

## Effect of Olive Leaf (*Olea europaea L.*) Extract-Enriched Coating on the Sensory and Microbial Quality of European Seabass (*Dicentrarchus labrax*) Fillets During Cold Storage

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**Abstract:** This study investigated the effects of gelatin-based coatings containing 1%, 2%, and 3% ethanolic olive leaf extract (EOLE) on the quality of European sea bass (*Dicentrarchus labrax*) fillets stored at +4 °C. Fish fillets were coated using the dipping method. In addition to sensory analysis, physicochemical and microbiological analyses were performed on days 1, 2, and 4 of cold storage. GC-MS results showed that di-(9-octadecenoyl)-glycerol (48.08%), dipalmitin (24.68%), glycidol stearate (11.69%), and 15-hydroxypentadecanoic acid (5.01%) were the primary constituents of EOLE. Sensory scores increased with higher coating concentrations, with the highest texture score observed in the 3% EOLE group ( $p < 0.05$ ). No significant differences were detected among the groups in pH and color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) on the same storage days ( $p > 0.05$ ). These changes were mainly attributable to storage duration. Total viable bacterial counts and psychrotrophic bacterial counts were similar in all experimental groups ( $p > 0.05$ ) and increased over the storage period. Coliform bacteria were detected only in the control group on day 2, while the coated groups remained below detectable levels. By day 4, the 3% EOLE group demonstrated significantly lower coliform counts than to other treatments ( $p < 0.05$ ). In conclusion, coatings containing EOLE contributed to the preservation of sensory qualities and showed a suppressive effect on coliform bacteria during refrigerated storage.

**Keywords:** Seafood, edible coating, agricultural by-product, sustainability, food safety

## Zeytin Yaprağı (*Olea europaea L.*) Ekstraktı ile Zenginleştirilmiş Kaplamanın Soğuk Depolama Süresince Avrupa Levreği (*Dicentrarchus labrax*) Filetolarının Duyusal ve Mikrobiyal Kalitesi Üzerine Etkisi

**Özet:** Bu çalışmada, %1, %2 ve %3 oranlarında etanolik zeytin yaprağı ekstresi (EOLE) içeren jelatin bazlı kaplamanın +4 °C'de saklanan Avrupa levreği (*Dicentrarchus labrax*) filetosunun kalitesi üzerindeki etkileri araştırılmıştır. Balık filetoları daldırma yöntemiyle kaplanmıştır. Duyusal analiz yanı sıra soğuk depolama sırasında 1. 2. ve 4. günlerde fizikokimyasal ve mikrobiyolojik analizler yapılmıştır. GC-MS sonuçları, di-(9-oktadekenoil)-gliserol (%48,08), dipalmitin (%24,68), glisidol stearat (%11,69) ve 15-hidroksipentadekanik asidin (%5,01) EOLE'nin ana bileşenleri olduğunu göstermiştir. Duyusal puanlar, kaplama konsantrasyonları arttıkça artmıştır. En yüksek doku puanı %3 EOLE grubunda belirlenmiştir ( $p < 0,05$ ). Aynı depolama günlerinde pH ve renk parametrelerinde ( $L^*$ ,  $a^*$ ,  $b^*$ ) gruplar arasında anlamlı bir değişiklik tespit edilmemiştir ( $p > 0,05$ ). Bu değişiklikler esas olarak depolama süresiyle ilgili olmuştur. Toplam canlı bakteri sayısı ve psikrotrofik bakteri sayısı depolama sırasında artmıştır, ancak deney grupları arasında benzerlik göstermiştir ( $p > 0,05$ ). Koliform bakteriler depolamanın 2. gününde yalnızca kontrol grubunda tespit edilmiş, kaplanmış gruplarda ise sayımlar tespit sınırının altında kalmıştır. 4. günde ise %3 EOLE uygulanan grupta, diğer uygulamalara kıyasla daha düşük koliform seviyeleri gözlenmiştir ( $P < 0,05$ ). Sonuç olarak, EOLE içeren kaplamalar duyu özelliklerinin korunmasına katkı sağlamış ve soğuk depolama süresince koliform bakteriler üzerinde baskılayıcı bir etki göstermiştir.

**Anahtar Kelimeler:** Deniz ürünleri, yenilebilir kaplama, tarımsal yan ürün, sürdürülebilirlik, gıda güvenliği

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## 1. Introduction

Today, the cultivation of European sea bass (*Dicentrarchus labrax*) farming represents one of the most significant sectors within the aquaculture industry (FAO, 2022; TÜİK, 2023). Sea bass is a highly valued fish globally and in Türkiye, and is widely preferred by consumers. This species is extensively farmed, particularly in the Mediterranean and Aegean regions. It is notably rich in protein, essential amino acids, omega-3 fatty acids, vitamins, and minerals (Küçükgülmez et al., 2006). The nutritional profile of sea bass makes it an excellent choice for a balanced, nutritious diet (Sikorski and Kolakowska, 2003). However, owing to its high nutritional content and inherent biochemical properties, sea bass is highly susceptible to quality deterioration during refrigerated storage. Rapid microbial proliferation and lipid oxidation have been reported as primary factors limiting its shelf life (Taliadourou et al., 2003). Consequently, implementing strategies to delay spoilage processes is crucial for maintaining the quality of the fillet.

Türkiye plays a significant role in global olive production. The number of olive (*Olea europaea*) trees grew from approximately 100 million in 2002 to 189 million in 2021 (Kılıç and Turhan, 2023). Consequently, olive production and exports increased. Notably, revenue from table olive exports rose from \$380,000 in 1982 to \$128 million in 2019 (Kılıç and Turhan, 2023). In addition to the contributions of olives and olive oil to the national economy, another valuable olive product is its leaf. Thanks to oleuropein and other phenolic compounds it contains, olive leaves have many pharmacological effects, such as antioxidant, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic, antiviral, hypoglycemic, and neuroprotective properties (Thielmann et al., 2017; Selim et al., 2022; Mumcu, 2023). Olive leaves are increasingly recognized as valuable byproducts in the food industry due to their antioxidant, antimicrobial, and natural preservative qualities (Ghanbari et al., 2012). The utilization of olive leaves as extracts promotes the utilization of an agricultural by-product, supporting economic sustainability by recycling waste materials into high-value natural ingredients (Obied et al., 2011).

Edible films and coatings have been extensively researched as novel protective methods that can potentially function as semi-permeable barriers and carriers of bioactive compounds, such as antioxidants and antimicrobials (Falguera et al., 2011). In seafood products, early research showed that biodegradable films made of gelatin and chitosan, which contained active ingredients, were effective in preventing microbial growth and enhancing the stability of fish fillet quality (Gómez-Estaca et al., 2010).

Olive leaf extract, due to its high content of phenolic compounds, is notable for its antioxidant and antimicrobial properties. It has been reported that, particularly when added to coatings, it helps to protect perishable foods against microbial activity and extends their shelf life (Bayram et al., 2020). Studies have shown that olive leaf extract, when utilized in coating solutions, decelerates spoilage by inhibiting microbial growth in fish fillets (Albertos et al., 2017). Similarly, limited studies have shown that olive leaf extract extends the shelf life and preserves the quality of fish and meat products (Meherpour et al., 2020; Famiglietti et al., 2022; Bilecen Şen and Güleç, 2024). Moreover, the application of olive leaves in biodegradable film and coating preparations has been identified as an environmentally sustainable approach (Fiorentini et al., 2024). This approach not only delivers safe, healthy food to consumers but also benefits the food industry economically by providing an innovative way of food preservation.

This study strived to preserve the freshness of fish meat and to sustainably utilize natural resources, such as olive leaves, in the food industry. The choice of sea bass was made to enhance preservation time and quality standards for this valuable species. Additionally, this research sought to increase the commercial potential to contribute to the national economy by offering consumers safe products with longer shelf lives. Although some studies in the literature have reported the use of olive leaf extracts in different fish species and packaging applications, no research has examined the effects of ethanolic olive leaf extract (EOLE)-containing coatings on the quality and shelf life of European sea bass fillets. Hence, this study aimed to evaluate the effects of EOLE-added coatings on microbial load, pH, color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ), and sensory quality of European sea bass (*D. labrax*) fillets under cold storage (+4 °C).

## 2. Materials and Methods

### 2.1. Preparation of EOLE for fillet coating applications

Olive leaf extract was prepared by macerating 20 g of dried, ground olive leaves in 200 mL of 70% (v/v) ethanol. The mixture was placed in a dark container and shaken twice daily for 15 days at room temperature (24–25 °C), and kept in the dark. After this period, the mixture was filtered through filter paper, and the extract was concentrated at 40 °C under low pressure using a rotary evaporator. The resulting liquid extract of EOLE was stored in light-proof, amber bottles at +4 °C until used for coating fish fillets.

## 2.2. Fish and olive leaves material

Fish (*D. labrax*) material was obtained from the local fish market at Çanakkale, Türkiye. The fish were transported to the Aquatic Products Processing Technology Laboratory in styrofoam boxes, maintaining the cold chain at +4°C. Filleting was performed under laboratory conditions, and the samples were stored under cold conditions prior to analysis. The olive leaves, gelatin, and glycerol were purchased from a commercial supplier.

## 2.3. Determination of the chemical composition of EOLE by GC–MS analysis

The chemical composition of the extract used in the study was analyzed using Gas Chromatography/Mass Spectrometry (GC-MS) (Kahraman Yılmaz et al., 2024). Analyses were performed on a Shimadzu GCMS QP 2010 ULTRA (Kyoto, Japan) instrument. The extract's components were separated using an Rxi-5 ms capillary column (30 m × 0.25 mm × 0.25 µm; Restek Corporation, Bellefonte, PA, USA) containing a low-polarity diphenyl-dimethyl polysiloxane phase. Helium served as the carrier gas. The oven was initially set to 40 °C and held for 3 minutes before increasing to 240 °C at 4 °C per minute. Injection was performed at 250 °C with a 1 µL volume in split mode (ratio 25). The mass spectra were scanned at 70 eV in the range 40–450 amu. The column flow rate was maintained at 1.78 mL/min, with a pressure of 100 kPa and a total flow rate of 49.3 mL/min. The obtained chromatograms were compared with the Wiley W9N11 library for component identification.

## 2.4. Preparation and application of the coating solution

The edible coating solution was prepared according to the method reported in the literature (Göktaş, 2022). Specifically, 20 g of gelatin was dissolved in 100 mL of distilled water, with 5% glycerol added as a plasticizer. EOLE was then added at concentrations of 1%, 2%, and 3% to form different experimental groups. No extract was added to the control group. The formulation ratios for EOLE were added based on previous studies (Göktaş, 2022; Lahreche et al., 2022). The coating solutions were homogenized using a magnetic stirrer. Fish fillets were then coated with solutions using the immersion method, ensuring an even coating across each fillet's surface. Fillets, both coated and uncoated with solutions containing different concentrations of EOLE, were placed in sterile plastic petri dishes to prevent air contact. All samples were stored at +4°C in a refrigerated incubator at +4°C, and color, pH, and microbiological analyses were performed at predetermined intervals. All experimental procedures were conducted in triplicate.

## 2.5. Sensory analyses

Sensory analyses of coated and uncoated sea bass fillets were conducted by 10 academic panelists trained in food sensory evaluation. Samples were cooked in a hot-air environment and evaluated after cooling to room temperature. A 5-point hedonic scale (1 = very poor, 5 = very good) was employed to assess color, odor, flavor, texture, and overall acceptability (Kurtcan and Gönül, 1987). Each panelist evaluated the samples in a randomized sequence, and mean scores were calculated.

## 2.6. pH analyses

During the analysis, the pH of the samples was measured by mixing 10 g of muscle tissue with 100 mL of distilled water. The pH meter (Model HI 2221; Hanna Instruments, USA) was used at room temperature by immersing the probe into the mixture, following Landvogt's (1991) method.

## 2.7. Color analysis of fish fillets

The color attributes of the fillet samples were assessed utilizing the Commission Internationale de l'Eclairage (CIELAB) color measurement system with a portable colorimeter (TCD100, China). The parameters L\* (brightness), a\* (red-green axis), and b\* (yellow-blue axis) were recorded during the assessment. Prior to data collection, the device was calibrated according to the manufacturer's instructions. A total of 9 readings were obtained from 3 different fillet samples within each group, with measurements taken at 3 different points on each sample. During measurement, the probe was positioned perpendicular to the fillet surface, and the values were recorded following stabilization. The mean value derived from these measurements for each sample was used to calculate the sample's color attribute, and the group results were analyzed based on these mean values.

## 2.8. Microbiological analyses

Total counts of aerobic, psychrophilic, and coliform bacteria, and yeast and mold, were conducted up to the last day to ensure compliance with edible safety standards. Under sterile conditions, a 10 g sample of fish muscle was collected and transferred into sterile stomacher bags containing 90 mL of 0.1% peptone solution. The samples were homogenized in the stomacher for 90 seconds. Serial dilution with 0.1% peptone was used (ISO, 2017). Subsequently, 1 mL samples were inoculated into sterile Petri dishes using the pour plate technique. Additionally, 100  $\mu$ L aliquots were surface-plated onto the respective agar media. Total counts of aerobes and psychrophiles were quantified on Plate Count Agar (PCA), following incubation at 30°C for 72 hours (ISO, 2001) and at 6.5°C for 10 days (ISO, 2013), respectively. Total yeast and mold counts were determined on Potato Dextrose Agar (PDA) plates acidified with 10% tartaric acid and incubated at 25°C for 5 to 7 days (Koburger & Marth, 1984). All results were expressed as  $\log_{10}$  colony-forming units per gram ( $\log_{10}$  cfu/g) of the sample.

## 2.9. Statistical analysis

The SPSS statistical software package (IBM SPSS Statistics, version 17.0) was used for the statistical analysis. All results are presented as mean  $\pm$  standard error (SEM). Although the experimental design consisted of two factors (coating concentration and storage time), initial tests of normality and homogeneity of variance indicated non-normality and/or heterogeneity of variance for certain parameters. Consequently, separate one-way analysis of variance (ANOVA) tests were performed: (i) within each storage day to evaluate the effect of coating concentration and (ii) within each coating group to examine changes over storage time. Tukey's test was applied to data that were normally distributed and homogeneous data, Tamhane's test for heteroscedastic data, and Dunn's test for non-parametric comparisons (Logan, 2010). Statistical significance was accepted at  $p < 0.05$ .

## 3. Results

### 3.1. Chemical composition of EOLE

GC-MS analysis revealed that the primary constituents of EOLE are 48.08% di-(9-octadecenoyl)-glycerol, 24.68% dipalmitin (hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester), 11.69% glycidol stearate, and 5.01% 15-hydroxypentadecanoic acid (Table 1). The results show that the extract has a substantial composition of fatty acids and glycerol esters.

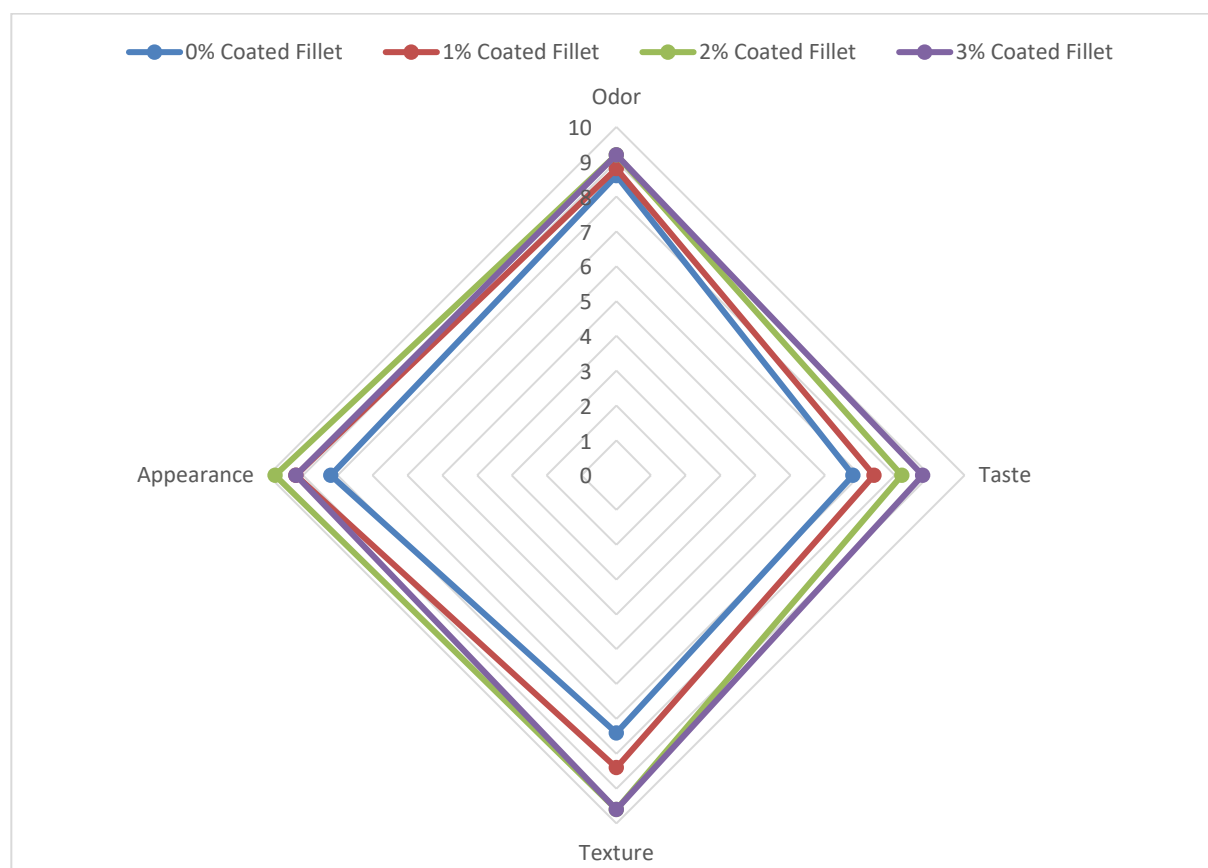
**Table 1.** Chemical composition of EOLE determined by GC–MS analysis

| Peak No. | Retention Time (min) | Area (%) | Identified compound                         |
|----------|----------------------|----------|---|
| 1        | 4.742                | 0.34     | 3-Hexanone                                  |
| 2        | 4.867                | 0.26     | 2-Hexanone                                  |
| 3        | 5.143                | 0.20     | 3-Hexanol                                   |
| 4        | 5.300                | 0.27     | 2-Hexanol                                   |
| 5        | 10.831               | 1.82     | Hydroperoxide, 1-ethylbutyl                 |
| 6        | 11.223               | 1.04     | Butanoic acid, 3-oxo-, 1-methylpropyl ester |
| 7        | 20.228               | 0.38     | Dodecane                                    |
| 8        | 40.568               | 1.32     | Neophytadiene                               |
| 9        | 47.036               | 5.01     | 15-Hydroxypentadecanoic acid                |

| Peak No. | Retention Time (min) | Area (%) | Identified compound   |
|----------|----------------------|----------|---|
| 10       | 51.708               | 24.68    | Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (dipalmitin) |
| 11       | 54.371               | 4.89     | Squalene  |
| 12       | 55.879               | 48.08    | Di-(9-octadecenoyl)-glycerol                                    |
| 13       | 56.585               | 11.69    | Glycidol stearate   |

### 3.2. Sensory results

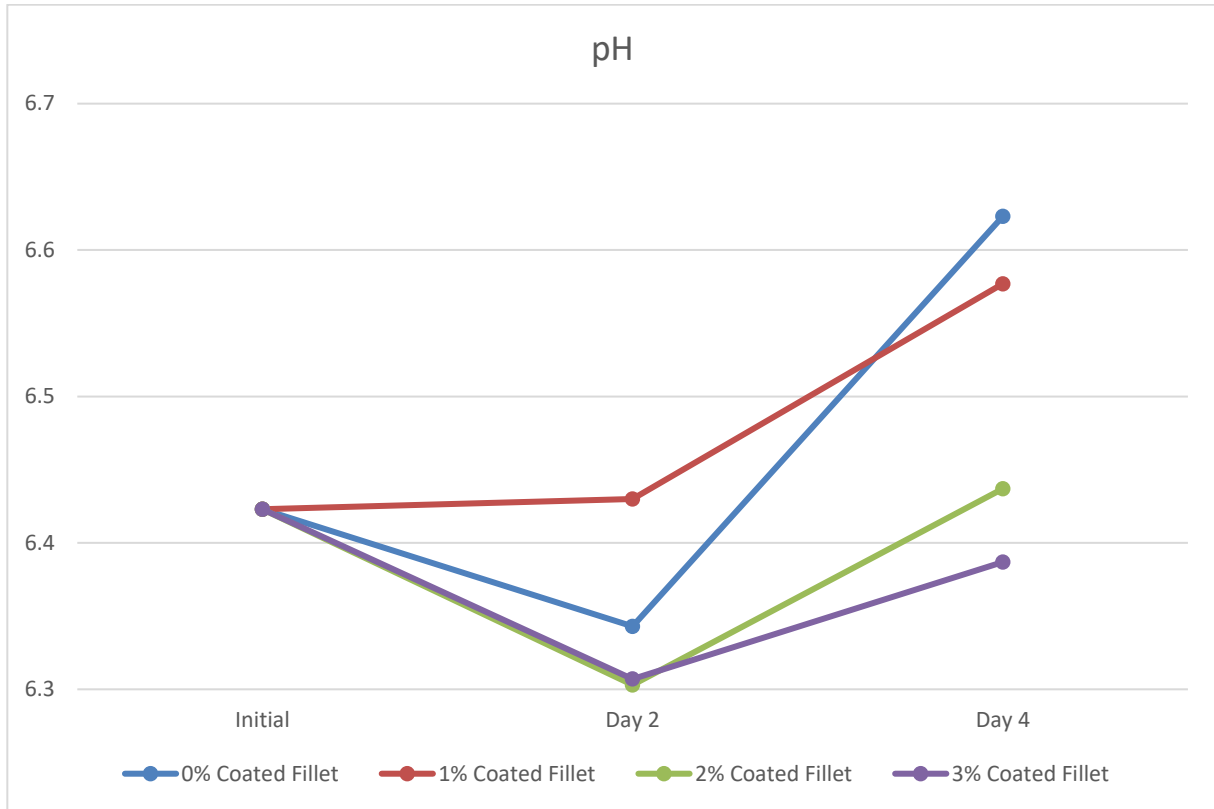
It was determined that total sensory preference increased in a dose-dependent manner with increasing coverage (Figure 1), with average overall preference scores of 7.75 for 0% coverage, 8.45 for 1%, 9.20 for 2%, and 9.20 for 3% coverage, respectively. However, statistical analysis revealed a significant difference only in the texture parameter. Specifically, the group with 0% coverage received the lowest texture score, whereas the groups with 2% and 3% coverage attained the highest texture scores, giving the best results ( $p < 0.05$ ). The 1% coverage group demonstrated an effect similar to that of the 0% coverage group and the other experimental groups ( $p > 0.05$ ).



**Figure 1** The effect of EOLE-containing coating applications on the sensory properties of fish fillets.

### 3.3. pH results

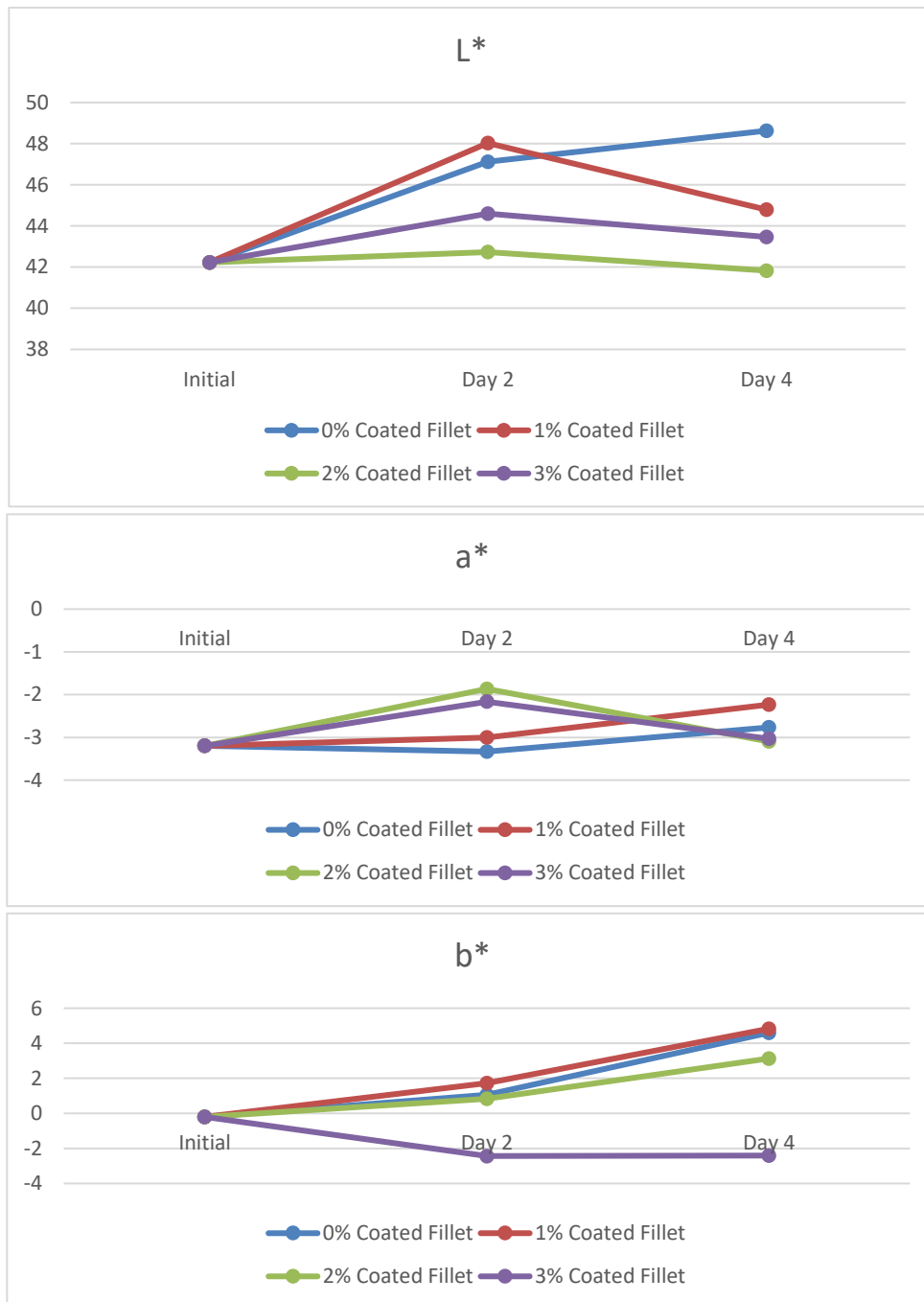
The effects of EOLE-containing coating applications on the pH values of fish fillets were evaluated during cold storage, and the results are presented in Figure 2. Initially, the pH was  $6.42 \pm 0.04$ . On the second day, slight reductions in pH values in the experimental groups. The lowest pH values were recorded in the 2% and 3% coating groups. On the fourth day of storage, a statistically significant difference was noted between the 0% and 3% coating groups, with the 0% coating group exhibiting a higher pH value ( $p < 0.05$ ). No significant differences were observed among the remaining groups on the same day ( $p > 0.05$ ).



**Figure 2.** The effect of EOLE-containing coating applications on the pH values of fish fillets during cold storage.

### 3.4. Color analysis results

The coating applications containing EOLE did not show any statistically significant differences among the treatment groups or with storage time ( $p > 0.05$ ) in the  $L^*$ ,  $a^*$ , and  $b^*$  color values of fish fillets during cold storage (Figure 3).



**Figure 3.** Effect of EOLE-containing coatings on the L\*, a\*, and b\* color parameters of fish fillets during cold storage.

### 3.5. Microbiological results

Changes in the microbiological quality of coated and uncoated fish fillets during cold storage are presented in Table 2. A significant increase in the total viable bacterial count (TVC) was observed in all groups, correlating with storage duration. Initially, TVC ranged from 2.12–2.36 log CFU g<sup>-1</sup>, rising to 5.06–5.17 log CFU g<sup>-1</sup> by day four, with no significant group differences on the same days ( $p > 0.05$ ). Within-group comparisons showed a significant increase in TVC over storage time ( $p < 0.05$ ). Total coliform counts were below the detection limit ( $<10^1$  CFU g<sup>-1</sup>) in all groups on the first day of storage. On the second day of storage, low levels of coliform growth were observed only in the control group, while counts remained below the detection limit in the coated groups. On the 4th day of storage, total coliform counts increased in all groups; however, the 3% coating group exhibited significantly lower counts than the other groups ( $p < 0.05$ ).

In terms of psychrotrophic viable bacterial counts, an increasing trend was observed across all groups

with increasing storage time. Although numerical differences were evident between the groups on the 1st and 2nd days of storage, they did not reach statistical significance ( $p > 0.05$ ). On the 4th day of storage, data showed that psychrotrophic bacterial counts ranged from 5.09 to 5.19 log CFU g<sup>-1</sup> in the experimental groups. There were significant increases in all groups over time ( $p < 0.05$ ). The levels of yeast and mold remained below the detection threshold ( $<10^1$  CFU g<sup>-1</sup>) throughout the storage period in all groups. This suggests that the application of coatings and cold storage conditions effectively inhibited the growth of yeast and mold. In general, it was determined that storage time, rather than coating concentrations, was the determining factor for microbiological parameters, and no statistically significant differences were observed among the groups on the same storage day.

**Table 2.** Effect of different concentrations of EOLE coatings on microbial counts of fish fillets during refrigerated storage (log CFU g<sup>-1</sup>)

| Media                       | Group            | Day                       |                           |                           |
|-----------------------------|------------------|---------------------------|---------------------------|---------------------------|
|                             |                  | 1                         | 2                         | 4                         |
| Total viable count          | 0% Coated Fillet | 2.12±0.07 <sup>aA</sup>   | 2.86 ± 0.14 <sup>aB</sup> | 5.17 ± 0.05 <sup>aC</sup> |
|                             | 1% Coated Fillet | 2.25±0.03 <sup>aA</sup>   | 2.99 ± 0.34 <sup>aB</sup> | 5.08 ± 0.02 <sup>a</sup>  |
|                             | 2% Coated Fillet | 2.28±0.05 <sup>aA</sup>   | 3.07 ± 0.12 <sup>aB</sup> | 5.06 ± 0.03 <sup>a</sup>  |
|                             | 3% Coated Fillet | 2.36±0.03 <sup>aA</sup>   | 3.03 ± 0.15 <sup>aB</sup> | 5.11 ± 0.02 <sup>a</sup>  |
| Total coliform              | 0% Coated Fillet | <10 <sup>1</sup>          | 0.49 ± 0.12               | 5.27 ± 0.06 <sup>a</sup>  |
|                             | 1% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | 5.20 ± 0.08 <sup>a</sup>  |
|                             | 2% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | 5.29 ± 0.05 <sup>a</sup>  |
|                             | 3% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | 4.81 ± 0.40 <sup>b</sup>  |
| Psychrotrophic viable count | 0% Coated Fillet | 2.21 ± 0.05 <sup>aA</sup> | 2.94 ± 0.12 <sup>aB</sup> | 5.19 ± 0.06 <sup>aC</sup> |
|                             | 1% Coated Fillet | 2.30 ± 0.04 <sup>aA</sup> | 3.08 ± 0.24 <sup>aB</sup> | 5.10 ± 0.03 <sup>aC</sup> |
|                             | 2% Coated Fillet | 2.15 ± 0.13 <sup>aA</sup> | 2.67 ± 0.21 <sup>aB</sup> | 5.09 ± 0.03 <sup>aC</sup> |
|                             | 3% Coated Fillet | 2.24 ± 0.05 <sup>aA</sup> | 2.94 ± 0.24 <sup>aB</sup> | 5.12 ± 0.03 <sup>aC</sup> |
| Yeast and Molds             | 0% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | <10 <sup>1</sup>          |
|                             | 1% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | <10 <sup>1</sup>          |
|                             | 2% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | <10 <sup>1</sup>          |
|                             | 3% Coated Fillet | <10 <sup>1</sup>          | <10 <sup>1</sup>          | <10 <sup>1</sup>          |

Values are expressed as means ± S.E.M (n = 3). Different lowercase letters within the same column denote statistically significant differences between coating concentrations on the same storage day ( $p < 0.05$ ). Different uppercase letters within the same row indicate significant differences over storage time within the same treatment group ( $p < 0.05$ ).

#### 4. Discussion

Specifically, derivatives of oleic and palmitic acid, as well as glycerol esters, have been reported as natural components of the lipid fraction in *O. europaea* leaves and extracts (Bianchi et al., 1992; Syed et al., 2022; Clodoveo et al., 2021). Contrary to existing literature, free oleic acid was not detected in the GC-MS fingerprint. Nonetheless, the predominance of di-(9-octadecenoyl)-glycerol may indicate the presence of lipid structures derived from oleic acid, potentially attributable to the extended extraction duration and the absence of high-temperature extraction procedures. These findings suggest the formation of esterified or bound lipid forms rather than free fatty acids.

This experiment demonstrated that the overall preference for the organoleptic properties of coating preparations incorporating EOLE increased proportionally with the coating ratio. Notably, a statistically significant difference was observed in the texture attribute. The absence of significant differences among groups regarding odor and taste attributes suggests that the olive leaf ethanolic extract did not impart any undesirable effects on the fish fillets, even at elevated coating ratios. These findings are aligned with prior research. For instance, Lahreche et al. (2020) observed that the application of olive leaf extract enhanced the textural qualities of vacuum-packed mackerel (*Auxis thazard*) fillets during cold storage. Similarly, Sánchez-García et al. (2025) reported that the sensory properties of fresh whiting fillets remained unaffected in active packaging containing olive leaf extract. Mirza et al. (2023) studied tilapia (whole fish) and found that edible coatings based on green tea and olive leaf extract improved chemical and microbial quality; however, sensory scores were significantly lower than those of the control group, possibly due to the external coloration of the fish.

This study observed an enhancement in the texture parameter in the 2% and 3% EOLE groups. This can be attributed to the barrier effect imparted by the coating on the surface of the fish fillets, thereby restricting water loss and preventing structural deterioration of the muscle tissue.

Previous studies have indicated that pH serves as a significant biomarker of freshness in fish products, with an increase in pH during storage correlating with biochemical and microbial spoilage processes (Abbas et al., 2008). It has been reported that a pH exceeding 6.8-7.0 indicates a loss of quality and the onset of spoilage in fish products (Vyncke, 1981). Mirza et al. (2023) reported that olive leaf extract was used at a single dose of 2% (w/v) via immersion without any coating material, resulting in a significant increase in pH on the fifth day of storage on ice ( $1\pm 2$  °C) compared to the control group. The present study demonstrates that pH values remained below the 6.8 threshold across different doses of EOLE-containing coating application, indicating that the application method and dosing approach may be critical factors influencing pH variation. The observed time-dependent pH changes are consistent with existing literature (Lahreche et al., 2020; Sánchez-García et al., 2025).

In the present study, EOLE did not exhibit significant variation in the L\*, a\*, or b\* color components. The variation was dependent on storage time rather than the coating process. Sánchez-García et al. (2025) also indicated that storage time was the most important factor affecting color variation in fish fillets. They explained that packaging with olive leaf extract can augment yellow components, particularly by increasing b\* values, but the fish would not assume a green coloration.

Meanwhile, in storage, time was likely the primary factor affecting the variation in microbial growth. The counts of total viable and psychrotrophic bacteria increased progressively with storage time, and no definitive concentration-dependent effect of EOLE on these parameters was observed. Nevertheless, on the second day, coliform bacteria were detected only in the control group, whereas the EOLE-coated groups had lower counts below the detection limit. Moreover, on the fourth day, the group with a 3% EOLE coating had lower coliform counts than the other groups, suggesting that elevated EOLE may exert a suppressive effect on coliform bacteria. Similarly, Sánchez-García et al. (2025) reported that packaging systems incorporating olive leaf extract could help in microbial suppression during the initial stages of storage. Additionally, as noted by Mirza et al. (2023) and Lahreche et al. (2020), the application of olive leaf extract generally exhibits a suppressive rather than an inhibitory effect on microbial growth during storage.

## 5. Conclusion

This study has shown that the application of ethanolic olive leaf extract (EOLE) as a coating may serve as a natural method for preserving European sea bass fillets. The results indicated that EOLE enhanced the texture of the fillets without impacting the pH and color values. Although no statistically significant differences were observed in microbial counts, coliform bacteria were only found in the control samples, whereas the coated samples exhibited values below the detection threshold. These findings suggest a limited short-term antimicrobial effect of EOLE. Further investigations are required to optimize application techniques and evaluate long-term efficacy.

## 6. Compliance with Ethical Standard

### a) Author Contributions

1. D.K.Y.: Conceptualization, Investigation, Validation, Data Curation, Formal analysis, Methodology, Writing—review and editing; 2. Ö.Ç.: Methodology, Investigation; The published version of the manuscript has been read and approved by both authors.

#### **b) Conflict of Interests**

There is no conflict of interest, according to the authors.

#### **c) Statement on the Welfare of Animals**

Not relevant

#### **d) Statement of Human Rights**

Sensory evaluation involving trained panelists was approved by the Institutional Ethics Committee (Approval No: 2025-YÖNP-0034).

#### **e) Funding**

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