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Microbial, chemical and sensory properties of coated rainbow trout Fillets by Fenugreek Grain containing Essences of Thyme and Oregano during Refrigerated storage

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ABSTRACT

This study was conducted to investigate the effect of polysaccharide coating on fenugreek seeds containing essential oil of thyme and oregano (1, 1.5 and 2%) as natural preservatives on shelf life of rainbow trout kept at refrigerated temperature based on microbial load, chemical evaluations and sensory properties done. For this purpose, fish fillets under 8 treatments were kept refrigerated at room temperature for 16 days and pH, peroxide, thiobarbituric acid, thymethylamine, volatile nitrogen vapor, microbial analysis and sensory evaluations were performed.Regarding to the general standards of bacteria, the total number of volatile nitrogen levels, the amount of thymethylamines, as well as sensory evaluation, the control samples exceeded the standard levels after 4 days (After 16 days of storage TVC 9.3 log cfu/g, PTC 8.3 log cfu/g, PV 6.4 meq/kg, TBA 0.95 mgMDA/kg, TVB-N 41 N/100g, TMA-N 13 mg/100g and total acceptance 2.5), while the samples were covered with 2% oregano coatings for 12 days (After 16 days of storage TVC 7 log cfu/g, PTC 6.8 log cfu/g, PV 4 meq/kg, TBA 0.43 mgMDA/kg, TVB-N 21 N/100g, TMA-N 6.5 mg/100g and total acceptance 5 respectively) and Covered specimens containing 2% Thyme essential oil were 16 days old (After 16 days of storage TVC 7 log cfu/g, PTC 6.5 log cfu/g, PV 4.6 meq/kg, TBA 0.49 mgMDA/kg, TVB-N 15 N/100g, TMA-N 5.2 mg/100g and total acceptance 6 respectively).

1- Introduction

Seafood plays a crucial role in human diets, possessing high nutritional value due to its easily digestible protein, low-calorie content, and beneficial Omega-3 fatty acids [1]. However, the perishability of fish presents challenges due to the presence of unsaturated fatty acids and enzymes that can lead to oxidation, resulting in changes to flavour and quality [2]. Oxidation leads to the production of volatile compounds such as hydroperoxides, fatty acids, ketones, and others, which alter the organoleptic properties [3-4]. Rainbow trout (*Oncorhynchus mykiss*) is one of the most desirable farmed fish in Iran, with consumption demand increasing daily. Notably, one of this fish's most important features is its fresh, non-frozen presentation. Given increasing consumer awareness, the demand for fresh fish has generally risen compared to frozen fish [5].

However, due to fish's high perishability, non-frozen storage significantly reduces its shelf life, as temperature reduction is one of the most effective factors in increasing fish shelf life [6]. Rainbow trout is classified as a fatty fish, and quality degradation in fatty fish primarily occurs due to the activity of microorganisms and lipid oxidation [7]. Therefore, for short-term storage of fish meat, freezing not only imposes a high cost but also decreases its marketability. Considering the high production and consumption of rainbow trout in Iran, actions to diversify the product and increase its shelf life while maintaining quality have become necessary. In this regard, one of the innovative preservation methods is the use of polysaccharide biocoatings in packaging.

Packaging through edible coatings and biodegradable polymers containing antioxidant and antimicrobial compounds can protect seafood products against water vapour, gases, mechanical and chemical damage, and microbial activity [8]. The use of extracted polysaccharides is now widely developed, mainly due to their safety, low cost, and availability. Polysaccharides are high molecular weight compounds that can be easily dissolved or dispersed in water under suitable conditions [9]. These compounds can be extracted from various plant and animal sources. However, using single-coating materials may not provide the necessary practical requirements for protection, and combining multiple materials with polysaccharides enhances the coating's functional and physical properties [10].

Plant sources are the most widely used for the extraction of hydrocolloid compounds, and one such source is the Fenugreek seed. Fenugreek (*Trigonella foenum-graceum* L.) is an annual herbaceous plant belonging to the Leguminosae family, native to the Eastern Mediterranean. The main components of the seed include saponins, alkaloids, and mucilaginous fibres (50%). Fenugreek is neutral and contains galactomannan and some xylan [11]. Given the drawbacks, such as the carcinogenic effects of chemical preservatives, and increased public awareness, a negative perception of synthetic food additives has developed among consumers, leading to an increased desire for natural preservatives to extend food shelf life.

For this reason, the use of herbal extracts and essential oils as preservatives has recently received particular attention. The importance of using these products for food packaging is 1ncreased by enriching edible films and

coatings with antimicrobial and antioxidant agents [12]. Multilayer coating systems have emerged as a promising approach as a type of active packaging. This technique provides improved coating properties such as reduced textural degradation, increased mechanical strength, extended shelf life, and higher barriers to water and gases [13-14]. Two of the most important plants with significant antioxidant, antimicrobial, and antifungal properties is thyme and oregano. Therefore, the objective of this research is to investigate the effect of fenugreek seed polysaccharide coating containing thyme and oregano essential oils on increasing the shelf life of refrigerated rainbow trout fillets.

2- Materials and Methods

2-1- Fish Sample Collection and Preparation

Rainbow trout (*Oncorhynchus mykiss*) were procured from a local fish farm in Babolsar County (Iran). The fish were immediately transported to the Food Science Laboratory in insulated boxes containing ice to minimize time and temperature variations. Upon arrival, the fish underwent manual processing, including gutting and deheading. Three fillets were subsequently prepared from each fish.

2-1-1- Preparation of Fenugreek Seed Polysaccharide (FSP) Coating

2.2.1. Polysaccharide Extraction

For polysaccharide extraction, fenugreek seeds (Babol, Iran) were processed according to the optimized method of Sabaghpoor et al. [15]. First, defatting was performed by mixing 100 g of dried fenugreek seed powder with hexane at 50°C for 2 hours. For subsequent ultrasound-assisted extraction (using a GTsonic ST13 ultrasound bath, China), the following optimized parameters were applied: a liquid-to-solvent ratio of 14.6 mL/g, an extraction temperature of 65°C, and an extraction time of 43 minutes.

Post-extraction, the solution was filtered through a single layer of 40-mesh cloth. The filtrate was then concentrated using a rotary evaporator (102/202, Steroglass S.R.L., Italy) at 50°C. Polysaccharide precipitation was induced by adding ethanol (Merck, Germany) to a final concentration of 80%. The precipitate was collected via centrifugation (HeHich, Universal 320R, Germany) at 5000×g for 20 minutes, washed with absolute ethanol and acetone, and finally dried to a constant weight at 50°C.

Preliminary tests indicated that FSP alone did not form a stable film; thus, the polysaccharide was formulated in combination with gelatin to ensure proper film formation [16-17]. The plasticizers used were glycerol and sorbitol (Merck, Germany). Initially, the solvent and the FSP/gelatin polymer mixture were combined and stirred on a magnetic hot plate at 45°C–50°C for 30 minutes. The plasticizers and the required essential oil (EO)—either thyme or oregano essential oil (Kimia Osareh Shargh, Iran)—were subsequently added. This mixture was stirred for an additional 15 minutes at 45°C, cooled, and then placed in a vacuum oven for 15 minutes to facilitate deaeration (bubble removal).

2-1-2- Fish Fillet Coating and Storage

The fish fillets were randomly divided into eight experimental groups:

1. Group 1: Uncoated control samples.
2. Group 2: Fillets coated with the FSP/gelatin base (without EO).

3. Groups 3, 4, and 5: Fillets coated with FSP/gelatin containing 1.0%, 1.5%, and 2.0% Thyme Essential Oil (TEO), respectively.

4. Groups 6, 7, and 8: Fillets coated with FSP/gelatin containing 1.0%, 1.5%, and 2.0% Oregano Essential Oil (OEO), respectively.

All microbiological, chemical, and sensory analyses were performed on all eight groups on days 1, 4, 8, 12, and 16 of the storage period at 4◦C.

2-2- Sample Analysis

2-2-1- pH Measurement

The pH was determined by homogenizing 10 g of the sample in 90 mL of distilled water. The pH of the mixture was then measured by inserting the electrode of a pH meter (ISTEK.Inc, South Korea) [18].

2-2-2- Peroxide Value (PV) Determination

The PV analysis began by mixing 150 g of the sample with 250 mL of chloroform (Merck, Germany). The mixture was filtered, and the filtrate was passed through a second filter half-filled with dry sodium sulfate (Merck, Germany). A 25 mL aliquot of the resulting solution was taken, and 37 mL of glacial acetic acid (Merck, Germany) and 1 mL of saturated potassium iodide (Merck, Germany) were added. After 1 minute, 30 mL of distilled water and a small amount of starch indicator were added. The liberated iodine was titrated against 0.01 N sodium thiosulfate solution (Merck, Germany) until a milky colour appeared. The PV was calculated using the following equation (as milliequivalents per kg of fat) [19]:

$$PV = \frac{1000 \times N \times \text{sodium thiosulfate } V}{Sample\ Weight}$$

2-2-3- Trimethylamine (TMA) Content

The fish extract was prepared using 5% trichloroacetic acid (Merck, Germany) followed by centrifugation (2500×g for 10 minutes). One milliliter of the extract was combined with 1 mL of 10% formaldehyde (Merck, Germany) in a test tube, and the volume was adjusted to 5 mL with distilled water. Next, 3 mL of 25% potassium hydroxide solution was added, followed by 10 mL of toluene, bringing the final volume to 18 mL. The tube was placed in a water bath (Memmert GmbH, Germany) at 30◦C for 5 minutes, agitated 40–60 times, and left at room temperature for 10 minutes to allow complete phase separation. A 2 mL aliquot of the upper phase was mixed with 2 mL of 0.02% picric acid solution. The resulting color change was measured spectrophotometrically (UV2100, Taiwan) at a wavelength of 410 nm [20].

2-2-4- Thiobarbituric Acid Reactive Substances (TBARS)

Ten grams of the sample were homogenized with 50 mL of distilled water for 2 minutes. This mixture was transferred to a distillation flask, washed with 47.5 mL of rinsing water, and 2.5 mL of 4 M hydrochloric acid (Merck, Germany) was added to adjust the pH to 1.5. Antifoam agent was also added. The flask was heated to collect 50 mL of distillate within 10 minutes from the start of boiling. Five milliliters of the distillate and 4 mL of TBA reagent (Merck, Germany) were transferred to a screw-cap tube and heated in a boiling water bath for 30 minutes. After cooling in water for 10 minutes, the absorbance was read at

538 nm using a spectrophotometer (UV2100, Taiwan) [21].

2-2-5- Total Volatile Basic Nitrogen (TVB-N)

The TVB-N content was determined using the Kjeldahl method. Ten grams of the sample, plus 2 g of magnesium oxide (Merck, Germany) and 50 mL of distilled water, were placed in a distillation flask. The volatile nitrogenous bases were collected in a receiver solution containing 2% boric acid (Merck, Germany) and methyl red (Merck, Germany) as an indicator. The resulting solution was then titrated against sulfuric acid (Merck, Germany) until a purple color was achieved. Results were expressed as mg nitrogen per 100 g of fish [22].

2-2-6- Bacterial Analysis

For microbial testing, 10 g of the fish fillet meat was aseptically homogenized with 90 mL of 0.85% sodium chloride solution. Serial decimal dilutions (up to 10^6) were prepared using peptone water. One milliliter of each dilution was used for bacterial plating via the pour plate method [23].

- Total Viable Counts (TVC): Cultured on Plate Count Agar (Sigma-Aldrich, Germany) at 37°C for 2 days (INB400, Memmert GmbH, Germany).
- Psychrotrophic Bacterial Counts (PBC): Cultured on Plate Count Agar at 7°C for 10 days.

Colony counting on the plates was used to determine bacterial numbers.

2-2-7- Sensory Evaluation

The sensory quality of the fish fillets was assessed by five trained panelists from the food science laboratory staff. Evaluations were conducted blind, with samples identified only by randomized three-digit codes. Panelists evaluated the samples for overall acceptability using a 9-point Hedonic scale (1 = lowest score, 9 = highest score). Samples receiving a score below 4 were defined as unacceptable for consumption [24].

2-3- Statistical Analysis

All experiments were conducted with three replicates, and results were expressed as mean values. Differences between samples were analyzed using Analysis of Variance (ANOVA) at a significance level of $P<0.05$. SPSS software was used for analysis, and EXCEL software was used for plotting graphs. Duncan's multiple range test was used for mean comparison when the overall effect of treatments was significant. Kruskal-Wallis H test was used to determine significant differences among the sensory test results for the tested treatments [25].

3-Results and Discussion

3-1- Total and Psychrotrophic Microbial Counts

The Total Viable Counts (TVC) of the fish fillets are presented in Figure 1. The initial microbial load was 3.5 log cfu/g. After day 4 and throughout the rest of the storage period (16 days), the TVC in the pure (unloaded) polysaccharide-coated samples was consistently lower than that of the uncoated control, though this difference was not statistically significant. After 8 days of storage, the TVC in the uncoated rainbow trout fillets reached 7.6 log cfu/g, which is often considered the spoilage threshold, while

the level for the pure polysaccharide-coated fillets remained below 7 log cfu/g. For samples coated with varying concentrations of essential oil (EO), the TVC also increased with storage time, similar to the control, but at a significantly slower rate. The most pronounced inhibition of microbial growth was observed in samples coated with 2% Thyme Essential Oil (TEO). The overall bacterial count in the 2% TEO samples remained below the acceptable standard limit of 7 log cfu/g throughout the entire storage period. While the TVC for fillets coated with 2% Oregano Essential Oil (OEO) was higher than the 2% TEO group, the difference was not statistically significant ($P<0.05$). Gtiniti and Koyun (2013) reported similar results regarding microbial growth inhibition by chitosan films containing TEO in silver carp [26]. Furthermore, Harpaz et al. (2003) reported a reduction in total bacterial counts in Asian sea bass [27]. The synergistic effect of carvacrol and thymol present in the thyme and oregano EOs is attributed to their ability to destabilize the bacterial outer membrane by degrading the lipopolysaccharide layer and increasing the permeability of the cytoplasmic membrane to ATP molecules, ultimately leading to bacterial death [28]. Both TEO and OEO are rich in phenolic compounds like thymol and carvacrol, and contain lower levels of terpenic compounds such as p-cymene and γ-terpinene.

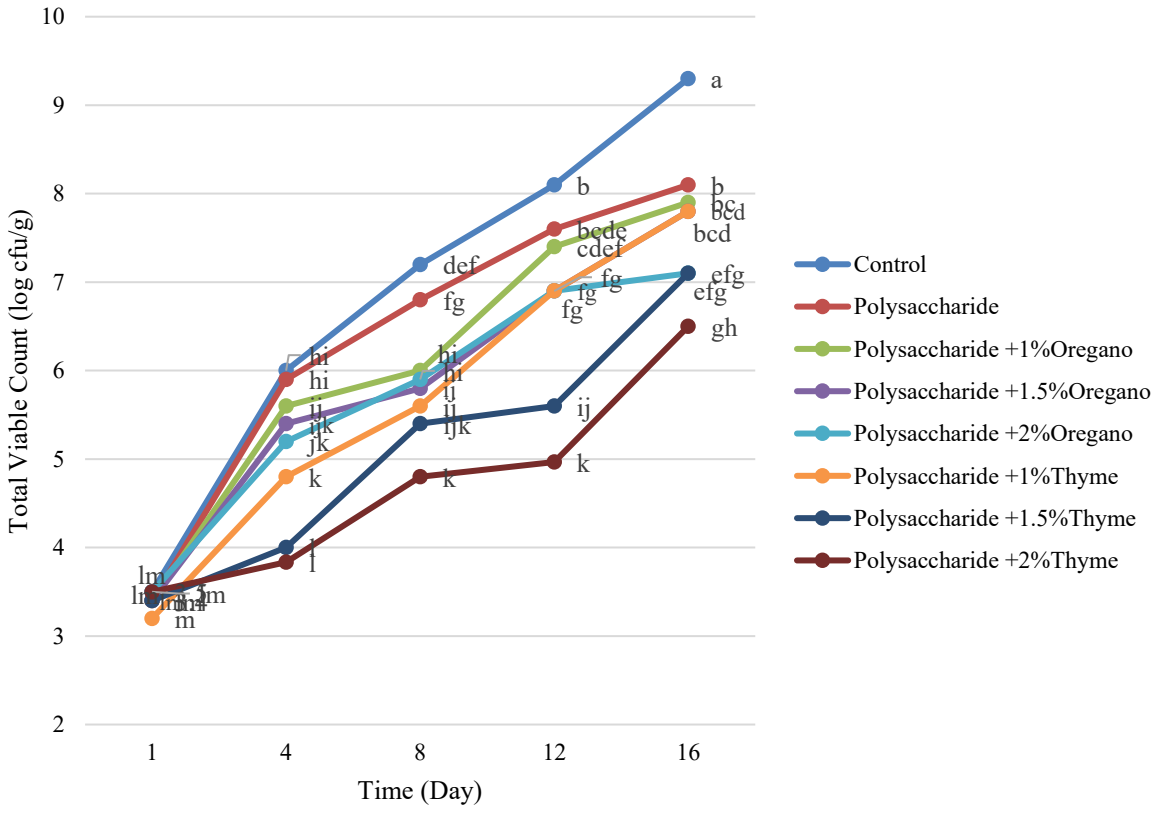

Fig 1. Changes in TVC of fish samples during storage.

The initial count for Psychrotrophic Bacterial Counts (PBC) was 3.1 log cfu/g, which rapidly increased to 5.6 log cfu/g and 8.3 log cfu/g on days 8 and 16, respectively (Figure 2). Polysaccharide-coated samples showed a smaller logarithmic cycle reduction in PBC compared to the control. Notably, the PBC in samples coated with 2% OEO or 2% TEO were statistically significantly lower than the control ($P<0.05$). Similar inhibitory effects against PBC were reported by Ojagh et al. (2009) using chitosan coatings combined with cinnamon essential oil on rainbow trout fillets [23].

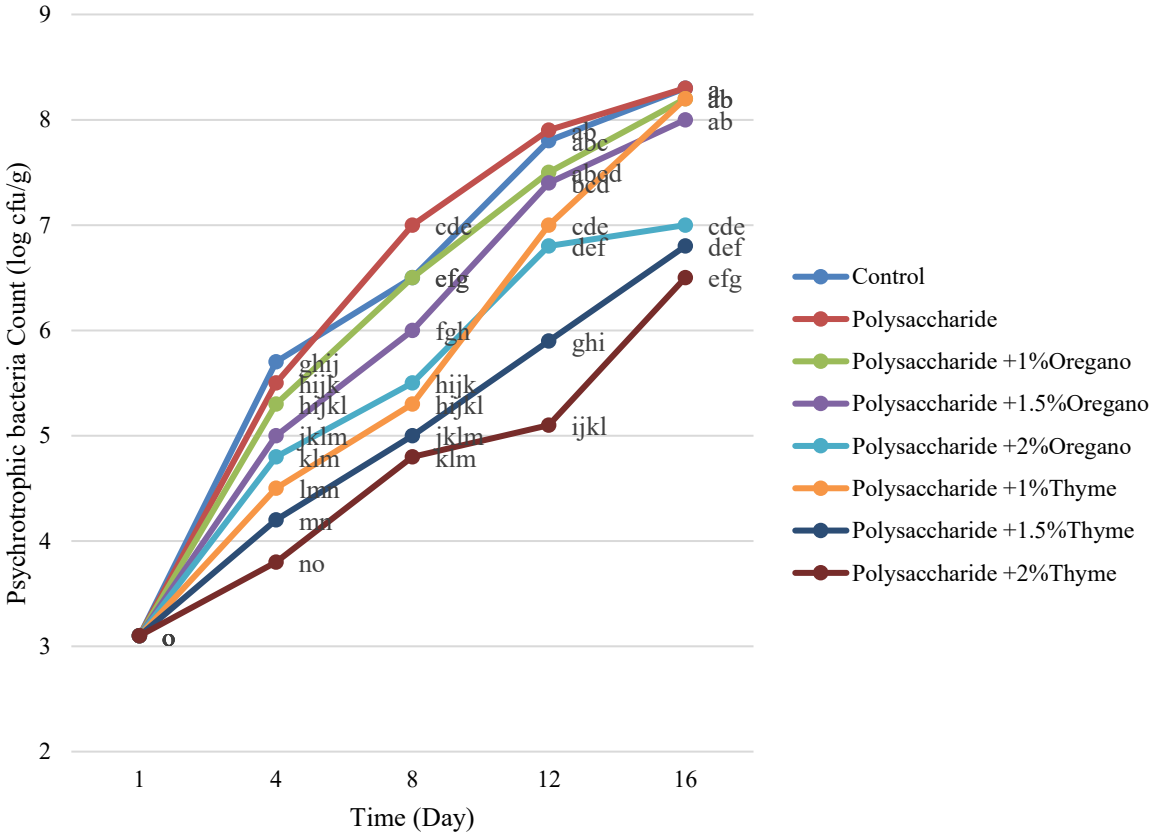

Fig 2. Changes in PTC of fish samples during storage.

3.2. Changes in Fillet pH

pH changes in the fish fillets are illustrated in Figure 3. The initial pH was 6.4, consistent with values reported by Arashisar et al. (2004) and Gimenez et al. (2002) [29,30]. The pH initially decreased until day 4, a reduction typically associated with post-mortem glycolysis in fish muscle [31]. Similar trends have been observed in studies on gilt-head sea bream and clams [32,33]. After 4 days, the pH of all fillets significantly increased with extended storage time ($P<0.05$). This pH increase is caused by the accumulation of volatile basic compounds, such as ammonia and trimethylamine (TMA), which are metabolic products of endogenous and bacterial enzymes within the fish tissue [33]. This rising pH negatively impacts quality

attributes, particularly odor, color, and texture, throughout the storage period [32]. The least change in pH was observed in the samples coated with 2% TEO, confirming the superior antimicrobial efficacy observed in the microbial tests, as 2% TEO coatings effectively inhibited the microorganisms responsible for producing basic metabolites.

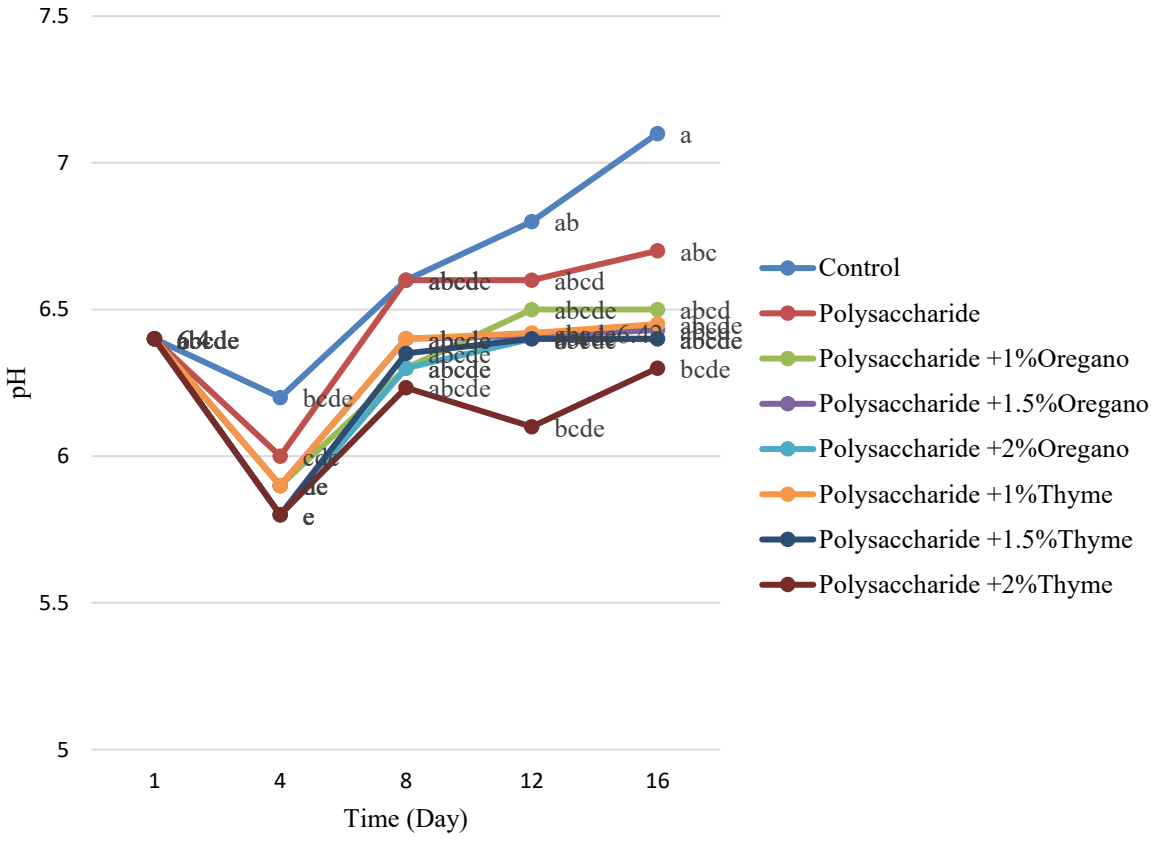

Fig 3. Changes in pH of fish samples during storage.

3-3- Changes in Peroxide Value (PV)

The changes in the PV of the fish fillets are shown in Figure 4. The initial PV of the rainbow trout fillets was 1.26 mEq peroxide/kg, with no significant difference between samples on day 1 ($P<0.05$). PV significantly increased in all samples during storage ($P<0.05$). Fillets coated with the pure polysaccharide coating exhibited a statistically significant lower PV compared to the control, suggesting that the fenugreek seed polysaccharide itself possesses inherent antioxidant activity that delays initial lipid oxidation. The results further showed that the 2% OEO coating was the most effective treatment for reducing the rate of oxidation during the primary phase. On day 16, the PV in the 2% OEO-coated samples was 43% lower than that of the control. This high efficacy is likely attributable to the high concentration of carvacrol (81.85%) in oregano essential oil. The PV of the control samples remained below 10 mEq/kg throughout the 16 days, possibly because the

fillets were stored in the dark and away from light, a condition that allows even low EO concentrations (1%) to effectively impede oxidation progression. A decrease in PV was noted in control samples after day 12, potentially due to the breakdown of hydroperoxides or their reaction with fish muscle proteins [34]. Storage time had a significant effect on PV ($P<0.05$). These findings align with the work of Ojagh et al. (2009), who reported that chitosan films containing cinnamon EO were effective in preventing the initial progression of fish lipid oxidation [23].

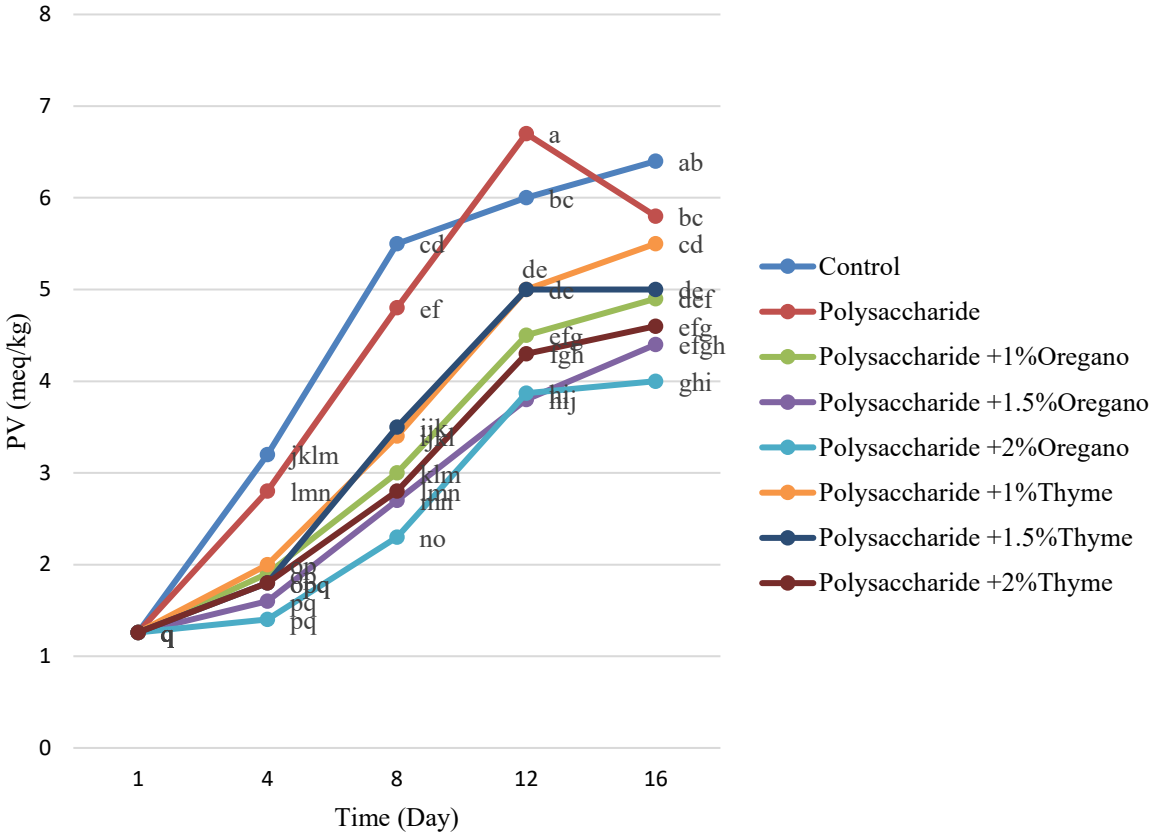

Fig 4. Changes in PV of fish samples during storage.

3-4- Changes in Thiobarbituric Acid Reactive Substances (TBARS)

TBARS changes are shown in Figure 5. The initial TBARS value was 0.12 mg MDA/kg, consistent with reports by Ojagh et al. (2010) and Rezai et al. (2008) [23,35]. The TBARS values for both control and coated samples increased over the storage period. By the end of storage (day 16), the TBARS values for the control, pure polysaccharide, 2% TEO, and 2% OEO groups were 0.95, 0.89, 0.49, and 0.43 mg MDA/kg, respectively. The antioxidant activity of the EOs is attributed to multiple mechanisms, including preventing radical initiation, limiting the transfer of metal ion catalysts, peroxide decomposition, and interaction with free radicals [36,37]. The increase in TBARS was significantly lower in

samples coated with OEO compared to the control. Oregano possesses high antioxidant activity due to its elevated content of phenolic acids and flavonoids [38,39]. Fernandez et al. (1997) suggested that TBARS may not accurately reflect the actual rate of lipid oxidation over the entire shelf life due to interactions between malondialdehyde, amino acids, proteins, and glucose in the fish fillets [39]. This could account for the observed decrease in TBARS after day 12 in the fillet samples.

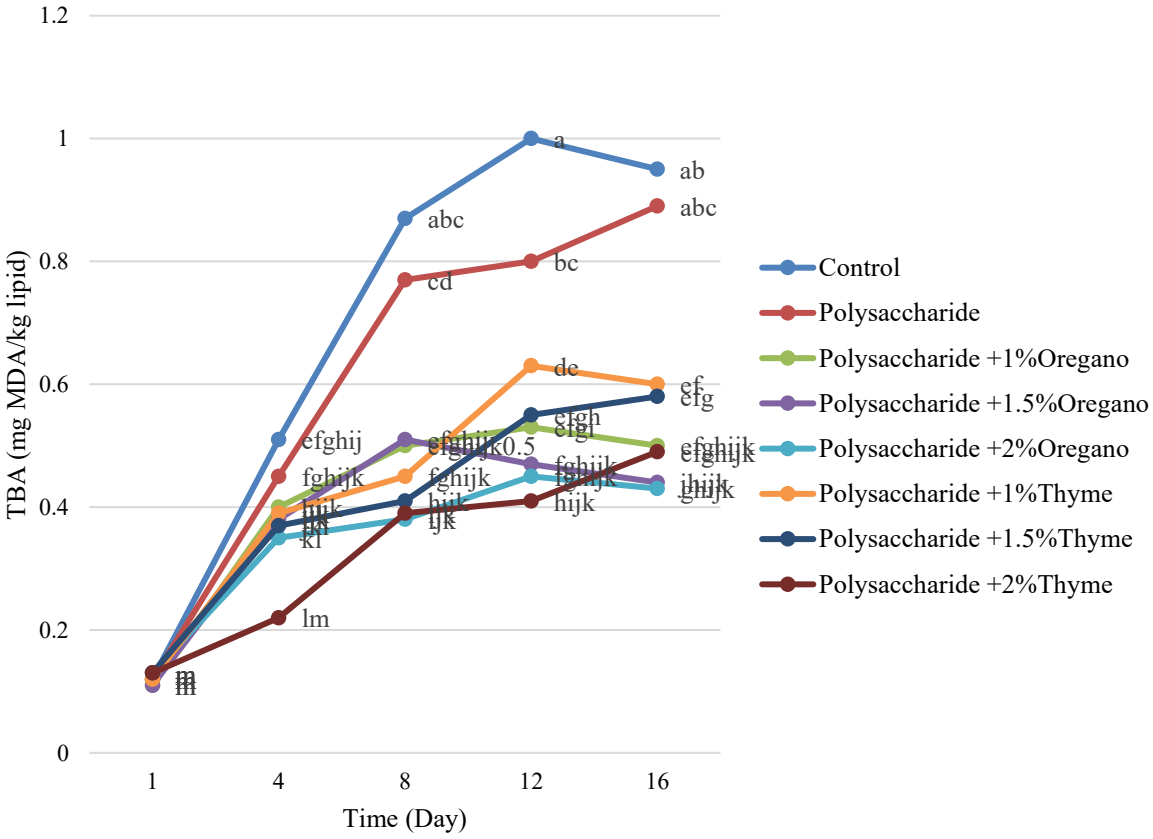

Fig 5. Changes in TBA of fish samples during storage.

3.5. Changes in Total Volatile Basic Nitrogen (TVB-N)

The increase in TVB-N for all samples during storage is shown in Figure 6. The initial TVB-N value (9.23 mg N/100g) suggests good quality of the rainbow trout fillets, correlating with the relatively low initial TVC (3.5 log cfu/g). The initial TVB-N in this study was higher than that reported by Arashisar et al. (2004) (<8 mg N/100g) [24] but lower than the range reported by Ojagh et al. (2009) (9.33–12.13 mg N/100g) [23]. The European Commission suggests a TVB-N level of 35 mg N/100g as the limit indicating the start of primary spoilage in fresh fish [41]. However, Gimenez et al. (2002) and Masniyom et al. (2002) proposed 25 mg N/100g as the point of initial spoilage for rainbow trout fillets and sea bass slices, respectively [30, 42]. In this study, the TVB-

N level for samples coated with various EO concentrations remained below 25 mg N/100g throughout the storage period, whereas the control and pure polysaccharide-coated samples reached this acceptable limit after 8 and 12 days, respectively (Figure 6). At the end of storage (day 16), the lowest TVB-N (15 mg N/100g) was observed in the 2% TEO-coated samples compared to the control (41 mg N/100g) ($P<0.05$). The lower TVB-N in the TEO-coated samples correlates with the superior microbial activity of the coating [43]. TEO and OEO mitigate the increase in TVB-N by reducing microbial growth and/or reducing the bacteria's capacity for oxidative deamination of non-protein nitrogenous compounds [44].

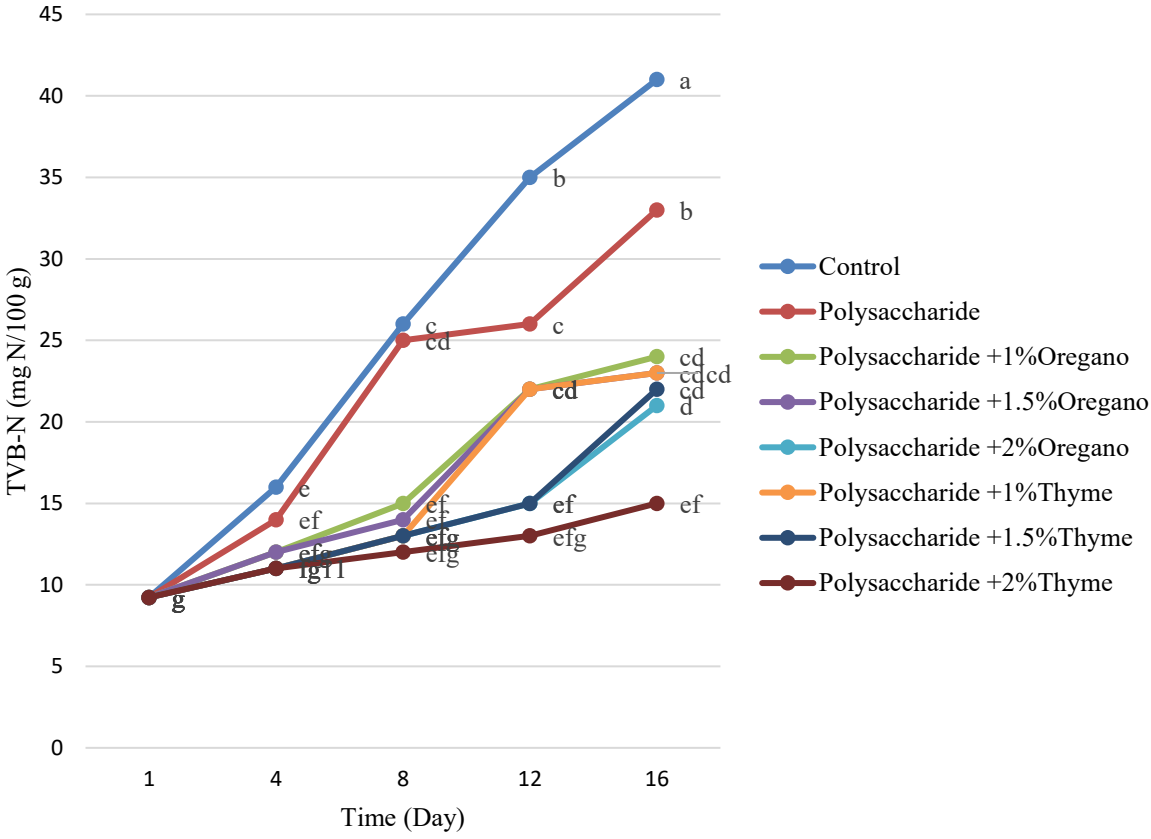

Fig 6. Changes in TVB-N of fish samples during storage.

3.6. Changes in Trimethylamine (TMA-N)

The changes in TMA-N during storage are presented in Figure 7. The initial TMA-N value (1.5 mg N/100g) confirms the good initial quality, aligning with the low initial microbial load (3.5 log cfu/g). TMA-N increased in all samples over time. Masniyom et al. (2002) reported 5 mg N/100g as the acceptable limit for sea bass fillets [42], while Kilinc et al. (2007) reported 8 mg N/100g [44]. Based on sensory and microbiological data, 5 mg N/100g was considered the limit for rainbow trout fillets in this study. Applying this limit, the control and pure polysaccharide-coated samples were deemed unacceptable by

day 8, while the 2% TEO-coated samples failed by day 16. TMA-N levels in the pure polysaccharide-coated samples showed no significant difference compared to the control throughout the storage period, indicating that the polysaccharide alone had no inhibitory effect on TMA-N accumulation. Dalgaard (2000), Connell (1990), and Souza et al. (2010) reported that TMA-N and TVB-N accumulation directly correlate with microbial spoilage and bacterial activity in various fish species during storage [40,45,46].

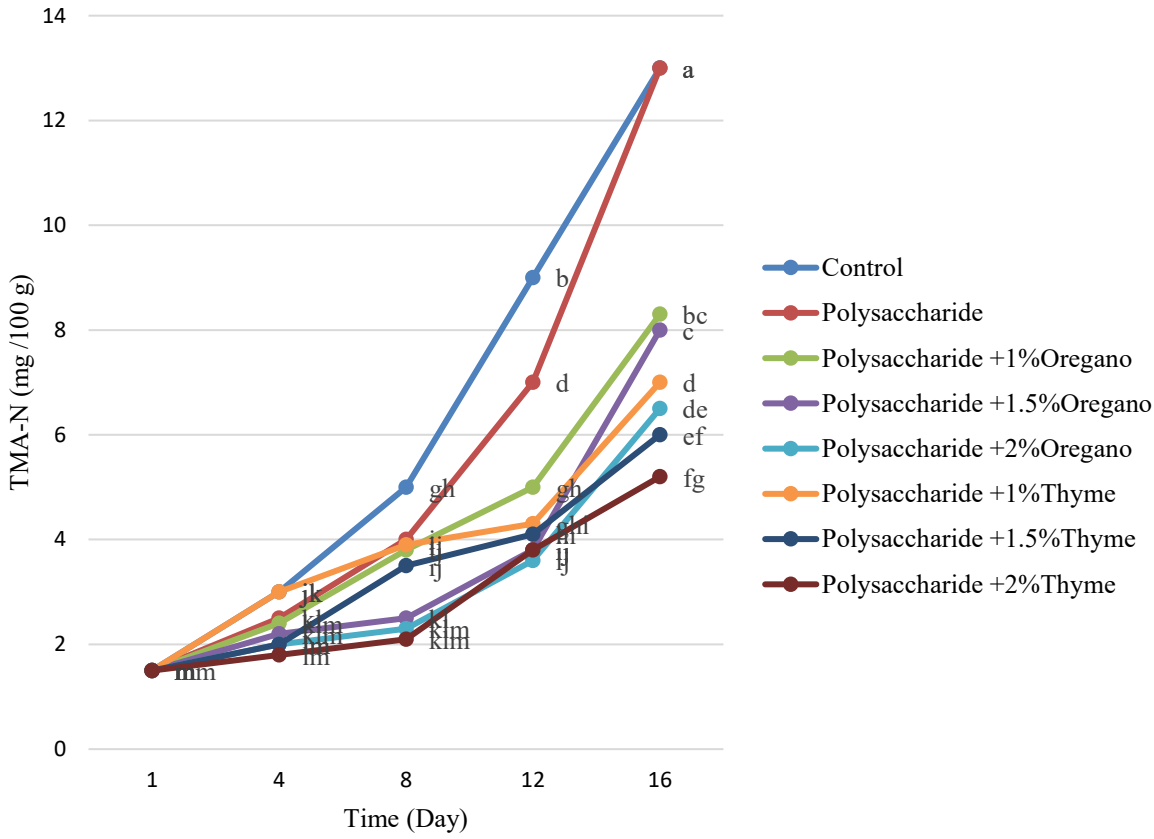

Fig 7. Changes in TMA-N of fish samples during storage.

3.7. Changes in Sensory Properties

The results of the sensory evaluation (overall acceptability) are shown in Figure 8. The application of TEO or OEO coatings did not negatively impact the sensory properties ($P>0.05$). Samples with a sensory score greater than 4 were considered acceptable [24,44,47]. Sensory scores (specifically odor) dropped below the unacceptable threshold of 4 for the control samples after 8 days and for the pure polysaccharide-coated samples after 12 days. After 8 days, the odor of the control samples became unacceptable, while it remained acceptable for all EO-coated samples. Based on sensory analysis, the shelf life of the fillets was determined to be 8 days for the control, 12 days for the pure polysaccharide coating, and 16 days for the

2% TEO and 2% OEO coatings. The coated samples retained a more pleasant odor and firmer texture compared to the control, likely due to the antioxidant and antimicrobial properties of the incorporated EOs. These findings are consistent with Ojagh et al. (2010), who reported that cinnamon-containing films maintained fish quality without detrimental effects on texture, color, odor, or overall acceptability for 16 days [23]. Similar results were reported by Kykkidou et al. (2009) for Mediterranean sea bass [48]. Coatings containing 1.5% and 2% TEO and 2% OEO effectively inhibited the microbial and chemical activities responsible for spoilage odors due to their high antimicrobial and antioxidant characteristics. Mexis et al. (2009) reported a sensory shelf life of 4 days for control rainbow trout fillets, 7–8 days for oregano EO-coated samples, and 17 days for samples coated with oregano EO plus an oxygen absorber [47].

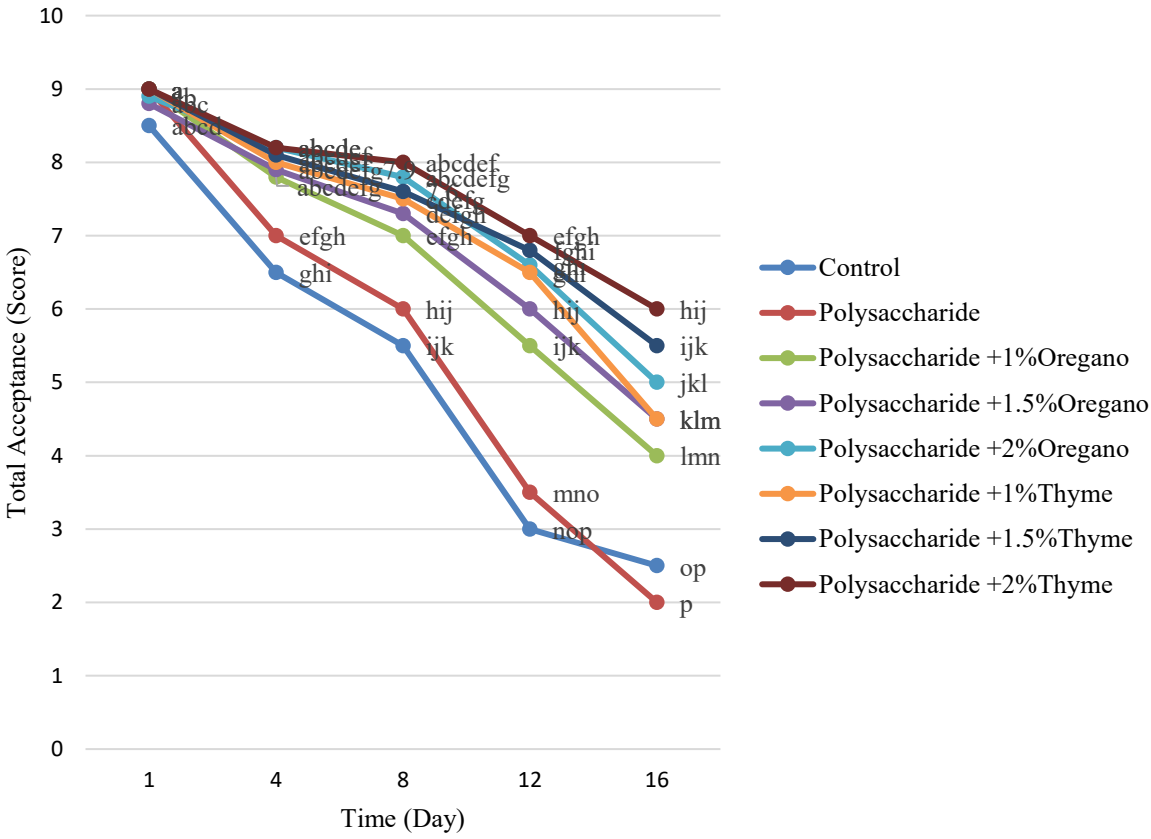

Fig 8. Changes in Sensory properties of fish samples during storage.

4- Conclusion

The results of this study demonstrated that coating rainbow trout fillets with fenugreek seed polysaccharide coatings containing thyme and oregano essential oils significantly extended the shelf life by inhibiting lipid oxidation, microbial spoilage, and chemical reactions. The use of the coating containing 2% TEO extended the shelf life of the fish fillets by at least 12 days. Based on the

standards for TVC, TVB-N, TMA-N, and sensory evaluation, the control samples exceeded the acceptable limits after 4 days. In contrast, the samples coated with 2% OEO remained acceptable for 12 days, and those coated with 2% TEO remained acceptable for 16 days. In conclusion, this research successfully introduced a novel coating with desirable antioxidant and antimicrobial properties to the field of food packaging, significantly enhancing the post-harvest quality and preservation of fresh rainbow trout.

5-References

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ویژگی‌های میکروبی، شیمیایی و حسی فیله‌های پوشش داده‌شده قزل‌آلای رنگین‌کمان با دانه شنبلیله حاوی اسانس آویشن و پونه کوهی طی نگهداری در یخچال

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چکیده

این پژوهش جهت بررسی اثر پوشش پلی‌ساکارید دانه شنبلیله حاوی عطرمایه آویشن و پونه کوهی به میزان (۱، ۱/۵، ۲٪) به‌عنوان مواد نگه‌دارنده طبیعی بر ماندگاری فیله ماهی قزل‌آلای رنگین‌کمان نگهداری شده در دمای یخچال بر اساس بار میکروبی، ارزیابی‌های شیمیایی و خصوصیات حسی انجام پذیرفت. بدین منظور فیله ماهی تحت ۸ تیمار به مدت ۱۶ روز در دمای یخچال نگهداری و تغییرات pH، پراکسید، تیوباربیتوریک اسید، تری‌متیل‌آمین، بازهای نیتروژنی فرار، آنالیز میکروبی و ارزیابی‌های حسی صورت پذیرفت. با توجه به استانداردهای شمارش کلی باکتری‌ها، مجموع بازهای ازته فرار، میزان تری‌متیل‌آمین و هم‌چنین ارزیابی حسی، نمونه‌های شاهد بعد از ٤ روز از حدود استاندارد تجاوز کردند (پس از ۱۶ روز نگهداری شمارش کلی باکتری‌ها ۹/۳ log cfu/g، شمارش کلی باکتری‌های سرمادوست ۸/۳ log cfu/g، پراکسید ٦/٤ meq/kg، تغییرات TBA معادل ۰/۹۵ mgMDA/kg، مجموع بازهای ازته فرار ٤۱ N/100g، میزان تری‌متیل‌آمین ۱۳ mg/100g و پذیرش کلی ۲/۵) درحالی‌که این میزان برای نمونه‌های پوشش دهی شده با پوشش‌های حاوی ۲٪ عطرمایه پونه کوهی ۱۲ روز (پس از ۱۶ روز نگهداری به ترتیب ۷ log cfu/g، ٦/۸ log cfu/g، ٤ meq/kg، ۰/٤۳ mgMDA/kg، ۲۱ N/100g، میزان تری‌متیل‌آمین ٦/۵ mg/100g و پذیرش کلی ۵) و برای نمونه‌های پوشش دهی شده با پوشش‌های حاوی ۲٪ عطرمایه آویشن، ۱۶ روز بود (پس از ۱۶ روز نگهداری به ترتیب ۷ log cfu/g، ٦/۵ log cfu/g، ٤/٦ meq/kg، ۰/٤۹ mgMDA/kg، ۱۵ N/100g، میزان تری‌متیل‌آمین ۵/۲ mg/100g و پذیرش کلی ٦).