber film (AS/Z), and bilayer acorn starch/zein nanofiber contain 10%(v/w) CEO (AS/ZCEO). The films were applied to both the top and bottom surfaces of the fillets in a sandwich model. The samples were placed in polyethylene boxes, and the lids were then securely closed. The treated samples stored at 4°C for 12 days, and microbial, chemical and sensory tests were performed on the treatments on days 0, 3, 6, 9, and 12.

Chemical analysis

Proximate analysis

AOAC official methods (AOAC, 2005) were utilized to determine the proximate composition of fish samples including moisture, crude fat and protein as well as ash contents ²⁷.

pH measurement

of the fish fillet (10 g) was homogenized in 90 ml of distilled water for 30 min. The pH values of the homogenate was measured using a digital pH meter (Zag-Chemi Co., Tehran, Iran) ²⁸.

Total volatile basic nitrogen (TVB-N) content

TVB-N level of fish through storage was determined using Kjeldahl distillation method (AOAC. (1995). Initially, 10 g of ground fish was mixed with 300 mL of distilled water and 2 g of magnesium oxide. The mixture, along with some boiling stones, was heated for 15 min until boiling. The apparatus was sealed and steam distillate was collected in a flask containing 25 mL of boric acid (2%) and indicators (Alcoholic solution of methyl red and methylene blue (0.1 g in 100 ml of ethanol). The steam distillation persisted for 25 min. The boric acid solution was titrated with 0.1 N H₂SO₄ until green color of the solution change to dark pink. TVB-N values based on Eq. 1 were reported in mg N₂ per 100g of fish muscle ².

$$\text{TVBN} = 14 \times V_1 \quad \text{Eq. 1}$$

In which V_1 (mL) is the volume of the H₂SO₄ used in the titration.

Microbiological analysis

To prepare decimal dilutions of samples, 10 g of each sample was transferred into individual stomaching bags containing 90 mL of sterile peptone water (0.1%), and homogenized using a stomacher (Stomacher 400, Circulator, Seward.) at 160 rpm for 60 s. After that, the decimal dilutions were prepared in the tubes. The appropriate dilutions were inoculated onto the plate count agar (for psychrotrophic bacteria count), de Man Rogosa and Sharpe agar (MRSA) (incubated anaerobically for lactic acid bacteria), violet red bile glucose agar (VRBGA) (for *Enterobacteriaceae*), and Rose Bengal Chloramphenicol Agar for yeast and mold count. The incubation conditions were 37°C for 1 day for mesophilic bacteria, 7°C for 10 days for psychrotrophic bacteria, 30°C for 2 days for lactic acid bacteria, 37°C for 1 day for *Enterobacteriaceae* count and 25°C for 5 days for Yeast and Mold. All counts were executed in triplicate and give as log₁₀ CFU/g ³⁰.

Sensory analysis

Each fish fillet sample was evaluated by a semi-trained 10-member panelist chosen among students and staff members. The sensory questionnaires measured intensity on a 9-point points hedonic scale (weak to strong) for the following attributes color, odor, texture and overall acceptance. The judges were not aware about the experimental approach and the samples were blind-coded. Samples that receiving overall scores of more than 5 were considered acceptable. Assessment was performed below white fluorescent light in the laboratory ³¹.

Color analysis

Additionally, color parameters of fish fillets were measured using a Lovibond® Model LC 100 Spectrocolorimeter (Tintometer® Group, Lovibond house, UK) at five random points and their mean values were used to represent the color values. The color parameters were reported as the lightness (L^*), redness (a^*), and yellowness (b^*), and ΔE (color difference). The values were calculated using Eq. 2

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad \text{Eq. 2}$$

L_0 , a_0 , and b_0 are the color parameters related to the control indicator on day 0 ³².

Statistical analysis

All measurements were conducted in triplicate. The Statistical Package for the Social Sciences (SPSS, Version 27) was used for data analysis. One-way analysis of variance was performed at 5% significant level Tukey's multiple range test using SPSS.

Results and discussion

Proximate composition

The proximate composition of rainbow trout fillets showed mean ($\pm$ SD) values of approximately $69.8 \pm 1.14\%$ moisture, $21.6 \pm 0.56\%$ protein, $4.5 \pm 0.85\%$ lipid, and $3.4 \pm 0.19\%$ ash (g/100 g fish fillet), consistent with findings from previous studies ^{5,31}. However, some variations were observed, particularly in lipid and ash contents. Such differences in the chemical composition of fish are largely influenced by factors including feeding, season of capture (especially spawning cycles), sex, fish size, and living environment. These compositional variations can significantly affect sensory qualities such as color, texture, odor, taste, and surface appearance, which determine consumer acceptability of fish products ³³. Additionally, they may influence microbial growth and, consequently, the overall quality and shelf life of the fish ³⁴.

Microbiological analysis

Fish deterioration is primarily induced by microbial and enzymatic activities, conducting to increase in pH and total volatile nitrogen compounds (TVB-N) through protein decomposition³⁵. Rainbow trout spoilage trail was assessed by quality parameters, including total psychrotrophic bacteria, *Enterobacteriaceae*, yeast and mold counts, pH, and TVB-N.

Psychrotropic bacteria

Gram-negative psychrophilic bacteria known for their rapid reproduction capabilities within a short timeframe and their possession of potent proteolytic enzymes³⁶. They are the major group of bacteria that responsible for spoilage in fresh fish cold stored in an aerobic environment. Fish spoilage and slime production can be attributed to the proteolytic activities of psychrotolerant Gram-negative bacteria such as *Shewanella* spp and *Pseudomonas* spp., therefore, enumeration of these bacteria could be a good reflection of the quality of cold stored fish flesh. This bacterial group produces various metabolic compounds, including aldehydes, ketones, alcohols, biogenic amines such as putrescine, histamine and cadaverine, and sulfides, which alter the taste, texture, and odor of fish meat^{3,34}.

As presented in Fig. 1, on the first day, the TPC of control was about 3.29 log CFU/g, and the lowest TPC number was 3.16 log CFU /g for the AS/ZCEO treatment groups which is consistent with data from Shahbazi and Shaveisi who reported initial value of 3.41 for TPC in rainbow trout fillets³⁷. Throughout the storage time, the T levels of all fish fillets display a gradual increase ($P \leq 0.05$), culminating in final levels of 9.6, 9.4, 9.53, 8.4, 9.1 and 7.28 log₁₀ CFU/g for the control, AS, Z., ZCEO, AS/Z film and AS/ZCEO film groups, respectively. As shown in Fig. 1, the AS/ZCEO treatment has the lowest number of TPC compared to the control (significantly, $p < 0.05$) on day 12 of storage period, which was almost 2.8 log CFU/g lower than control treatment.

Control, AS, ZCEO and AS/Z treatments exceeded the recommended limit of 7 log₁₀ CFU/g for refrigerated raw fish³⁸. On the 9th day of storage, whereas the TPC value of AS/ZCEO sample exceeded this limit on day 12. The comparison between TPC and Total Viable Count of rainbow trout samples during the storage time indicated that predominant spoilage-related bacterial flora in fresh fish were are aerobically stored at 4°C, is the psychrotrophic bacteria, which is in line with previous findings³⁹. In consistent with the present findings, a similar TPC pattern was reported by other studies that evaluated the effect of food packaging systems containing thymoquinone and resveratrol on the bacterial quality of fresh fish fillets (Ahmadi et al., 2024). As a consequence of these results, it can be postulated that the AS/ZCEO film slow down the microbial growth in Rainbow trout fillets under cold storage.

Lactic acid bacteria (LAB)

LAB are facultative anaerobic bacteria that can grow under both aerobic and anaerobic conditions. LAB were also part of the natural microflora of fresh rainbow trout fillets (Ghaly et al., 2010). LAB grow slowly at refrigeration temperatures. Changes in the LAB counts of fish fillets are shown in Fig. 2. The initial LAB count was 2.00 log cfu/g (on day 0) and reached a count of 6.12 log cfu/g on day 12 of storage for the control samples. This results in line with Mexis et al that reported initial of LAB in rainbow fillet 2.2 and reach 6.00 in day 6 for the air packaged samples storage at 4°C⁴⁰. The lowest initial amount of LAB (1.69 logCFU/g) was measured in AS/ZCEO, indicating the rapid action of CEO. At the end of storage time (day 12). AS/ZCEO reduced LAB count by 1.8 log CFU/g compared to control, and CEO significantly ($p < 0.05$) had effective role in declining LAB in rainbow trout fillet. In line with this, Jokar et al. investigated the effect of adding cinnamon and Shirazi thyme essential oils loaded in Persian gum film on rainbow trout fillets stored in the refrigerator and reported that Farsi gum film incorporated CEO reduced LAB count almost 1.30 log CFU/g compared to control group³.

Another study reported that Farsi gum coating incorporated with clove and Shirazi thyme essential oils emulsions caused a 2.8 log CFU/g reduction in LAB count of rainbow trout fillet stored at refrigerator².

Enterobacteriaceae

Enterobacteriaceae, a hygiene indicator, are also part of the microflora of fresh rainbow trout³⁹. Figure 3 shows that a significant increase was observed in the number of *Enterobacteriaceae* during the storage period with higher increase for the control group. The *Enterobacteriaceae* count on day 0 varied between 2.5–2.62 log CFU/g among the different treatments, which was lower than the value (3.19 log CFU/g) reported by Mehdizadeh et al. for the control sample. This indicates better water hygiene conditions or faster refrigeration of the fish fillet samples⁴¹. On day 12, *Enterobacteriaceae* counts reached the value of 7.65 log CFU/g for control samples. This is in line with Drum and coworker who reported *Enterobacteriaceae* count of control sample almost 7.8 log CFU/g in the day 12⁴². On day 12, *Enterobacteriaceae* count was 1.85 log CFU/g lower in AS/ZCEO ($p < 0.05$) compared to control. The role of *Enterobacteriaceae* to the microflora of fish and its possibility to spoilage must be taken into attention in the case of water pollution or delay in chilling after fishing⁴⁰.

Although *Enterobacteriaceae* can grow at low temperatures, the data in Fig. 3 shows that the number of *Enterobacteriaceae* is lower compared to psychophilic bacteria. This could be due to their slower growth rate compared to psychrophilic Gram-negative spoilage bacteria.

Yeast and molds

Yeasts and molds, which typically originate from soil, are not part of the natural microflora of fish. Their presence usually indicates contamination from water during fishing or from subsequent processing steps such as skinning, evisceration, or filleting. Among the studied groups, there was a significant difference in yeast and mold counts, as well as a significant effect of storage time ($p < 0.05$). As shown in Fig. 4, the yeast and mold counts fluctuated across the samples throughout the storage period. No

yeasts or molds were detected on day 0; however, their numbers increased significantly during the storage period, with the control group exhibiting the highest rate of increase ($p < 0.05$). AS/ZCEO was the best treatment which could reduce 2 log CFU/g of yeast and mold compared to control group in the last day (day 12) of storage rainbow trout fillet in refrigerator. This is in line with ⁴³, who reported yeast and mold count increase to near 6 log CFU/g in the control group. Therefore, interestingly, AS/ZCEO could inhibit a broad spectrum of bacteria and yeast and mold. Yaru et al. investigated the action mechanism of CEO against fungi. Their observations revealed that CEO collapsed hypha, disrupted plasma membrane, cytoplasmic matrix leakage, prevented the biosynthesis of ergosterol and influenced the energy metabolism of fungi by reduction the activities of malate dehydrogenase and succinate dehydrogenase in tricarboxylic acid cycle ⁴⁴.

pH

The pH can function as an indicator of fish freshness. Changes in the pH values of fish fillets during storage are shown in Fig. 5. A pH values varying between 6.3–6.42 (in all groups in day 0); which is in agreement with that reported by others ⁴⁵, and increasing during cold storage ($p < 0.05$). AS/ZCEO had a lower pH than the other samples due to cinnamon essential oil, which contains acidic compounds such as cinnamic acid. This may be due to the antibacterial and antioxidant ability of CEO, which can delay the microbial and oxidation proliferation in fish. Significant differences were observed between pH values in different treatments at the end of the storage period ($P < 0.05$). The pH value of control samples increased faster than other sample, and reached to the maximum value (7.34) at the end of 12-day storage in refrigerator. pH value increase owing to the formation of basic nitrogenous compounds in fish like ammonia and biogenic amines as a consequence of autolytic activity of fillet endogenous enzymes and the proteolytic activity of psychotropic bacteria ³⁰. In line with this, it was reported that CEO could reduce the amount of most biogenic amines in fish fillet ⁴⁶.

The increasing trend in pH was dependent on the coating film type. There is a correlation between pH values and microbial counts, such that AS/ZCEO had a maximal ($p < 0.05$) inhibitory effect against microbial growth, and the lowest pH value was measured in this treatment. These results are similar to previous study, which found Farsi gum coating contain clove and *Zataria multiflora* essential oil effectively delayed the pH increase of rainbow trout fillets ². The transformation of pH value can be used as the quality testing standard for fish products ^{47,48}.

TVB-N

TVB-N value reflects the freshness of fish muscle. Throughout the spoilage in meat and meat products, the production of nitrogenous compounds with low molecular weight, such as dimethylamine, trimethylamine and ammonia, collectively known as TVB-N, is the primary reason for off-flavor and sensorial rejections. TVB-N produced by degradation of nitrogenous compounds of protein and non-proteins by microorganisms and enzymes during storage of fish muscle ⁴⁹.

The initial amount of TVB-N in the treatments ranged from 7.21 ± 1.01 in the fillet coated with AS/ZCEO to 9.33 ± 0.8 mg N/ 100 sample in control, show no significant difference ($p > 0.05$) among the different groups at the beginning of storage (Fig. 8). TVB-N value shows the freshness of the fillet and agrees with other studies ^{5,36,49}.

TVB-N values of samples increased significantly ($p < 0.05$) accompanied by microbial growth, with storage time; however, the TVB-N value of the ZCEO and AS/ZCEO groups was significantly ($P < 0.05$) lower than the other groups during all the storage period, which was chiefly assigned to the presence of CEO. This result is consistent with other pH and microbial analysis studies.

TVB-N reached a value of 25.67 ± 0.80 mg N/100 g on the 6th day of storage, indicating that the fish fillet had reached the acceptability threshold for fish flesh, considered to be 25 mg N/100 g ⁵⁰.

Until the day of 12th, index of TVB-N of coated samples with AS/ZCEO was not beyond the acceptable limit (25.2 ± 1.4 mg N/100 g). However, at the same time, the amounts of TVB-N in other treatments were higher than 25 mg N/100 g. The presence of CEO in bilayer film significantly ($p < 0.05$) retarded the increasing rate in TVB-N index. Based on the plausible explanations, the slower increasing trend in TVB-N index in sample with the presence of CEO may be due to a reduction in the bacterial count or their ability to deamination of nitrogenous compounds ^{2,5}. These results demonstrated that bilayer film loaded with CEO could inhibit loss of fish quality through acting as a semi-permeable barrier. In line with these results, Joukar et al. found that coating of rainbow trout fillets with 2% Farsi gum enriched with 1% CEO could retard the increasing rate in the TVB-N index ³.

Sensory evaluation

When using natural preservatives to enhance food quality, it is crucial to consider their potential impact on sensory attributes ⁵¹. The variations in sensory characteristics in the rainbow trout fillets during 12 days storage at 4 °C are represented in and Tables 1. The sensory scores for all evaluated attributes were declined throughout the storage period ($P \leq 0.05$). The color, odor, texture and overall acceptability scores were declined in all treatment throughout storage days. In general, the control samples received the lowest scores by panelists throughout the entire storage time except for that at day 0. Regarding to the acceptable sensory score for consumption, 5 and above it ³¹, Panelists recognized the color, odor, texture and overall acceptability scores of the control samples were lower than this limit at the 6th day, while this time for AS/ZCEO were the 9th days.

These findings consisted with the chemical findings of samples (Figs. 5 and 6). In fact, the sensory scores have been reduced by increasing the physicochemical values (such as pH, TVB-N,) during storage period. Signs of spoilage, including a slimy texture, visible discoloration, and off-odor, may be the result the decomposition of proteins, oxidation of lipids, and degradation of non-protein nitrogen products such as ammonia, hypoxanthine, lactans, furans, esters, ketone, TMA, aldehyde, and dimethylamine which cause the off-odor and off-flavor of fish flesh induce the low score for the samples ^{4,34}. The fillets treated

with films contain CEO were preferred more by the panelists ($p < 0.05$). The observed phenomenon is likely attributable to the antibacterial and antioxidant properties of CEO, which had positive effect on fish by removing the fishy odor and effectively delayed the onset of spoilage symptoms compared to the control group. Antioxidant and gas barrier impacts by coating could be the causes decreasing the oxidation and enhancing the shelf life of the fish along with quality preservation. Therefore, it can be concluded that the treatments kept the rainbow trout fillets sensory quality due to their antimicrobial and antioxidant properties ³⁰.

Adding cinnamon oil to chitosan coating enhanced significantly ($p < 0.05$) the beneficial effects on color and overall acceptability of the fish in final days ⁵. A study reported that the application of marinade emulsion contained CEO, oregano, and thyme essential oils led to significant increase in overall acceptability of fish and meat products ⁵².

Some studies have mentioned that rainbow trout fillets packaged with zein nanofibers, loaded with natural preservatives, could improve sensory properties and shelf life ^{4,53}.

Table 1
Sensory scores (mean $\pm$ SD) of different treatments of rainbow trout fillet during storage at 4°C for 12 days

Parameters	Storage period (day)					
	Treatments	0	3	6	9	12
Color	Control	8.90 $\pm$ 0.48 ^{Aa}	6.90 $\pm$ 0.45 ^{ABb}	5.00 $\pm$ 0.47 ^{Cc}	3.00 $\pm$ 0.65 ^{Bd}	1.21 $\pm$ 0.14 ^{De}
	AS	7.56 $\pm$ 0.61 ^{Aa}	7.19 $\pm$ 0.29 ^{ABb}	5.43 $\pm$ 0.62 ^{BCc}	3.77 $\pm$ 0.52 ^{Bd}	1.50 $\pm$ 0.08 ^{Ce}
	Z	8.43 $\pm$ 0.62 ^{Aa}	6.73 $\pm$ 0.66 ^{Bb}	5.15 $\pm$ 0.45 ^{Cc}	3.05 $\pm$ 0.58 ^{Bd}	1.21 $\pm$ 0.06 ^{De}
	ZCEO	8.77 $\pm$ 0.52 ^{Aa}	7.09 $\pm$ 0.43 ^{ABb}	6.04 $\pm$ 0.34 ^{ABc}	3.54 $\pm$ 0.43 ^{Bd}	2.31 $\pm$ 0.19 ^{Be}
	AS/Z	8.34 $\pm$ 0.74 ^{Aa}	6.48 $\pm$ 0.68 ^{Bb}	5.42 $\pm$ 0.33 ^{BCc}	3.36 $\pm$ 0.73 ^{Bd}	1.41 $\pm$ 0.14 ^{Ce}
	AS/ZCEO	8.89 $\pm$ 0.43 ^{Aa}	7.74 $\pm$ 0.47 ^{Ab}	6.21 $\pm$ 0.43 ^{Ac}	5.45 $\pm$ 0.38 ^{Ad}	3.30 $\pm$ 0.25 ^{Ae}
Odor	Control	8.69 $\pm$ 0.52 ^{Aa}	6.75 $\pm$ 0.55 ^{Bb}	4.86 $\pm$ 0.30 ^{Dc}	2.31 $\pm$ 0.32 ^{Ed}	1.08 $\pm$ 0.10 ^{De}
	AS	8.36 $\pm$ 0.52 ^{Aa}	6.89 $\pm$ 0.27 ^{Bb}	5.74 $\pm$ 0.23 ^{Bc}	3.51 $\pm$ 0.40 ^{Cd}	1.56 $\pm$ 0.12 ^{Ce}
	Z	8.84 $\pm$ 0.42 ^{Aa}	6.80 $\pm$ 0.24 ^{Bb}	5.06 $\pm$ 0.42 ^{CDc}	2.69 $\pm$ 0.39 ^{DEd}	1.18 $\pm$ 0.15 ^{De}
	ZCEO	9.02 $\pm$ 0.43 ^{Aa}	7.13 $\pm$ 0.47 ^{ABb}	5.78 $\pm$ 0.24 ^{Bc}	4.36 $\pm$ 0.62 ^{Bd}	1.95 $\pm$ 0.11 ^{Be}
	AS/Z	8.51 $\pm$ 0.55 ^{Aa}	6.51 $\pm$ 0.35 ^{Bb}	5.51 $\pm$ 0.38 ^{BCc}	3.34 $\pm$ 0.43 ^{CDd}	1.60 $\pm$ 0.11 ^{Ce}
	AS/ZCEO	8.76 $\pm$ 0.55 ^{Aa}	7.68 $\pm$ 0.49 ^{Ab}	6.64 $\pm$ 0.49 ^{Ac}	5.34 $\pm$ 0.35 ^{Ad}	2.98 $\pm$ 0.14 ^{Ae}
Texture	Control	8.61 $\pm$ 0.46 ^{Aa}	6.94 $\pm$ 0.47 ^{ABb}	4.90 $\pm$ 0.24 ^{Dc}	2.53 $\pm$ 0.51 ^{Cd}	1.40 $\pm$ 0.19 ^{De}
	AS	8.41 $\pm$ 0.48 ^{Aa}	6.65 $\pm$ 0.50 ^{Bb}	5.78 $\pm$ 0.37 ^{ABc}	3.40 $\pm$ 0.32 ^{BCd}	1.40 $\pm$ 0.18 ^{De}
	Z	8.55 $\pm$ 0.47 ^{Aa}	6.66 $\pm$ 0.33 ^{ABb}	5.19 $\pm$ 0.29 ^{CDc}	2.81 $\pm$ 0.55 ^{Bd}	1.42 $\pm$ 0.21 ^{De}
	ZCEO					
	AS/Z					
	AS/ZCEO					

Parameters		Storage period (day)					
		Treatments	0	3	6	9	12
		8.40 ± 0.36 ^{Aa}	7.09 ± 0.34 ^{ABb}	5.80 ± 0.35 ^{ABc}	3.49 ± 0.38 ^{Bd}	2.13 ± 0.26 ^{Be}	
		8.44 ± 0.41 ^{Aa}	7.00 ± 0.56 ^{ABb}	5.56 ± 0.42 ^{BCc}	3.36 ± 0.65 ^{Bd}	1.81 ± 0.60 ^{Ce}	
		8.79 ± 0.54 ^{Aa}	7.54 ± 0.40 ^{Ab}	6.15 ± 0.26 ^{Ac}	4.85 ± 0.47 ^{Ad}	3.51 ± 0.20 ^{Ae}	
	Overall acceptability	Control	8.95 ± 0.52 ^{Aa}	6.71 ± 0.26 ^{Bb}	4.93 ± 0.26 ^{Cc}	2.61 ± 0.48 ^{Dd}	1.11 ± 0.12 ^{De}
		AS	8.05 ± 0.59 ^{Ba}	6.78 ± 0.50 ^{Bb}	5.35 ± 0.35 ^{BCc}	3.34 ± 0.29 ^{BCd}	1.54 ± 0.11 ^{Ce}
		Z	8.25 ± 0.42 ^{ABa}	6.78 ± 0.19 ^{Bb}	5.08 ± 0.45 ^{Cc}	2.73 ± 0.55 ^{CDd}	1.16 ± 0.17 ^{De}
		ZCEO	8.67 ± 0.55 ^{ABa}	6.90 ± 0.34 ^{Bb}	5.74 ± 0.50 ^{Bc}	3.71 ± 0.32 ^{Bd}	2.00 ± 0.17 ^{Be}
		AS/Z	8.40 ± 0.33 ^{ABa}	6.70 ± 0.40 ^{Bb}	5.53 ± 0.28 ^{BCc}	2.83 ± 0.91 ^{CDd}	1.60 ± 0.70 ^{CDe}
		AS/ZCEO	8.75 ± 0.38 ^{ABa}	7.53 ± 0.42 ^{Ab}	6.44 ± 0.40 ^{Ac}	5.33 ± 0.46 ^{Ad}	3.70 ± 0.20 ^{Ae}
Different small and capital letters indicate statistical differences among times and treatments, respectively (P < 0.05).							

Different small and capital letters indicate statistical differences among times and treatments, respectively (P < 0.05).

Color parameters of fish fillets

Color is one of the fastest visual indicators of fish freshness, as changes in pigment and muscle proteins, and microbial growth alter appearance over time. Fresh rainbow fillets display a bright and ruddy shine, but storage in refrigeration leads to browning linked to myoglobin oxidation, protein denaturation, and the progressive disruption of myofibrils through enzymatic and non-enzymatic reactions³¹. Because color depends on heme pigments, muscle structure, and free water content, even small biochemical alterations can produce noticeable shifts in L*, a*, and b* values, therefore increasing ΔE ⁵⁴.

Figure 7 shows that the control samples exhibited the fastest rate of discoloration, reaching a ΔE of 17.8 by day 12. In contrast, fillets coated with AS, Z, ZCEO, AS/Z, and especially AS/ZCEO films maintained better color stability throughout storage. The AS/ZCEO-coated group demonstrated the lowest overall color change ($\Delta E \approx 6.01$), likely due to its strong antioxidant and antimicrobial activity. These results

align with earlier findings that storage increase ΔE and coating with films that have antioxidant and antibacterial effect can have good effect on change in color and increase color quality^{2,55}.

Conclusion

In this study, single and bilayer of acorn starch and zein electrospun nanofiber with or without CEO were applied to control microbiological, chemical, and sensory changes in rainbow trout fillets stored at 4°C for 12 days. According to the results, the acorn starch–zein bilayer film containing CEO (AS/ZCEO) was the best treatment, and inhibited spoilage microorganisms, reduced TVB-N, and extended shelf life by about three days compared to control. These effects were attributed to the combined antimicrobial, antioxidant, and barrier properties of the film. Overall, this natural and biodegradable packaging offers a sustainable alternative to petroleum-based packaging materials. Further studies are needed to evaluate the efficacy of this bilayer film in preserving other foods such as meat and cheese.

Declarations

Funding Declaration

The authors declare that no funding was received for the conduct of this study.

Ethical approval

Written informed consent was obtained from all participants prior to sensory evaluation of the food samples. The sensory assessment was conducted solely for evaluating the quality and overall acceptability of the rainbow trout fillet.

Author Contribution

"M. K": Formal analysis, Methodology, Software, Writing – original draft"J. A": Conceptualization, Data curation, Supervision, Validation, Visualization, Writing – review and editing"L. L": Supervision, Writing – review and editing

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

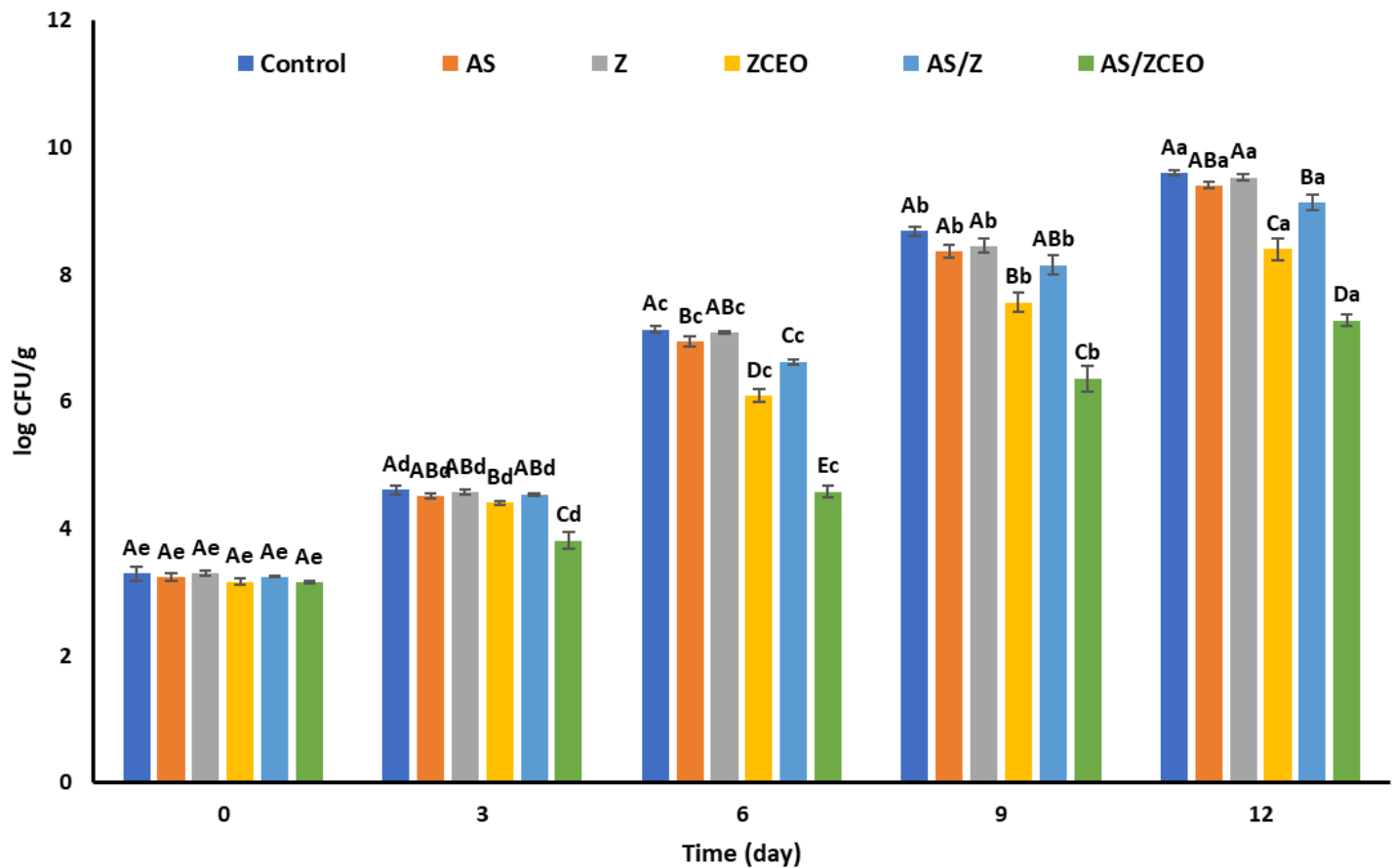


Figure 1

Psychrophilic bacteria count in different treatments of fish fillets during storage at 4°C for 12 days. Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film film incorporated with cinnamon essential oil (CEO); AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zain film incorporated with CEO 10%(v/v). A-D: different uppercase letters show differences between treatments for same storage times ($p < 0.05$). a-e: different lowercase letters show differences between the storage times within same analysis group ($p < 0.05$).

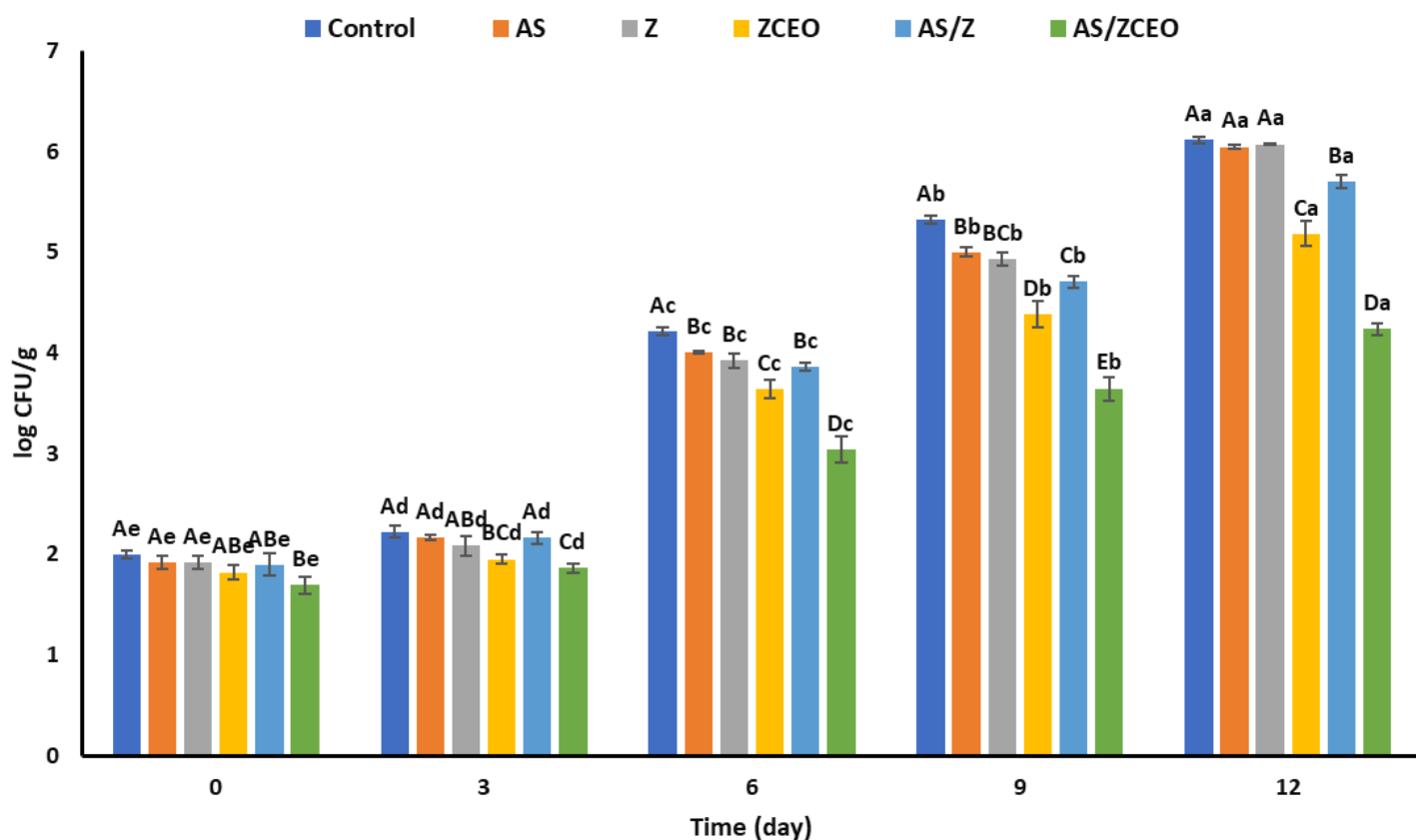


Figure 2

Lactic acid bacteria count in different treatments of fish fillets during storage at 4°C for 12 days.

Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film incorporated with cinnamon essential oil (CEO); AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zain film incorporated with CEO 10%(v/v). A-D: different uppercase letters show differences between treatments for same storage times ($p < 0.05$). a-e: different lowercase letters show differences between the storage times within same analysis group ($p < 0.05$).

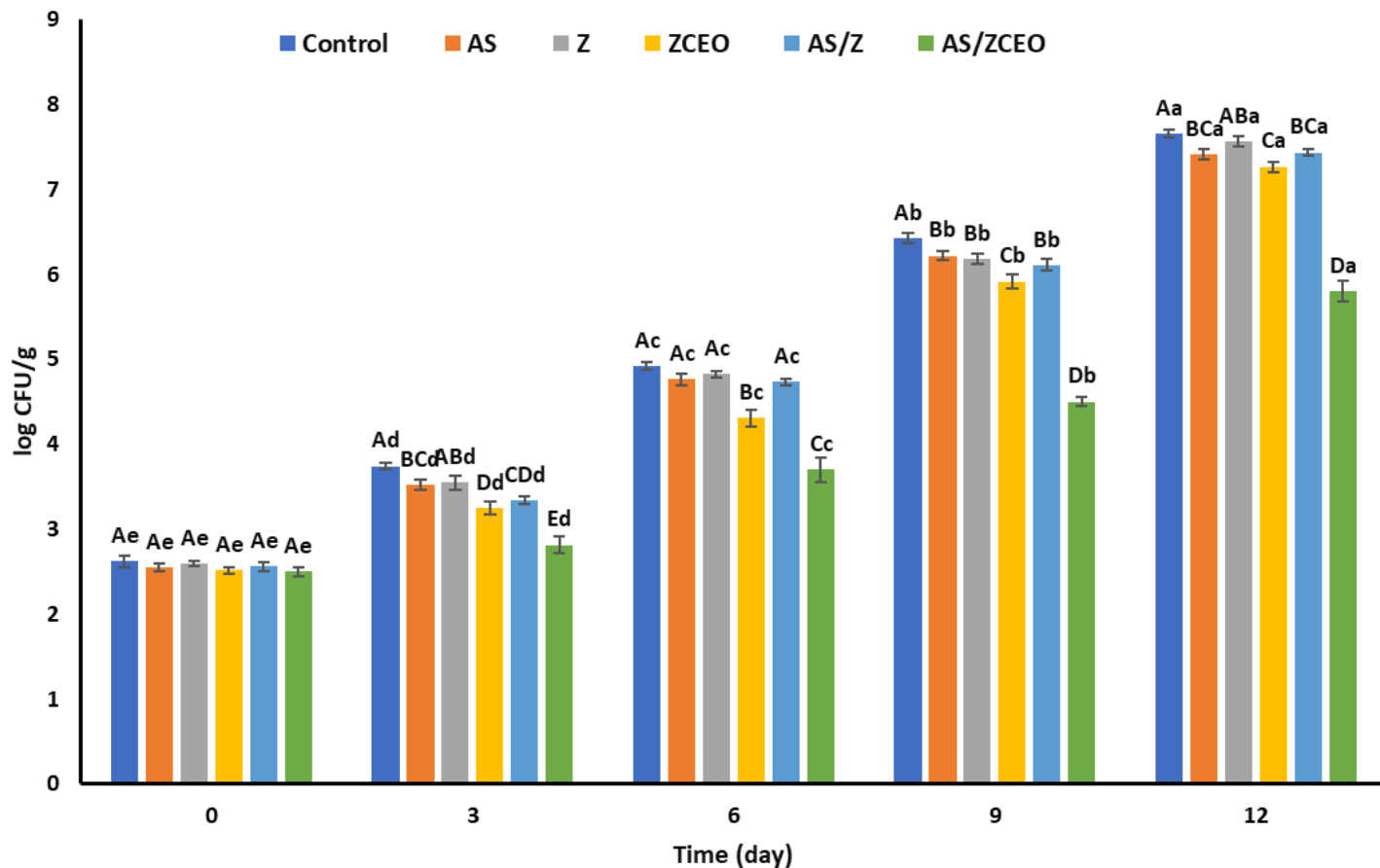


Figure 3

Enterobacteriaceae population in different treatments of fish fillets during storage at 4°C for 12 days. Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film incorporated with cinnamon essential oil (CEO) ; AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zain film incorporated with CEO 10%(v/v). A-D: different uppercase letters show differences between treatments for same storage times ($p < 0.05$). a-e: different lowercase letters show differences between the storage times within same analysis group ($p < 0.05$).

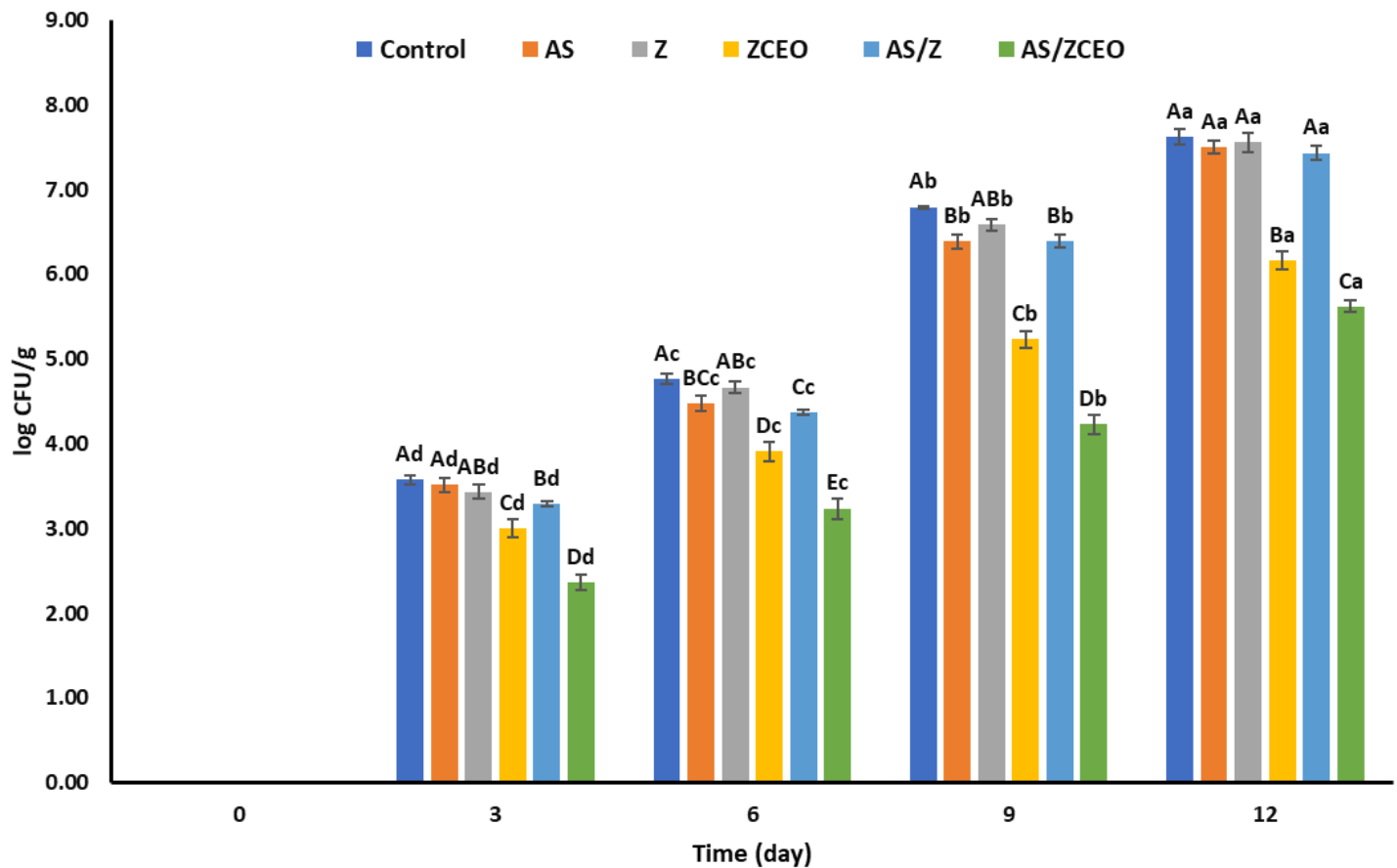


Figure 4

Yeast and mold count in different treatments of fish fillets during storage at 4°C for 12 days. Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film incorporated with cinnamon essential oil (CEO) ; AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zain film incorporated with CEO 10%(v/v). A-D: different uppercase letters show differences between treatments for same storage times ($p < 0.05$). a-e: different lowercase letters show differences between the storage times within same analysis group ($p < 0.05$).

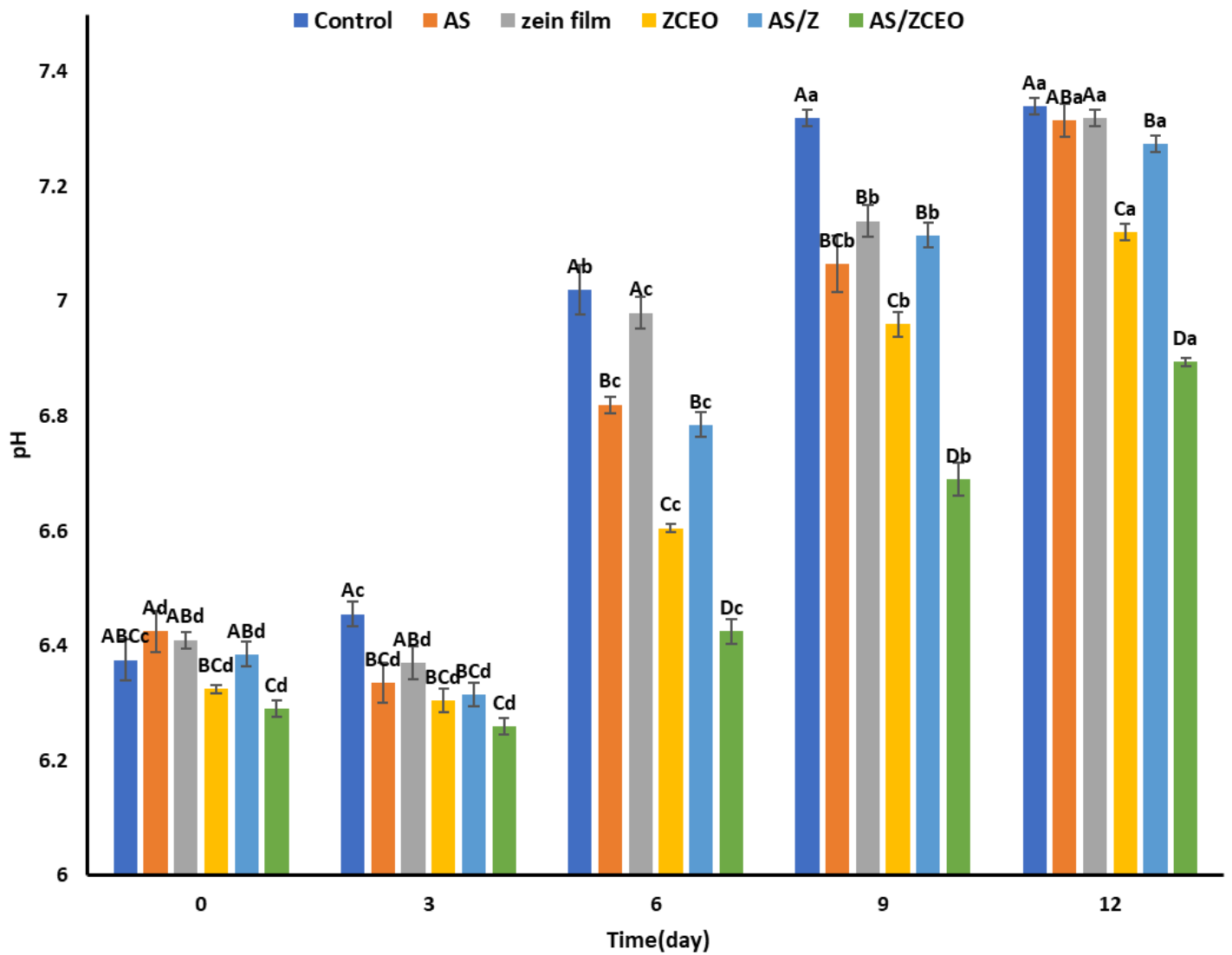


Figure 5

pH value of different treatments of fish fillets during storage at 4°C for 12 days. Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film incorporated with cinnamon essential oil (CEO); AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zain film incorporated with CEO 10%(v/v). A-D: different uppercase letters show differences between treatments for same storage times ($p < 0.05$). a-e: different lowercase letters show differences between the storage times within same analysis group ($p < 0.05$).

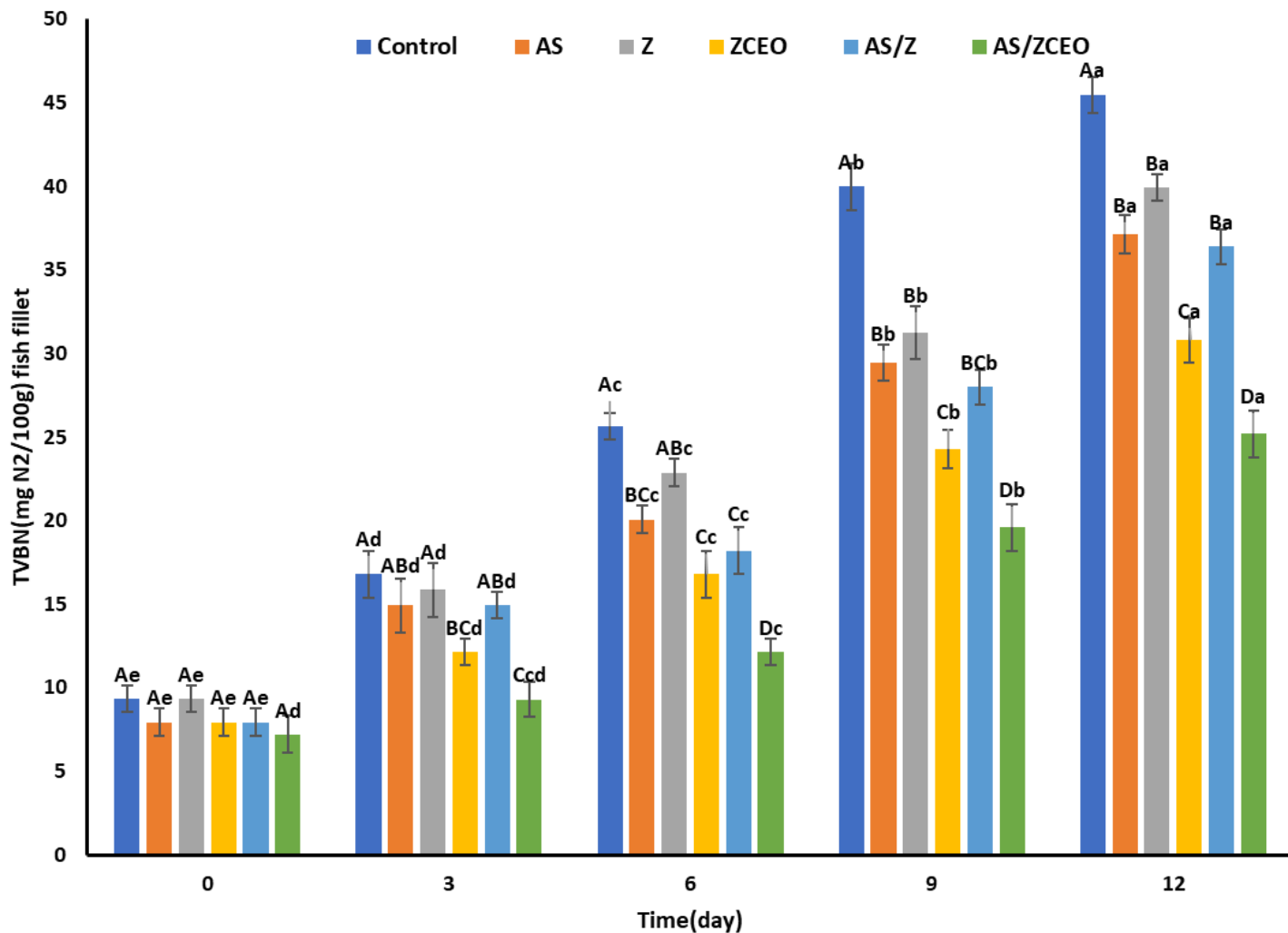


Figure 6

TVB-N value of different treatments of fish fillets during storage at 4°C for 12 days. Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film incorporated with cinnamon essential oil (CEO) ; AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zain film incorporated with CEO 10%(v/v). A-D: different uppercase letters show differences between treatments for same storage times ($p < 0.05$). a-e: different lowercase letters show differences between the storage times within same analysis group ($p < 0.05$).

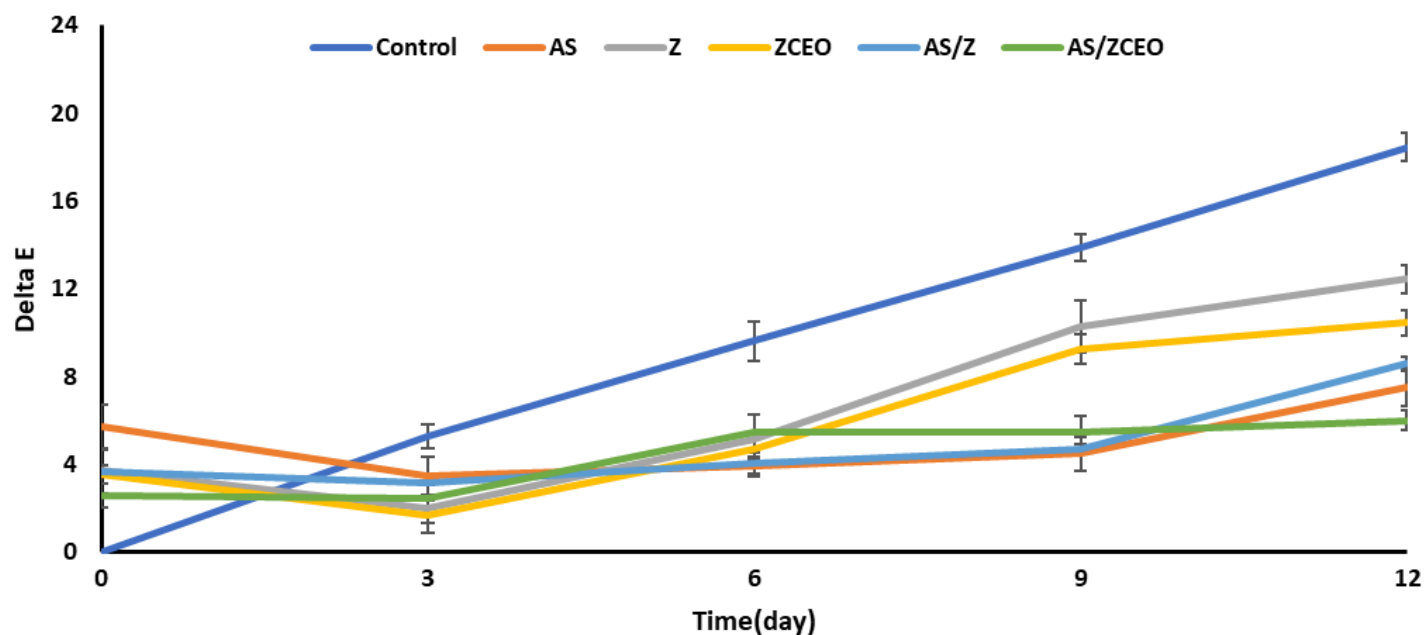


Figure 7

Changes in the color of different treatments of fish fillets during storage at 4°C for 12 days. Control: non-coated samples; AS: samples coated with acorn starch film; Z: samples coated with zein electrospun film; ZCEO: samples coated with zein electrospun film incorporated with cinnamon essential oil (CEO); AS/Z: samples coated with bilayer acorn starch and zein film, and AS/ZCEO: sample coated with bilayer acorn starch and zein film incorporated with CEO 10%(v/v).

Supplementary Files

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