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The role of laurel, oregano, and thyme essential oils on the oxidative stability and microbiological quality of sea bass fillets *(Dicentrarchus labrax)* during refrigerated storage

Die Rolle von ätherischen Lorbeer-, Oregano- und Thymianölen auf die oxidative Stabilität und mikrobiologische Qualität von Wolfsbarschfilets (Dicentrarchus labrax) während der gekühlten Lagerung

Kubra Unal

Summary

This study aimed to investigate the physicochemical (pH, Thiobarbutiric acid number (TBARS), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, L*, a*, b*) and microbiological (total psychrotrophic aerobic bacteria (TPAB), total mesophilic aerobic bacteria (TMAB), *Enterobacteriaceae* and *Pseudomonas* counts) and sensory properties in European sea bass *(Dicentrarchus labrax)* fillets containing laurel, oregano, and thyme essential oil (EO) over 6 days. Treatment with laurel, oregano, and thyme EO had significantly ($P < 0.05$) higher DPPH levels and lightness values, but lower ($P < 0.05$) TBARS value. The laurel EO treatment generally did not affect the microorganisms counts of the samples on the 6th day ($P > 0.05$), while the highest odor score was determined in the groups of laurel EO. The lowest *Enterobacteriaceae* and *Pseudomonas* spp. counts were obtained from thyme EO added samples with a 4.59±0.07 log CFU/g and 5.19±0.07 log CFU/g, followed by samples treated with oregano EO with 4.82±0.02 log CFU/g and 5.21±0.05 log CFU/g respectively, at the end of the 6 days of storage.

Keywords: Fish fillet; oxidative stability; natural additive

Introduction

Fish consists of very important nutritional components like essential amino and fatty acids, which can show important effect in the elimination of critical diseases such as cancer and diabetes. However, fish is highly perishable due to its weak connective tissue and higher moisture content (Kiessling, Ruohonen, & Bjørnevik, 2006; Petricorena, 2015). Thus, some food preservation methods are applied in order to delay microbiological spoilage, and undesired physicochemical changes, as well as sensory deterioration of fish meat (Arvanitoyannis, Stratakos, & Mente, 2009; Oğuzhan & Angiş, 2012). The use of synthetic preservatives has a negative perception by consumers in relation to possible toxicological effects and health damages. With this, the use of natural additives, which are extracted from natural, renewable and sustainable sources, is an important alternative in the preservation of foods (da Silva et al., 2021). In this sense, while consumers desire food consisting of naturally-based materials, the use of natural additives such as probiotic bacteria, chitosan, liquid smoke, thymol, nisin, and curcumin within different formulations and applications have been applied for improvement of microbiological quality, oxidative stability, and sensory characteristic (odor, color, taste, texture) of fish fillets (Ceylan, Meral, Alav, Karakas, & Yilmaz, 2020; Ceylan, Unal Sengor, Basahel, & Yilmaz, 2018; Meral et al., 2019; Šimat et al., 2020).

Considered as one of the main sources of natural additives used in fish meats, essential oil (EO) has included bioactive components such as terpenes, terpenoids, phenolic acids, aldehydes, esters (Raut et al., 2014). The main antimicrobial activity mechanism of EO have been described as interaction of their hydrophobic components with the lipids of the cell membrane (Nazzaro et al., 2013). da Silva et al (2021) also stated that the hydrophobic nature of EO damaged to the structural and functional properties of the cell membrane of microorganisms. On the other hand, to mitigate the effects of free radicals in food systems, EO as compounds with antioxidant activity are utilized. The antioxidant compounds of EO act mainly as free radical scavengers, blocking the oxidation process in the stage of initiation (Moon and Shibamoto, 2009).

Among the essential oils, laurel *(Laurus nobilis)* EO are used in the food technology for flavoring and food preservative (Vilela et al., 2016). The high amount in oxygenated monoterpenes in laurel EO indicated antimicrobial activity towards foodborne pathogens (da Silveira et al., 2014). Some studies have indicated the antimicrobial and antioxidant activities of *L. nobilis* EO in fresh chicken (Djenane et al., 2012), in fresh sausage (da Silveira et al., 2014), in anchovy, mackerel and sardine (Y. Özoğul et al., 2022), in fish oil (Yeşilsu and Özyurt, 2019). Thyme EO and oregano EO have an interest of researchers and food processors as a potential natural additive as antimicrobial and antioxidant agent in all times. They include high amount of phenolic compounds such as carvacrol and thymol. The antimicrobial activity and lipid oxidation stability is due to the preservative effect of polyphenols including inhibition of some enzymes and free radical scavenging ability (Bensid et al., 2014; Tajkarimi et al., 2010). However, no studies were made to analyze laurel EO effects directly in sea bass fillets. To knowledge, there have been also no previous studies in which essential oils of laurel, oregano and thyme were added simultaneously to sea bass fillets as natural food additives for controlling oxidative and microbial spoilage. Hence, the purpose of the current study was to evaluate the influence of using laurel, oregano, and thyme essential oils on the microbiological spoilage, and physcochemical deterioration of sea bass fillets during the refrigerated storage period for 6 days.

Material and methods

Material

The laurel *(Laurus nobilis),* oregano *(Origanum vulgare),* and thyme *(Thymus vulgaris)* EOs were provided by company (Mellys' Nature) in Turkey. Fish samples *(Dicentrarchus labrax)* were obtained from an international supermarket (Metro) in Konya, Turkey.

Samples preparation

The sea bass samples were gutted, filleted without skin. Four types of treatment groups were prepared. The fillets were homogenized with 1 % (w/w) laurel, oregano, or thyme EO, and then massaged by hand for uniform distribution of the EO. The control group had no EO added. Each fillet was packed in low density polyethylene pouches individually. Afterwards, the fillet samples were aerobically packaged and held at 4 ± 1°C for 6 days. Sampling was carried out at beginning of the storage day, 2, 4 and 6 days.

Determination of volatile compounds of EO

The EO were studied using a gas chromatography-mass spectrometer (Agilent Technologies, 7890B GC System, USA) with a mass spectrometric detector (Agilent Technologies, 5977BN MSD, USA). The mass scan range was m/z 30-600, the source and the quadruple temperature was 230 °C and 150 °C, respectively. A HP-5MS capillary column (30 m × 250 µm × 0.25 µm, Agilent, Belgium) was used with helium gas (flow rate of 1 ml/min). Initial oven temperature was 50 °C, then it was raised to 150 °C (rate 3 °C/min), finally the temperature was 250 °C for 2 min. Samples were inserted into the device using a splitless injection technique. Identification of compounds was performed by comparing recorded mass spectra with reference spectra in the computer library (NIST 14 Mass Spectral Library).

Proximate composition

The moisture, protein, fat, and ash contents of the fish were measured by standard methods of the AOAC (2000). Moisture (g water/100 g sample) was determined after drying 3 g sample at 105°C in order to provide constant weight. Protein (g protein/100 g sample) was analyzed according to the Kjeldahl method. Factor 6.25 was used for conversion of nitrogen to crude protein. Fat content (g fat/100 g sample) was determined by using a Soxhelet fat extraction apparatus. Ash content (g ash/ 100 g sample) was determined at 550°C for 2 h. The pH values were determined with a pH meter (Testo 205, pH-Temperatur-Messgerat, AG, Lenzkirch, Germany).

Color measurements

Color (L^*:lightness), a^*:redness, and b^*:yellowness) measurements were carried out on the surface of the samples using a chromameter CR-400 (Konica Minolta, Inc., Osaka, Japan) (Hunt et al., 1991). Measurements were made directly upon the fillet samples and carried out 4 times, 1 in the middle and 3 on different parts of the samples.

Journal of Food Safety and Food Quality 73, Heft 4 (2022), Seiten 109–138

Thiobarbituric acid (TBARS) number determination

The method described by Gökalp, Kaya, Tülek, and Zorba (2012) and Tarladgis et al (1960) was used to measure the level of oxidative rancidity. Minced fish fillets were blended (Waring Commercial Blendor) with distilled water (50 °C). They were mixed with HCl and heated. The collected distillate was transferred to 5 mL of TBA reagent and the mixture was incubated in a boiling water. The absorbance was read at 530 nm (Shimadzu-UV mini 1240, Kyoto-Japon). The number of TBARS values were expressed as milligrams of malonaldehyde per kilogram samples (mg MDA kg^{-1}) by multiplying the absorbance values by the coefficient 7.03.

DPPH radical scavenging activity

Antioxidant capacity of the treatment groups was measured according to Brand-Williams, Cuvelier, and Berset (1995) with minor modification. Ground fish fillet was homogenized with methanol (25 ml), centrifuged, and filtered (Whatman No. 1). The supernatant was blended with methanol, then 50 µl were taken and added to 2950 µl of 100 µM DPPH. Methanol with 2950 µl of a DPPH solution were prepared as a blank solution. The samples were vortexed and kept in the dark. The absorbance of the solution was read at 517 nm (Shimadzu-UV mini 1240, Kyoto-Japon). The results were expressed as a percentage of free radical scavenging activity (%).

Microbiological enumeration

Minced fish samples were aseptically and taken to a 225 mL sterile ringer solution and mixed using a stomacher (Lab Blender, Seward, London). After homogenization, serial decimal dilutions were made using the ringer solution as the diluent, and 0.1 mL samples for each dilution were spread and duplicated on selective agar plates. Enumeration of total mesophilic aerobic bacteria (TMAB) was performed on standard plate count agar (PCA, Merck) with incubation at 30 °C for 2 days; meanwhile total psychrotrophic aerobic bacteria count (TPAB) was conducted at 7 °C for 10 days on the same medium (Arashisar, Hisar, Kaya, & Yanik, 2004). *Pseudomonas* spp. were enumerated on CFC agar (supplemented with SR 103, Oxoid, Basingstoke, UK) and incubated at 20 °C for 48 hours (Mead & Adams, 1977). For *Enterobacteriaceae* enumeration, a 1.0 mL sample was inoculated onto 10 mL of molten (45 °C) violet red bile glucose agar (VRBGA, Oxoid code CM 485) and incubated at 30 °C for 1 day (Mossel, Eelderink, Koopmans, & Van Rossem, 1979).

Sensory evaluation

Sensory evaluation was conducted on storage days 2 and 6. The sensory panel was performed by semi-trained panellists from the Department of Food Engineering at Selçuk University. The samples were cooked individually in an electric grill at 170 °C for 10 min. Cooked samples coded with random 3-digit numbers were immediately served to the panellists with water and breads. Panellists attended two sessions and four samples were presented to each panellist for each session. The appearance, odor, and texture properties of the samples were evaluated using a 9-point hedonic scale (9: extremely liked, 5: moderately liked, 1: disliked) (Yıldız-Turp & Serdaroglu, 2010).

Statistical analysis

All data was subjected to one-way analysis of variance to determine the TBA, DPPH, and microbiological assays in all groups. MINITAB for Windows Release 20.0 was used to reveal significant differences between treatment, and also comparisons of all differences among them were evaluated by the Tukey's Multiple Range Test ($P < 0.05$). Each measurements were repeated in duplicate with three replications.

Results and discussion

Proximate composition

The moisture, fat, ash, and protein content of sea bass meats were found as 72.15±0.02, 8.07±0.79, 1.85±0.81, and 19.65±0.13,%, respectively. The average pH value (6.05±0.21) of fish samples was lower than the pH (6.43) found by Alparslan, Gürel, Metin, Hasanhocaoğlu, and Baygar (2012) for fresh sea bass. Moisture content (72.15%) was similar (74.02%) to that determined by Boulares, Ben Moussa, Mankai, Sadok, and Hassouna (2018). Similar fat and protein content were determined by Alparslan et al. (2012), who evaluated that the fat and protein levels of fish were 8.36% and 19.43 %, respectively. Attouchi and Sadok (2012) determined the ash value of farmed sea bream as 1.42% and were comparable with these values. Factors such as feeding, catching season, characterization of sea water, and even gender can produce variability in the composition of the fish (Alparslan et al., 2012; Attouchi & Sadok, 2012; F. Özogul, Kuley, & Özogul, 2007).

Composition of essential oils (EO)

The major compounds of the EO used in this study are shown in Table 1. The main components for laurel EO were eucalyptol (44.75%), α-pinene (18.46%), and α-terpinyl acetate (14.50%); for oregano EO, carvacrol (51.19%), terpinen-4-ol (15.66%) and γ-terpinene (14.95%); and for thyme EO, were thymol (40.35%), linalool (29.76%) and γ-terpinene (4.57%). Some differences were seen between these results and other researches. Stefanova et al. (2020) determined the principal EO components of the laurel EO were 1,8-cineole, α-terpinyl acetate, terpinen-4-ol, α-pinene and methyleugenol 30.8, 14.9, 6.0, 5.3 and 3.6%, respectively. Shange, Makasi, Gouws, and Hoffman (2019) evaluated the EO composition of the oregano used

TABLE 1: *Major components of different essential oils identified by GC-MS.*

Essential oil	Compounds	RP (%)
Laurel	Eucalyptol	44.75
	α pinene	18.46
	α Terpinyl acetate	14.50
	Terpinen-4-ol	2.75
	Methyleugenol	1.38
	δ Terpineol	1.06
Oregano	Carvacrol	51.19
	Terpinen-4-ol	15.66
	γ Terpinene	14.95
	ρ-Cymene	5.52
	Thymol	3.63
	Linalyl acetate	3.26
Thyme	Thymol	40.35
	Linalool	29.76
	Benzene, 1,2,3,4-tetramethyl	6.52
	γ Terpinene	4.57
	Caryophyllene	2.50
	β-Myrcene	2.05

GC-MS, gas chromatography-mass spectrometer; RP, relative percentages.

TABLE 2: *pH, TBARS number and DPPH content of sea bass fillets treated with essential oils.*

Parameters	Treatments	Storage time (days) 0	2	4	6
pH	Control	6.10±0.03^{aD}	6.19±0.01^{aC}	6.26±0.02^{aB}	6.38±0.01^{abA}
	Laurel	6.10±0.03^{aC}	6.19±0.01^{aB}	6.27±0.02^{aAB}	6.33±0.01^{cA}
	Oregano	6.10±0.03^{aC}	6.21±0.01^{aB}	6.26±0.01^{aB}	6.36±0.01^{bcA}
	Thyme	6.10±0.03^{aC}	6.23±0.01^{aB}	6.29±0.03^{aAB}	6.40±0.01^{aA}
TBARS (mg MA/kg sample)	Control	0.24±0.02^{aC}	0.49±0.05^{aB}	0.62±0.05^{aA}	0.89±0.04^{aA}
	Laurel	0.24±0.02^{aAB}	0.14±0.01^{bB}	0.23±0.00^{bAB}	0.38±0.06^{bA}
	Oregano	0.24±0.02^{aAB}	0.18±0.01^{bB}	0.25±0.01^{bA}	0.30±0.01^{bA}
	Thyme	0.24±0.02^{aAB}	0.10±0.00^{bB}	0.12±0.01^{cB}	0.24±0.02^{bA}
DPPH (%)	Control	0.23±0.08^{aA}	0.22±0.07^{bA}	0.18±0.04^{cA}	0.15±0.05^{cA}
	Laurel	0.23±0.08^{aC}	7.81±0.42^{aA}	6.51±0.14^{bA}	3.60±0.22^{bB}
	Oregano	0.23±0.08^{aD}	7.86±0.09^{aA}	7.08±0.23^{abB}	4.36±0.31^{bC}
	Thyme	0.23±0.08^{aD}	8.16±0.24^{aA}	7.22±0.10^{aB}	5.93±0.11^{aC}

Data are expressed as mean ± standard deviation. Means with different lower case letter in the column for each storage day are significantly different ($P < 0.05$). Means with different uppercase letter in the line for each treatment are significantly different ($P < 0.05$)

in their research, and reported the main components were carvacrol (42.94%), thymol (17.40%), ρ-cymene (8.04%), and γ-terpinene (1.82%). Y. Özogul et al. (2020) determined the important EO components of thyme in their study included carvacrol (71.8%), γ-terpinene (6.7%), and caryophyllene (1.2%) obtained from the MS libraries. They also reported that carvacrol had the main phenolic constituent of thyme EO. Comparing these results with different studies, the several variations in chemical structure could be attributed to the geographic origin, harvest time, climatic conditions of the plant, and the extraction methods (Rezaei & Shahbazi, 2018).

pH, TBARS value, DPPH radical scavenging activity

Table 2 indicates pH levels of sea bass fillets treated with different EO during 6 days of storage. Initially, the pH of the fillet had 6.10± 0.03. pH indicated no significant differences ($P > 0.05$) between no added EO and samples treated with EO during refrigerated storage except day of 6. The pH values increased with storage time and this might be associated with increasing microbial count and production of ammonia, trimethylamine, etc. (Jeon, Kamil, & Shahidi, 2002; F. Özogul et al., 2007).

TBARS method is the most widely used process to evaluate the degree of oxidation, while the DPPH analysis is used to measure the absorption capacity of oxygen radicals (Amorati, Foti, & Valgimigli, 2013; Hassoun & Çoban, 2017). Table 2 shows the effects of EO treatment and storage day on the TBARS and DPPH values of sea bass fillets throughout 6 days of refrigerated storage. Treatment with laurel, oregano, and thyme EO increased ($P < 0.05$) antioxidant activity levels than the control group. Antioxidant activity decreased significantly with storage day. This antioxidant effect is probably due to the presence of phenols in the EO (Saricoban & Ozcan, 2004). Jayasena and Jo (2014) and Amorati et al. (2013) found that carvacrol and thymol was the primary component responsible for the antioxidant activity of EOs. Rodriguez-Garcia et al. (2016) also reported that antioxidants derived from essential oils played a role directly or indirectly and inhibited chain initiation. They showed free radical scavenging activity, besides other mechanisms.

As illustrated in Table 2, using essentil oil had a significant influence on lipid oxidation ($P < 0.05$). Initially, the TBARS value was found to be 0.24 mg MDA kg^{-1}, similar to published data (Jouki, Yazdi, Mortazavi, Koocheki, & Khazaei, 2014; Y. Özogul et al., 2017). However, Bensid, Ucar, Bendeddouche, and Özogul (2014) reported a substantially higher initial TBARS value (3.08 mg MDA kg^{-1}) for anchovy samples. TBARS values for treated sea bass fillets increased from 0.24 to 0.89 mg MDA kg^{-1} and generally increased with storage day. The increase in TBARS value showed the formation of secondary oxidation products (Pezeshk, Rezaei, & Hosseini, 2011). Moini et al. (2009) claimed that TBARS values of 1–2 mg MDA kg^{-1} were commonly considered the upper most limit for typical odor. In current research, lower TBARS levels were found for all samples with lower lipid oxidation. Laurel, oregano, and thyme EO treatments showed lower oxidation stability compared to the control group. My results agreed with Huang, Liu, Jia, Zhang, and Luo (2018), who reported that adding EO in general, lowered the TBA value for grass carp fillet, while Erkan, Tosun, Ulusoy, and Üretener (2011) determined bluefish treated with 1% laurel or thyme EO were high in phenol compounds, high in DPPH values, and displayed lower oxidation on the 6th day compared with other treatments, especially the control groups. The prevention of oxidation by EO from *Origanum* plants was associated with the concentration of the main contents such as carvacrol and thymol (Lagouri, Blekas, Tsimidou, Kokkini, & Boskou, 1993).

Color properties

Table 3 demonstrates that the effect of EO treatment and storage time on the color properties (L^*, a^*, b^*) of sea bass fillet during refrigerated storage for 6 days. Laurel, oregano, and thyme EO treatments show higher L^* values, which indicate lightness, compared to the control group. Most likely, the protective effect of EO influence the color lightness. The initial L^*, a^*, and b^* values of filleted sea bass were determined as 45.53 ± 0.08, 1.13 ± 0.21, and –0.57 ± 0.28, respectively. At the end of the storage time, L^* values significantly ($P < 0.05$) increased, and a^* values significantly ($P < 0.05$) decreased in the control group. These results were similar to those of Cakli, Kilinc, Cadun, Dincer, and Tolasa (2007), who also reported higher L^* values, but no significant differences in a^* and b^* values. However, the decrease in a^* values (red color) might be related to the connection between oxidation and

TABLE 3: *Color parameters of sea bass fillets treated with essential oils.*

Parameters	Treatments	Storage time (days) 0	2	4	6
L^*	Control	45.53±0.08^{aC}	46.57±0.03^{cB}	49.34±1.18^{bAB}	53.26±0.90^{bA}
	Laurel	45.53±0.08^{aA}	51.49±0.48^{bA}	52.70±0.07^{abA}	54.93±1.19^{aA}
	Oregano	45.53±0.08^{aA}	52.95±0.29^{bA}	53.42±1.26^{abA}	55.77±0.70^{aA}
	Thyme	45.53±0.08^{aA}	58.38±1.01^{aA}	55.32±1.52^{aA}	55.26±1.94^{aA}
a^*	Control	1.13±0.21^{aA}	1.01±0.23^{aAB}	0.87±0.56^{aAB}	0.79±0.23^{aB}
	Laurel	1.13±0.21^{aA}	–0.05±0.59^{aA}	0.11±0.72^{aA}	0.16±0.67^{aA}
	Oregano	1.13±0.21^{aA}	–0.04±0.26^{aA}	–0.29±0.26^{aA}	–0.67±0.06^{aA}
	Thyme	1.13±0.21^{aA}	0.17±0.21^{aA}	–0.23±0.06^{aA}	–0.87±0.51^{aA}
b^*	Control	–0.57±0.28^{aA}	–0.72±0.41^{aA}	2.71±1.89^{aA}	1.43±1.02^{aA}
	Laurel	–0.57±0.28^{aA}	–0.84±0.28^{aA}	2.35±1.26^{aA}	1.95±1.31^{aA}
	Oregano	–0.57±0.28^{aA}	–1.83±0.47^{aA}	0.48±0.92^{aA}	0.31±0.64^{aA}
	Thyme	–0.57±0.28^{aA}	–1.28±0.52^{aA}	0.53±1.01^{aA}	0.25±0.62^{aA}

Data are expressed as mean ± standard deviation. Means with different lower case letter in the column for each storage day are significantly different ($P < 0.05$). Means with different uppercase letter in the line for each treatment are significantly different ($P < 0.05$)

Journal of Food Safety and Food Quality 73, Heft 4 (2022), Seiten 109–138

color parameters in meat samples (Lynch & Faustman, 2000). Given that color oxidation might catalyze lipid oxidation, the formation of free radicals oxidized iron atoms or denatured myoglobin molecules, essentially changing their color. As the TBARS values of samples in this study increased slightly during the storage period, this trend of decreasing *a** values might be due to interference with the lipid oxidation during myoglobin oxidation. In this study, all EO treatments had no significant ($P > 0.05$) differences in the *a** and *b** values of samples.

Microbiological enumeration

The initial TMAB of 2-day sea bass fillets were found to be 4.86±0.17 log CFU/g in control groups, while the numbers for laurel, oregano, and thyme EO treated fillets were 4.25±0.53, 3.29±0.03, and 3.14±0.09, respectively. This result was consistent with Harpaz, Glatman, Drabkin, and Gelman (2003)'s findings which indicated that oregano and thyme EO reduced initial TMAB counts of Asian sea bass samples by 2 log CFU/g. The TMAB increased during refrigerated storage, and numbers reached 7.97±0.12 log CFU/g for controls while they were 7.16±0.08, 6.50±0.16, and 6.57±0.09 for sea bass fillets with laurel, oregano, and thyme EO, respectively. As it can be seen in table 4, statistically significant differences were observed in TMAB counts of sea bass fillets treated EO's compared to controls for the entirety of the refrigerated storage period. In controls and laurel EO-treated sea bass fillets, the TMAB values exceeded the upper tolerable microbiological limit of 7 log CFU/g for marine specimens after 6 days of storage, while the numbers were below the allowed limit in samples with oregano and thyme EO. Previously, Huang et al. (2018) reported that grass carp fillets with oregano EO had lower TMAB counts of 7.48 log CFU/g on the 6th day compared to those treated with thyme EO (7.75 log CFU/g). While these numbers were approximately 1 log CFU/g higher than my counts obtained after 6 day, their findings did correlate well with current TMAB numbers in samples with oregano EO which had higher counts compared to those with thyme on day 6 of storage.

On the other hand, total psychrotrophic bacteria counts were 4.99±0.07, 4.06±0.17, 3.03±0.05, and 3.50±0.20 log CFU/g at 2 day for control, laurel, oregano, and thyme, respectively, which increased quickly with an increase in storage period until day 6. As seen in TMAB, the highest numbers were obtained from control and laurel treated sea bass fillets during the entire storage period. The TPAB counts for control and laurel EO treated samples exceeded 7 log CFU/g on day 6 of storage, while those for oregano and thyme EO treated samples were below 7 log CFU/g showing reduced counts by approximately 2 log CFU/g compared to the controls. Similarly, an increase in psychrotrophic bacteria over time was also observed in a previous study conducted by Cai et al. (2015) in control and EO-treated samples. Present results are in agreement with those of Kostaki, Giatrakou, Savvaidis, and Kontominas (2009), who reported that aerobically packaged aquacultured sea bass fillets with thyme EO displayed extended shelf life. However, these researchers observed that TPAB counts reached approximately 7 and 6 log CFU/g values for control and thyme EO added fillets, respectively, by the 10th day of storage which is not in accordance with current findings.

TABLE 4: *The result of microbiological analysis of the sea bass fillets treated with essential oils.*

Parameters (log CFU/g)	Treatments	Storage time (days) 2	4	6
Total psychrotrophic aerobic bacteria counts	Control	4.99±0.07^{aB}	6.49±0.12^{aA}	8.11±0.19^{aA}
	Laurel	4.06±0.17^{aB}	6.02±0.12^{aA}	7.19±0.01^{aA}
	Oregano	3.03±0.05^{bB}	4.98±0.13^{bA}	6.09±0.07^{bA}
	Thyme	3.50±0.20^{bB}	5.20±0.07^{bA}	6.12±0.03^{bA}
Total mesophilic aerobic bacteria counts	Control	4.86±0.17^{aC}	6.32±0.03^{aB}	7.97±0.12^{aA}
	Laurel	4.25±0.53^{aB}	5.94±0.07^{abA}	7.16±0.08^{abA}
	Oregano	3.29±0.03^{bC}	4.81±0.29^{bB}	6.50±0.16^{bA}
	Thyme	3.14±0.09^{bC}	4.47±0.02^{bB}	6.57±0.09^{bA}
Enterobacteriaceae counts	Control	2.97±0.18^{aC}	5.16±0.27^{aB}	6.76±0.04^{aA}
	Laurel	2.64±0.65^{abB}	4.90±0.51^{abA}	6.03±0.07^{abA}
	Oregano	2.31±0.02^{abC}	3.89±0.62^{bB}	4.82±0.02^{bA}
	Thyme	1.99±0.26^{bC}	3.10±0.33^{bB}	4.59±0.07^{bA}
Pseudomonas counts	Control	2.69±0.05^{aC}	4.96±0.11^{aB}	6.36±0.02^{aA}
	Laurel	2.05±0.44^{bC}	4.14±0.04^{abB}	6.06±0.02^{aA}
	Oregano		3.49±0.07^{bB}	5.21±0.05^{abA}
	Thyme		3.20±0.03^{bB}	5.19±0.07^{bA}

Data are expressed as mean ± standard deviation. Means with different lower case letter in the column for each storage day are significantly different ($P < 0.05$). Means with different uppercase letter in the line for each treatment are significantly different ($P < 0.05$)

Regarding *Enterobacteriacaea,* the numbers in sea bass fillets for control and laurel, oregano, and thyme EO added samples were 2.97±0.18, 2.64±0.65, 2.31±0.02, and 1.99±0.26 log CFU/g after two days and increased over a storage period of 6 d for all groups. In this study, the lowest counts were obtained from thyme EO added samples with 4.59±0.07 log CFU/g followed by samples treated with oregano EO with 4.82±0.02 after 6 days. This result concurred with a previous study which indicated that the lowest counts of *Enterobacteriaceae* were obtained from thyme EO added rainbow trout groups during ice storage (Özogul et al., 2017).

Concerning *Pseudomonas,* their initial counts were 2.05±0.44 and 2.69±0.05 for laurel EO groups and control, respectively, while oregano and thyme EO added sea bass fillets had *Pseudomonas* spp. counts below 2 log CFU/g at the beginning of the storage. Over the 6 days of storage, no statistical differences were observed between laurel EO added and control groups in *Pseudomonas.* On the other hand, oregano and thyme EO added samples had similar *Pseudomonas* spp. counts (5.21±0.05 and 5.19±0.07, respectively) at the end of the 6 days of storage. In a previous study, Kostaki et al. (2009) showed that *Pseudomonas* numbers were approximately 6 log CFU/g for untreated sea bass fillets and around 4.5–5 log CFU/g for thyme added fillets at 6 day in parallel with my findings.

Interestingly, Huang et al. (2018) reported that *Pseudomonas* spp. counts for control, oregano, and thyme EO added grass carp fillets were approximately 8 log CFU/g at the 6th day and may be related to the inhibitory effect of the chemical composition of the essential oils. Indeed, significant components current EOs in this study were quite different from those reported in their study. Karabagias, Badeka, and Kontominas (2011) and Emiroğlu, Yemiş, Coşkun, and Candoğan (2010) also found a remarkable controlling effect of thyme and oregano EO on the *Pseudomonas* in line with these findings.

Overall, these results indicated that thyme and oregano EO reduced microbial load and produced a potent inhibitory effect in the way of TMAB, TPAB, *Enterobacteriaceae,* and *Pseudomonas* compared to control, whereas laurel EO demonstrated weak antibacterial behaviors throughout the entire cold storage in sea bass fillet samples. In fact, these findings agree well with the result of Ghabraie, Vu, Tata, Salmieri, and Lacroix (2016), who studied the antibacterial influence of thirty-two different EO against five indicator pathogenic microorganisms. These researchers showed

that laurel EO exhibited an antagonistic effect against only one pathogen, a 10.5±1.7 mm inhibition zone to *E. coli.* In contrast, common thyme and oregano EO were effective to 4 pathogens (between 18.1 mm and 35.6 mm inhibition zone) and against 3 pathogenic microbes (between 11.5 and 57.5 mm inhibition zone). The antibacterial activity of thyme and oregano EO has previously been attributed to their aromatic phenolic compounds, such as thymol and carvacrol, which caused structural and functional damage to the cytoplasmic membrane of bacteria (Jouki et al., 2014). Terpenes have several different chemical groups, including alcohol (linalool, geraniol, etc.), aldehyde (citral and citronellal), phenol (thymol and carvacrol), ketone (carvone and camphor), ether (eucalyptol), and hydrocarbon (cymene, pinene etc.) groups. However, it has been reported that the stronger antibacterial activity was related to the hydroxyl groups (phenolic and alcohol compounds), whereas ether and hydrocarbons provided less activity (Guimarães et al., 2019). This was supported by a previous study that found *S. putrefaciens,* considered as the strongest spoiler of seafood, to be the most resistant bacteria amongst all the tested microbes against eucalyptol (44.75%) and α-pinene (18.46%), the most active constituents of laurel EO (Zengin & Baysal, 2014). In this study, oregano and thyme EO managed to keep the microbiological load of sea ass fillets under 7 log CFU/g without the need for a different application or combination during 6 days of refrigerated storage. However, the same positive effect cannot be mentioned for laurel EO. This situation is probably due to the low concentration of laurel EO applied to the fillets.

Sensory scores

The sensory scores of cooked sea bass fillet samples on days 2 and 6 are indicated in Figure 1. Except for the odor scores on day 2 ($P < 0.05$), the essential oil treatment did not influence ($P > 0.05$) the appearance and texture properties on these days. The laurel EO group had the highest odor score on day 2, while oregano EO and thyme EO group had the lowest. Kostaki et al., (2009) reported that essential oils were preservative only at concentrations close to or exceeding 1% (v/w) required to extend shelf life, but generally imparted unpleasant sensory properties such as strong odor to foodstuffs. However, Bensid et al., (2014) reported that thyme, oregano and clove extracts could be used as easily accessible natural source since they had better consumer acceptance. In their study, the sensory scores were appreciated because the icing treatment to anchovy was applied indirectly with these plant extracts.

As seen in Figure 1, the sensory scores of the samples decreased as a result of microbial growth as refrigerated storage progressed. Similarly, Mexis et al (2009) found that the score for both odor and taste decreased over storage. Some researchers also reported that the bioactive phytochemicals of plant products degraded/oxidized, and their effectiveness decreased towards the end of storage (Bambeni et al., 2021; Manessis et al., 2020).

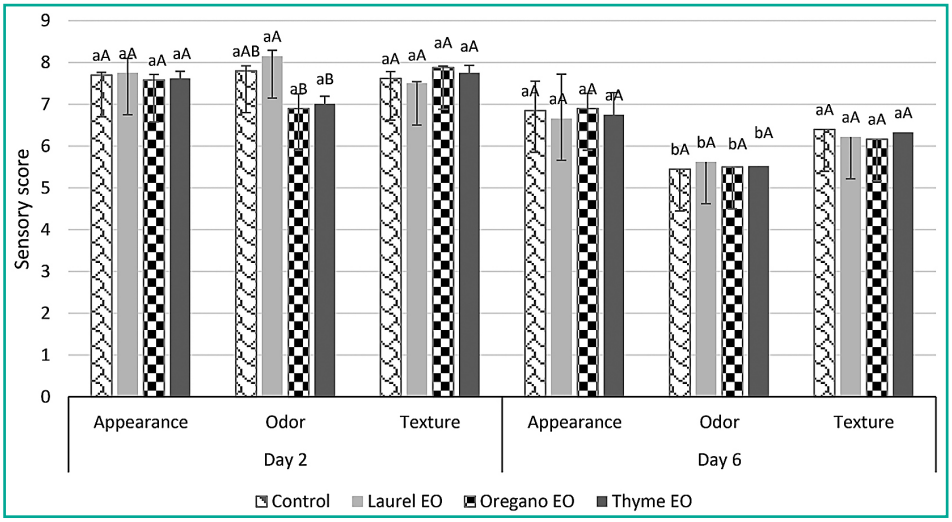

FIGURE 1: *Sensory scores of cooked sea bass fillets treated with different essential oils on days 2 and 6. Bar charts with different letters indicate significant differences between the treatments (A–B) on each storage day and storage days (a–b) in each treatment (P < 0.05).*

Conclusions

The treatment of sea bass fillet with laurel, oregano, and thyme EO at 1% showed lower TBA values, higher DPPH, and *L** values. Thyme and oregano EO reduced microbial load, potent inhibitory effect in TMAB, TPAB, *Enterobacteriaceae,* and *Pseudomonas,* compared to control. In contrast, laurel EO demonstrated weak antibacterial behaviors throughout the entire refrigerated storage. It can be concluded thyme and oregano EO can be recommended for sea bass fillet as a natural preservative.

Data Availability Statement

Research data are not shared.

Ethical Guidelines

Ethical Review: This study does not involve any human or animal testing.

Conflict of interest

The author declare no conflicts of interest.

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