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The impact of marine and terrestrial based extracts on the freshness quality of modified atmosphere packed sea bass fillets

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ABSTRACT

The extracts of *Padina pavonica* (a macroalgae, ME), *Rubus fruticosus* (blackberry) leaves (BE), *Juniperus oxycedrus* waste (JWE) and *Juniperus communis* needles (JE) were used to evaluate their potential effects on chemical, microbial and organoleptic qualities of sea bass (*Dicentrarchus labrax*). The sensory assessment showed that fish fillets treated with BE, JE and ME were preferred more by the panellists, and rejected at 18 days of storage compared to the control (12 days). All extracts (except JWE) together with modified atmospheric packaging (MAP) decreased the values of biochemical parameters of sea bass fillets. TVB-N, PV, FFA, TBA values remained lower in treatment groups. Microbiological results were consistent with sensory results. Although microbial growth showed a rapid increase in the control and JWE groups during storage, BE, ME and JE groups significantly inhibited the bacterial growth in fish meat ($p < 0.05$). Lactic acid bacteria in sea bass fillets under MAP condition increased slightly during storage and reached the highest levels in the control and JWE groups, respectively, with the value of 5.60 and 5.52 log cfu/g at the end of storage. This study showed that all extracts except JWE together with MAP application extended the shelf life of sea bass fillets up to 18 days compared to the control (12 days), exhibiting potential antioxidant and antimicrobial agents in seafood industry.

1. Introduction

Food and seafood wastes from agriculture or marine are generated in great quantities. The greater part of these wastes is underutilized and can result in environmental issues, causing loss of beneficial nutrients. Although food waste has been conventionally recovered as fertilizers and animal feed, the growing demand for environmental problems as well as resource conservation, are forcing to utilise food waste more efficiently for the fabrication of value-added food additives or chemicals through novel processes. The plant-derived wastes are generally rich in carbohydrates, lignocellulose, protein, fat, minerals and phytochemicals. Valorisation of these compounds not only decreases environmental problem but also promotes sustainability of agro-food industries (Jin et al., 2018).

Macroalgae are well known as an important source of food and they contain a variety of bioactive compounds. These bioactive compounds can be combined into food products for preservation since they are edible, safe and low-priced (Hayes, 2015). Thus, a considerable attention has been given to determine qualitative and quantitative phytochemical composition of macroalgae. Among them, *Padina pavonica* is widely spread from tropical to temperate waters. Due to its availability and biological properties (Al-Enazi et al., 2018; Generalić Mekinić et al., 2019, 2021), *P. pavonica* has potential to be applied in food manufacturing (Čagalj et al., 2022).

Mediterranean maquis is a natural ecosystem containing different kinds of plants with the capacity of the biosynthesis of secondary metabolites. Among them, *Rubus fruticosus* and *Rubus ulmifolius* belong to Rosaceae family and known as blackberry. In particular, blackberry leaves are widely used as a tea substitute and contain phenolic compounds with high antioxidant activity (Kolarević et al., 2021; Lebedev

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et al., 2022). Moreover, Halim et al. (2022) reported that immature leaves of blackberry (*Syzygium cumini* L.) had more phenolic metabolites, flavonoid, phenol and antioxidant activity than their mature leaves and seeds. A various parts of blackberry had different phenolic metabolites, including rosmarinic acid, gallic acid, catechin hydrate, 3, 4-DHBA (3,4-dihydroxybenzoic acid), epicatechin, caffeic acid, vanillic acid, rutin, syringic acid, *p*-coumaric acid, myricetin and *trans*-ferulic acid. Thus, all parts of the blackberry have potential as a promising source of bioactive components in the both pharmaceutical and food industries.

Juniperus is another class of the Mediterranean maquis and belongs to the Cupressaceae family. *Juniperus communis* and *Juniperus oxycedrus* are among the most widespread species and widely utilised as traditional medicine for cure of different infectious diseases (Karaman et al., 2003). Extracts/essential oils of juniper species have been extensively searched in relation to antioxidant activity and antimicrobial potential (Elshafie et al., 2020; Maeh et al., 2019; Meringolo et al., 2022; Miceli et al., 2020).

Fish are highly perishable products and rapidly deteriorate post-mortem as a results of a various biochemical and microbial breakdown mechanisms (Bondoc, 2002, 2014). Among different preservation methods, modified atmosphere packaging (MAP) can effectively delay the spoilage process and extend the shelf life of fresh fish (Tsironi & Taoukis, 2018). It is indicated that the selection of optimum MAP parameters such as gas composition and gas to product volume ratio is a very important step in the design of MAP for fish products. MAP, with the design of an appropriate atmosphere, can inhibit the oxidation of lipids and the growth of aerobic bacteria, both of which will prolong the shelf-life of fish products. A combination of O_2, CO_2, and N_2 are commonly used, varying concentrations in commercial applications of MAP technology (Nie et al., 2022).

Within the PRIMA (Partnership for Research and Innovation in the Mediterranean Area) project consortium (BioProMed), the qualitative and quantitative profile of the phenolic compounds contained in marine and terrestrial based plant species were characterized in previous studies (Barbieri et al., 2022, 2022agalj et al., 2022). Among many marine macroalgae and terrestrial plant extracts analysed, the extracts obtained from *Padina pavonica* (a macroalgae from Mediterranean coast of Croatia), *Rubus fruticosus* (blackberry) leaves, *Juniperus oxycedrus,* and *Juniperus communis* needles (collected in the Mediterranean maquis of Croatia) with the highest phenol contents were chosen to assess their potential use in seafood industry. In the current study, these extracts were employed to evaluate their potential effects on chemical, microbial and organoleptic qualities of modified atmosphere packed sea bass (*Dicentrarchus labrax*), which has high economic value and is commonly cultured in Europe.

2. Materials and methods

2.1. Marine and territorial materials

Macroalgae was collected in the Adriatic Sea off the southern coast of the island Čiovo (43.493389° N, 16.272505° E) whereas blackberry leaves and Juniper species were sampled at the mountain Kozjak, Croatia (43°58′15″N, 16°32′39″E) in 2020. Among the tested macroalgae and territorial plants, the samples with the highest total phenol content were chosen and they were *Padina pavonica* (a macroalgae, ME), *Rubus fruticosus* (blackberry) leaves (BE), *Juniperus communis* waste (after extraction of essential oil, JWE) and *Juniperus oxycedrus* needles (JE). Preparation of their extracts was described in detail in previous studies (Barbieri et al., 2022, 2022agalj et al., 2022). Briefly, the algal and plant extracts were prepared form dry material in 50% ethanol and extracted by microwave assisted extraction (advanced microwave extraction system ETHOS X, Milestone Srl, Sorisole, Italy) using 200 W and 60 °C over 15 min for algae and 600 W over 5 min for plant samples. The EtOH was evaporated and the extracts freeze dried until application in fish product.

2.2. Preparation of fish sample

Fresh sea bass (*Dicentrarchus labrax*) were obtained from a local fish retailer in Adana, Turkey. Their length and weight were 33.1 ± 1.36 cm and 357.06 ± 20.58 g, respectively. Fish were eviscerated, filleted and split into 5 lots. One lot was put on plates (two fillets per plate) without treatment (the control) whereas the other lots were applied with extracts at the level of 0.5%. Fish fillets were immersed in solution with different extracts (5 min). After that, fish fillets were placed in a plate with pads inside and then modified atmospheric packaged (60%CO_2 and 40%N_2) and stored at 2 ± 2 °C. Chemical, microbiological, sensory and analyses were carried out at regular intervals (0, 4, 8, 12, 14, 16, 18, and 20 days).

2.3. Sensory analyses

Sensory evaluation of raw fish was assessed by using the Quality Index Method (QIM) proposed by Bonilla, Sveinsdottir, and Martinsdottir (2007) with minor modifications. This scheme contains skin (skin brightness, skin mucus) and meat quality parameters (flesh texture, colour, odor, brightness, gaping and dripping loss). For each parameter, demerit points are given from 0 to a maximum point of 3, where 0 showed the finest quality and greater marks revealed inferior quality.

Cooked fish fillets were assessed in terms of taste, odor, and texture according to the Torry method (Howgate, 1982). A 10 to 3 scoring scheme was used, where 10 points indicate fresh fish and 3 indicate spoiled fish. To prepare the cooked fish samples, 2 fillets from each group were removed and cooked for 2 min in a microwave oven (Siemens HF24G241, Germany) at 600 W. Prepared fillets were served hot to the panellists.

2.4. Biochemical analysis

The total volatile basic nitrogen (TVB-N) analyses of sea bass was measured using the Antonocopoulus (1973) method and expressed as mg of TVB-N per kg of muscle. The TBA (thiobarbituric acid reactive substances) value was carried out by the method of Tarladgis et al. (1960) to measure the oxidation degree of lipid mg of malondialdehyde (MA) per kg of fish sample. Total lipid content was determined by Bligh and Dyer (1959). Free fatty acid (FFA) analysis (% oleic acid) was done by AOCS. Method Ca 5a-40 (1997). Peroxide value (PV) was measured in meq of peroxide oxygen per kg fat by the use of AOCS method Ja 8–87 (1994). The pH of fish fillets was determined by using a pH meter (315i/SET, Weilheim, Germany). The sample was homogenized using an Ultra-turrax (T25, Ika-Werke, Staufen, Germany) in distilled water at a ratio 1:10 (w/v) at its highest setting.

2.5. Microbiological analysis

Triplicate samples with double quantities from each party (n = 6) were removed for microbiological analysis. Ten grams of fish muscle was stomached (Masticator Nr S18/420, IUL Inst., Barcelona, Spain) using Ringer solution (90 ml, 1/4 strength) for 3 min. Further decimal dilutions were prepared and inoculated onto the surface of plate count agar (Fluka 70152, Steinheim, Switzerland) plates. Plates were then placed in an incubator for 10 d at 5 °C and 2 d at 30 °C for psychrophilic viable count and total mesophilic count, respectively (ICMSF, 1986). For total coliform count, Violet Red Bile Agar (VRBA, Oxoid, CM0107, Hampshire, England) was used (FDA, 1998). Aliquots of 1 ml of each dilution were moved to petri dishes with the two-fold pour plate method. They were developed for 24 h at 30 °C. For total lactic acid bacteria count, 1 ml of appropriate dilutions was taken and inoculated into petri dishes containing De Man, Rogosa-Sharpe Agar (MRS, Oxoid, CM1153, Hampshire, England) by pouring method. Petri dishes were

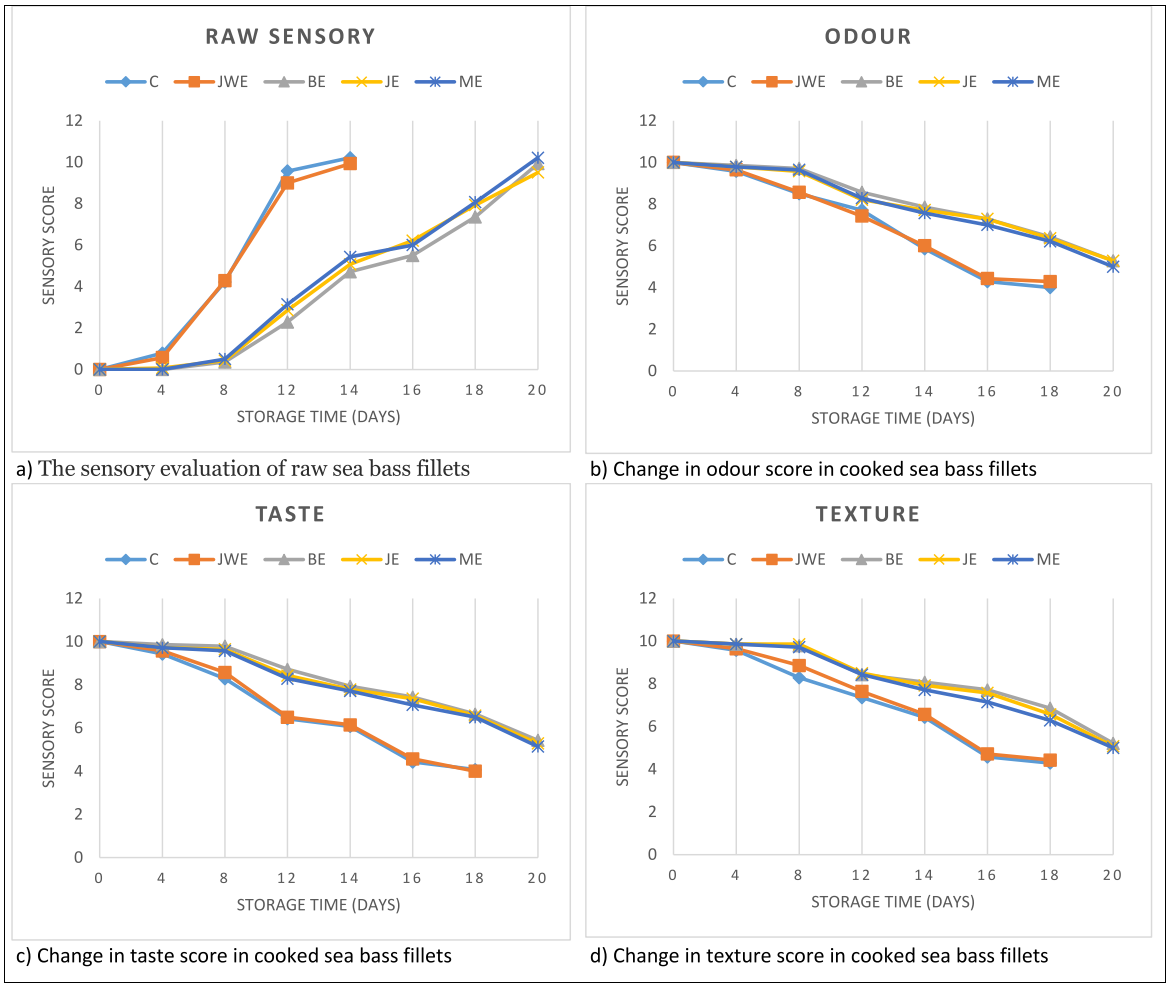


Fig. 1. Sensory change of raw and cooked sea bass fillets.

then placed at 30 °C for 72 h.

2.6. Statistical analysis

All experiments were carried out in triplicate and the results were given as the mean and standard deviation of these measurements. A one-way analysis of variance (ANOVA) was run using the SPSS version 19 software (SPSS, Chicago, Illinois, USA) and the Duncan's Multiple Range Test comparisons at P value of <0.05 were run to determine significant differences.

3. Results and discussion

3.1. Sensory analyses

Fig. 1 (a) shows the changes in sensory parameters of fish fillets applied with various extracts and packaged with modified atmosphere during cold storage (2 ± 2 °C). Organoleptic properties play a major role in consumer acceptance and marketability of fish. In this study, sensory scores increased during storage period. Significant differences ($p < 0.05$) were noted among the control and treatment groups in terms of sensory parameters. However, similar trend was noted between JWE and the control groups during storage. Except for the control and JWE groups, it was observed that the application of the other extracts had a positive effect on fish fillets. Fish fillets treated with BE, JE and ME were preferred more by the panellists, and these extracts extended the shelf life of the sea bass. In this regard, the control and JWE groups were rejected on the 12th day of storage, while the other groups were rejected on the 18th day. Overall acceptability sensory scores on day 12 of rejection of the sea bass fillet were 9.57 for the control, 9.93 for the JWE group, 2.29 for the BE group, 2.86 for the JE group and 3.14 for the ME group. BE, JE and ME groups were rejected on the 18th day and the general acceptability scores were determined as 7.36, 7.93 and 8.07, respectively.

In MAP application, changing the atmosphere in the package reduces the microbial growth that may develop in the packaged product and extends the shelf life by slowing down the enzymatic degradation. Such changes usually result in a decrease in the O_2 content of the air in the package and an increase in the CO_2 and N_2 ratios. In this study, the use of MAP (60% CO_2 and 40% N_2) and the extracts (especially BE, JE and ME) extended the shelf life of fish fillets. The effect of the MAP technique on the shelf life of the product relies on the product type, the initial quality of the raw material, the gas mixture, the storage temperature, the gas/product volume ratio, and the protection/barrier properties of the packaging material as well as the hygienic rules during processing and packaging (Alice et al., 2020; Das et al., 2021; Tsironi & Taoukis, 2018). Sivertsvik et al. (2002) indicated that Gram-positive microorganisms, for example; LAB resistant to CO_2, have an important part in the degradation of MAP-packed fish species caught from temperate waters. The capacity of LAB to prevent the growing of other microorganisms could be due to the production of bacteriocin and lactic acid in fish products under anaerobic conditions (Lim, 2016). LAB has been reported to be the predominant bacteria in the final population of sea bream fillets packed with MAP, and a significant decrease in bacterial

growth rate was found with increasing CO_2 content (20%–80%) (Alice et al., 2020; Tsironi & Taoukis, 2010).

It was reported that macroalgae and plant based extracts contain substances with many beneficial biological activities such as antimicrobial, antioxidant, anticancer, antiaging (Biris-Dorhoi et al., 2020; Cindana Mo'o et al., 2020; Kalasariya et al., 2021; Rathod et al., 2021; Salem et al., 2021). Plants contain phenols, flavonoid, alkaloid, terpenoid, tannin, berberine, quinine etc (Rahman et al., 2021). It is rich especially in phenolic substances known as the most important groups of natural antioxidants. The most common herbal phenolic antioxidants are flavonoids, coumarins, cinnamic acid derivatives, phenolic acids and tocopherols (Deveci et al., 2016). There are many studies on the effect of applying various plant and macro/microalgae extracts on different fish species and storage conditions on shelf life of fish products. Pan et al. (2021) reported that grass carp fish fillets employed with tea polyphenols had lower bacterial growth and increased shelf life from 10 to 16 days. Uçak (2020) investigated the effects of adding extract of pomegranate seed on the quality of trout burgers. It was determined that the addition of pomegranate seed extract had a positive effect on the sensory quality of the fish burgers and increased the shelf life by 9 days in comparison to the control group. Ozogul et al. (2021) studied the impacts of aqueous extracts (1%) of microalgae (*Spirulina plantensis* and *Chlorella vulgaris)* on microbiological, sensory, and chemical parameters on vacuum packed sardine (*Sardinella aurita*) fillets. As a result of the research, the shelf life of sardines was determined as 6 days for the control and 9 days for the Spirulina and Chlorella groups. Sáez et al. (2021) studied the effect of algae extracts (*Haematococcus pluvialis, Ulva ohnoi, Crassiphycus corneus, Arthrospira platensis*) on trout fillet and they found that these extracts delayed the firmness loss with longer the shelf life of the trout. Similar results have been found in relation to application of different plant extracts on different fish species and under different storage conditions (Abdel-Wahab et al., 2020; Ebadi et al., 2019; Tayel et al., 2018, 2021).

Fig. 1 (b,c,d) shows the changes in total sensory scores (smell, taste, and texture) of cooked sea bass fillets treated with various extracts over the storage period. A decrease was observed in all groups during storage. Significant variations in sensory indices were observed among the groups ($p < 0.05$). Plant and macroalgae extracts did not cause any negative effects on the taste, smell and flavour of the fish. However, in terms of general acceptability, BE, JE and ME groups received high scores. When cooked fish fillets were compared with raw fillets, the control and JWE groups were refused on day 14, and BE, JE and ME groups on day 20. Decreases in odor scores were noted during storage time (Fig. 1b). Although no difference was generally observed between JWE and the control groups, considerable differences were noted between these groups and BE, JE and ME groups. During storage a regular decrease in taste values was noticed for all groups (Fig. 1c). The control and JWE groups showed a faster decrease in taste score than those of the other groups. No difference were observed between the BE, JE and ME groups, with the BE group having a slightly higher value. Panellists could not detect a taste difference between the groups.

Texture is a significant index in evaluating the quality of fish meat. Soft fish meat is not accepted by the consumer and the fish processing industry is considered a loss of quality. During the storage period, decreases were observed in texture scores as well as in odor and taste scores (Fig. 1d). It was noticed that the control and JWE groups displayed similar trend throughout the storage and their texture scores were lower than the other groups. After the 4th day of storage, there was a substantial difference between the control and JWE groups and the other groups ($p < 0.05$). BE, JE, and ME applications on sea bass fillets were more preferred by panellists and texture, taste, and odor scores were higher during storage (Fig. 1b,c,d). JWE extract showed similar scores with the control group. BE, JE, and ME had a positive impact on the odor, taste, and texture of the sea bass fillets. In particular, the BE group had the highest value in terms of taste, smell and texture scores.

Heat treatment applications are known to be the most effective and practical method used to reduce the microorganism load in the product. As observed in this study, it was determined that the bad odor in the raw samples were suppressed by cooking and thus the product was still consumable. As a result of deterioration, alcohols, aldehydes, ketones, esters, sulphurous and amine-containing volatile compounds are formed in fish meat (Guan et al., 2019; Olafsdottir et al., 2005). Because the cooking process suppresses these putrid compounds, sensory scores of cooked products are higher than those of raw material during storage. Although the control and JWE groups were rejected on the 12th day and the other groups were rejected on the 18th day for raw fish, they were still within acceptable limits on the 14th and 20th days when fish were cooked, respectively. Similar findings have been reported for different fish and fish products treated with different plant extracts (Apang et al., 2020; Kenar et al., 2010; Ozogul et al., 2010, 2014; Raeisi et al., 2016; Viji et al., 2015).

Table 1
Changes in TVB-N values (mg/100 g) during cold storage of sea bass fillets.

Days/ Groups	C	BE	JE	JWE	ME
0	13.89 ± 0.30^{a}	13.83 ± 0.19^{a}	13.93 ± 0.04^{a}	13.97 ± 0.61^{a}	13.82 ± 0.04^{a}
4	19.35 ± 1.71^{a}	14.44 ± 0.00^{b}	15.33 ± 0.05^{b}	17.07 ± 1.02^{ab}	16.06 ± 0.99^{b}
8	24.19 ± 0.96^{a}	15.90 ± 0.81^{d}	17.97 ± 0.06^{c}	22.13 ± 0.08^{b}	18.46 ± 1.01^{c}
12	39.54 ± 0.95^{a}	20.20 ± 2.27^{b}	21.6 ± 1.03^{b}	38.30 ± 1.07^{a}	23.65 ± 0.83^{b}
14	48.79 ± 0.14^{a}	22.76 ± 1.01^{b}	23.58 ± 0.19^{b}	46.08 ± 2.85^{a}	25.52 ± 0.84^{b}
16	55.73 ± 0.25^{a}	27.36 ± 0.20^{d}	29.17 ± 0.12^{c}	56.72 ± 0.05^{a}	31.41 ± 0.92^{b}
18	70.39 ± 1.51^{a}	31.99 ± 1.96^{b}	33.29 ± 0.04^{b}	67.96 ± 1.83^{a}	35.23 ± 0.33^{b}
20	74.53 ± 2.02^{a}	40.99 ± 1.16^{c}	44.51 ± 0.03^{bc}	71.14 ± 1.88^{a}	46.74 ± 0.88^{b}

$^{a-d}$ shows significant differences ($p < 0.05$) between control and treatments for each group. C, Control; BE, *Rubus fruticosus* (blackberry) leaves; JE, *Juniperus oxycedrus* needles; JWE, *Juniperus communis* waste (after extraction of essential oil); ME, macroalgae (*Padina pavonica*) extract.

3.2. Biochemical changes

TVB-N (total volatile basic nitrogen) is formed due to microbial degradation of proteins and non-protein nitrogenous composites, including dimethylamine, ammonia, trimethylamine, as well as other volatile essential composites. It is one of the significant chemical indices to measure quality of fish during storage. Table 1 displays the variations in TVB-N levels of sea bass fillets treated with ME, BE, JWE and JE extracts in modified atmosphere package during storage. The initial values of TVB-N for the all groups were in the range of was 13.82–13.97 mg/100 g, which are lower than those obtained for sea bass (Messina et al., 2021; Yazgan et al., 2017, 2017orapci, 2022). The non-protein nitrogen content of the fish may vary with the diet of the fish, the fishing season, the size of the fish, various environmental factors and the microbiological quality of the fish tissue (Connell, 1995; Goulas & Kontominas, 2007; Kyrana et al., 1997). Generally, the TVB-N level increases during storage due to the activity of degrading bacteria, storage condition, endogenous enzymes, and hygienic procedures (Kykkidou et al., 2009). Statistically significant differences ($p < 0.05$) were found between the control and treatment groups on the 4th day of storage in sea bass fillets. On the 12th day of storage, the increase in TVB-N levels of the control and JWE groups reached to 39.45 and 38.30 mg/100 g, while in the other treatment groups, this increase was between 20.20 and 23.65 mg/100 g. The Commission of the European Communities (CEC, 1995) reported 35 mg/100 g as the acceptable upper limit for the TVB-N level. In this study, the control and JWE groups exceeded this permissible upper limit on day 12 of storage, but ME group on the 18th day of

Table 2
Changes in pH during cold storage of sea bass fillets.

Days/ Groups	C	BE	JE	JWE	ME
0	6.68 ± 0.09[a]	6.67 ± 0.09[a]	6.66 ± 0.08[a]	6.69 ± 0.06[a]	6.67 ± 0.10[a]
4	6.71 ± 0.06[a]	6.69 ± 0.07[a]	6.70 ± 0.10[a]	6.72 ± 0.11[a]	6.69 ± 0.05[a]
8	6.84 ± 0.06[a]	6.75 ± 0.09[a]	6.79 ± 0.06[a]	6.81 ± 0.10[a]	6.77 ± 0.02[a]
12	6.97 ± 0.05[a]	6.78 ± 0.07[b]	6.81 ± 0.02[b]	6.95 ± 0.03[a]	6.8 ± 0.08[b]
14	7.02 ± 0.02[a]	6.82 ± 0.06[b]	6.83 ± 0.12[b]	7.01 ± 0.07[a]	6.84 ± 0.10[b]
16	7.04 ± 0.01[a]	6.86 ± 0.05[b]	6.87 ± 0.05[b]	7.08 ± 0.12[a]	6.86 ± 0.10[b]
18	6.94 ± 0.03[a]	6.92 ± 0.07[a]	6.94 ± 0.05[a]	6.53 ± 0.04[b]	6.91 ± 0.06[a]
20	6.41 ± 0.03[c]	6.95 ± 0.12[a]	7.02 ± 0.07[a]	6.6 ± 0.02[b]	7.03 ± 0.05[a]

[a-d] shows significant differences ($p < 0.05$) between control and treatments for each group. C, Control; BE, *Rubus fruticosus* (blackberry) leaves; JE, *Juniperus oxycedrus* needles; JWE, *Juniperus communis* waste (after extraction of essential oil); ME, macroalgae (*Padina pavonica*) extract.

Table 3
Changes in PV (meq O_2/kg) during cold storage of sea bass fillets.

Days/ Groups	C	BE	JE	JWE	ME
0	4.14 ± 0.09[a]	4.11 ± 0.13[a]	4.07 ± 0.11[a]	4.15 ± 0.14[a]	4.12 ± 0.10[a]
4	4.30 ± 0.14[a]	3.45 ± 0.25[b]	2.73 ± 0.08[c]	4.37 ± 0.26[a]	2.52 ± 0.08[c]
8	4.57 ± 0.12[a]	4.38 ± 0.28[a]	3.26 ± 0.31[b]	4.47 ± 0.27[a]	4.37 ± 0.42[a]
12	6.01 ± 0.57[a]	4.05 ± 0.32[cd]	3.57 ± 0.28[d]	4.81 ± 0.33[b]	4.33 ± 0.35[bc]
14	6.96 ± 0.34[a]	3.47 ± 0.28[c]	3.05 ± 0.20[c]	4.86 ± 0.29[b]	4.62 ± 0.41[b]
16	4.60 ± 0.37[a]	3.60 ± 0.15[b]	3.06 ± 0.34[c]	3.96 ± 0.30[b]	2.44 ± 0.04[d]
18	4.91 ± 0.3[a]	2.15 ± 0.14[d]	2.07 ± 0.09[d]	4.22 ± 0.36[b]	2.95 ± 0.22[c]
20	5.88 ± 0.35[a]	3.32 ± 0.30[c]	4.29 ± 0.28[b]	4.74 ± 0.37[b]	3.61 ± 0.16[c]

[a-d] shows significant differences ($p < 0.05$) between control and treatments for each group. C, Control; BE, *Rubus fruticosus* (blackberry) leaves; JE, *Juniperus oxycedrus* needles; JWE, *Juniperus communis* waste (after extraction of essential oil); ME, macroalgae (*Padina pavonica*) extract.

storage, BE and JE groups on the 20th day of storage. The reason for the low TVB-N levels could be due to high phenol content and antimicrobial activity of *Padina pavonica* (ME) (Al-Enazi et al., 2018; Ben Ali et al., 2010; Keramane et al., 2020) and *Rubus fruticosus* (blackberry) leaves (BE) (Grabek-Lejko et al., 2022; Krzepiłko et al., 2021, 2021agalj et al., 2022), *Juniperus oxycedrus* needles (JE) (Barbieri et al., 2022; Ben Mrid et al., 2019; Yaglioglu & Eser, 2017).

The variations in pH values during cold storage of sea bass fillets applied with different plant extracts and packaged in a modified atmosphere are given in Table 2. Post-mortem changes in fish meat directly affect pH values initially (Ruiz-Capillas & Moral, 2001). With the cessation of cardiac activity and insufficient oxygen supply to the tissues, the energy required for metabolic events is provided by the anaerobic transformation of glycogen to lactic acid. The lactic acid accumulated due to glycogenolysis reduces the pH of fish meat to 5.5–5.6. The glycogenolysis that occurs after death ends when the enzymatic activity is inactive due to the depletion of glycogen reserves or low pH value. While the pH value is around 7 in live fish, this value changes in the range of 6–7.1 depending on various reasons after death (Simeonidou et al., 1998). The initial pH value was 6.67 ± 0.10. Çorapci (2022) reported similar initial pH values in sea bass fillets. There is no statistical difference between the pH values of all groups during the first eight days of storage. By the 12th day, it was observed that C and JWE groups had higher pH values than BE, JE and ME groups. This elevation continued until the storage's 16th day, and the difference between all groups was statistically insignificant on the 18th day except for JWE group. There was a sudden pH value decrease in C and JWE groups at the 18th day of storage. This decrease is thought to be due to carbonic acid formed due to the dissolution of CO_2 in the fillet surface and the aqueous phase of the muscle tissue (Ordonez et al., 2000). When all groups were considered, the pH values increased gradually until the 16th day of the experiment. The rise in pH during storage could be because of accumulation of basic composites (trimethylamine ammonia, and other biogenic amines), which are created due to the growth of bacteria and the resulting destruction of muscle tissue (Ruiz-Capillas & Moral, 2001). Olatunde et al. (2019) observed a gradual rise in pH of Asian sea bass slices with ethanolic coconut shell extract in modified atmosphere packaging. However, as observed in this study, fluctuations in pH values of modified atmosphere packed sea bass fillets were also reported in other studies, using diverse N_2 and CO_2 combinations (Provincial et al., 2010; Ruiz-Capillas & Moral, 2001; Torrieri et al., 2006).

The changes in the peroxide values (PV, meq/kg) of the sea bass fillets are shown in Table 3. PV of fillets treated with C, BE, JE, JPE and ME extracts at the beginning of storage were found to be 4.14, 4.11, 4.07, 4.15 and 4.12 meq O_2/kg, respectively, and fluctuations were detected in the following days. It was observed that different extracts had no effect on peroxide value at the beginning of storage ($p < 0.05$). PV values of C group were statistically higher than those of other treatment groups during storage ($p < 0.05$). The highest PV values were determined as 6.96 meq O_2/kg in the C group on the 14th day. Apart from the C group, statistically high PV values were noted in the JWE group during storage ($p < 0.05$). The highest PV value was determined as 4.74 meq O_2/kg value on the 20th day of storage in the JPE group whereas the lowest value was obtained as 2.07 meq O_2/kg in the JE group on the 18th day. PV values were determined as 5.88, 3.32, 4.29, 4.74 and 3.61 meq O_2/kg in C, BE, JE, JWE, and ME groups at the end of storage period, respectively.

One of the most important factors in the deterioration of fish quality is reduction in polyunsaturated fatty acids and therefore rise in peroxide (Pradhan et al., 2018; Tenyang et al., 2017). Lipid peroxidation is a highly complex autocatalytic process consisting of two steps. In the first stage, peroxides, the primary products of oxidation, are formed and in the second stage, hydroperoxides with high reactivity are broken down into aldehydes, ketones and carboxylic acids, which are secondary products of oxidation (Bakalinova et al., 2009). In some studies, it is reported that the fish is fresh when the peroxide value is lower than 5 meq O_2/kg, and it starts to deteriorate between 5 and 10 meq O_2/kg (Aberoumand & Baesi, 2020; Bannenberg et al., 2017). Pawar et al. (2020) reported that fish is not suitable for human consumption when its peroxide value exceeds 10 meq O_2/kg. Fıçıcılar and Gençcelep (2021) reported that the decomposition in flavor would take place when PV reached a value between 20 and 40 meq O_2/kg. In the study of Ucar et al. (2020) on sea bass fillets, it was observed that the PV values in the control group and nisin-treated fillets of the samples stored in the refrigerator at +4 °C in normal air varied between 2 and 7 meq O_2/kg. de Aguiar Saldana Pinheiro et al. (2021) observed that PV values of sardine fillets never exceed 4 meq O_2/kg under MAP conditions. They reported that the low PV values of the MAP samples might be due to the low levels of oxygen in the package. Messina et al. (2015) studied the effect of MAP and natural antioxidants on common dolphinfish and they observed that the samples with antioxidants were statistically lower oxidation parameters than the other groups. Kadem et al. (2020) applied the root extract (0.2% and 0.5%) of caper bush (*Capparis spinosa*) to rainbow trout fillets during storage at MAP conditions. In this study, it was noticed that the PV of the control were statistically higher than the

Table 4
Changes in FFA during cold storage of sea bass fillets FFA.

Days/ Groups	C	BE	JE	JWE	ME
0	3.77 ± 0.42^{a}	3.51 ± 0.30^{a}	3.37 ± 0.25^{a}	3.68 ± 0.12^{a}	3.80 ± 0.44^{a}
4	2.29 ± 0.15^{b}	2.77 ± 0.27^{a}	2.84 ± 0.23^{a}	2.90 ± 0.16^{a}	2.08 ± 0.15^{b}
8	3.63 ± 0.29^{a}	3.03 ± 0.22^{b}	3.48 ± 0.16^{a}	3.79 ± 0.08^{a}	2.60 ± 0.18^{c}
12	4.74 ± 0.02^{a}	2.67 ± 0.20^{c}	2.92 ± 0.27^{c}	4.70 ± 0.07^{a}	3.56 ± 0.06^{b}
14	3.16 ± 0.32^{a}	2.44 ± 0.17^{b}	2.95 ± 0.37^{ab}	3.05 ± 0.26^{a}	2.92 ± 0.20^{ab}
16	4.39 ± 0.34^{a}	2.58 ± 0.30^{c}	2.24 ± 0.19^{c}	3.95 ± 0.11^{b}	2.64 ± 0.20^{c}
18	7.55 ± 0.36^{a}	2.82 ± 0.02^{c}	4.42 ± 0.24^{b}	7.13 ± 0.41^{a}	4.62 ± 0.27^{b}
20	4.08 ± 0.41^{a}	3.20 ± 0.11^{b}	3.16 ± 0.22^{bc}	3.24 ± 0.22^{b}	2.73 ± 0.12^{c}

$^{a-d}$ shows significant differences ($p < 0.05$) between control and treatments for each group. C, Control; BE, *Rubus fruticosus* (blackberry) leaves; JE, *Juniperus oxycedrus* needles; JWE, *Juniperus communis* waste (after extraction of essential oil); ME, macroalgae (*Padina pavonica*) extract.

Table 5
Changes in TBA values (mg MA/kg) during cold storage of sea bass fillets.

Days/ Groups	C	BE	JE	JWE	ME
0	0.55 ± 0.03^{a}	0.40 ± 0.01^{d}	0.43 ± 0.01^{c}	0.51 ± 0.01^{b}	0.44 ± 0.01^{c}
4	0.40 ± 0.03^{a}	0.37 ± 0.00^{c}	0.39 ± 0.03^{ab}	0.38 ± 0.01^{ab}	0.39 ± 0.01^{ab}
8	0.44 ± 0.01^{a}	0.42 ± 0.01^{cd}	0.41 ± 0.01^{d}	0.43 ± 0.01^{b}	0.42 ± 0.01^{bc}
12	0.40 ± 0.00^{a}	0.36 ± 0.01^{c}	0.35 ± 0.00^{d}	0.38 ± 0.01^{b}	0.39 ± 0.02^{ab}
14	0.40 ± 0.01^{a}	0.37 ± 0.01^{c}	0.37 ± 0.00^{c}	0.37 ± 0.01^{c}	0.38 ± 0.01^{b}
16	0.48 ± 0.01^{a}	0.41 ± 0.01^{d}	0.42 ± 0.01^{cd}	0.44 ± 0.01^{b}	0.42 ± 0.00^{c}
18	0.46 ± 0.01^{a}	0.43 ± 0.02^{cd}	0.44 ± 0.01^{bc}	0.45 ± 0.01^{ab}	0.42 ± 0.01^{d}
20	0.44 ± 0.02^{a}	0.39 ± 0.01^{c}	0.40 ± 0.01^{bc}	0.42 ± 0.02^{b}	0.39 ± 0.01^{c}

$^{a-d}$ shows significant differences ($p < 0.05$) between control and treatments for each group. C, Control; BE, *Rubus fruticosus* (blackberry) leaves; JE, *Juniperus oxycedrus* needles; JWE, *Juniperus communis* waste (after extraction of essential oil); ME, macroalgae (*Padina pavonica*) extract.

other groups during the storage period (28 days), increasing above 6 meq O_2/kg on the 12th and 14th days only in the C group during storage. PV values in all other treatment groups did not exceed 5 meq O_2/kg.

Table 4 shows the changes in FFA (% oleic acid) values of sea bass fillets durin storage. FFA values of C, BE, JE, JWE and ME extracts at the beginning of storage were found to be 3.77%, 3.51%, 3.37%, 3.68% and 3.80%, respectively, and fluctuations were observed during storage as observed in PV. There was no statistical difference ($p > 0.05$) between the FFA values of the treatment groups at the beginning of storage. During storage, the highest FFA value was obtained as 7.55% for C group and 7.13% for JWE on the 18th day. FFA values of JWE and C groups were similar except for the 16th and 20th days, and generally higher than the other groups. The lowest value detected during storage was 2.08% in the ME group on the 4th day. In general, FFA values did not reach 5% in all groups during storage.

Hydrolytic degradation occurs by lipase enzymes and initiates the creation of free fatty acids (FFA). Free fatty acids are then oxidized to form low molecular weight compounds that cause bad odor and taste in fish and aquatic products (Otero et al., 2019). The development of lipid hydrolysis is highly dependent on the hydrolytic enzyme contents under the influence of different internal and external factors. Separation of free fatty acids from the triglyceride matrix can increase the rate of lipid oxidation and the development of bad odor (Prabhakar et al., 2020). Ucar et al. (2020) reported that the FFA values of sea bass fillets with nisin were in the range of 2%–8%, which are similar to the results of the current study. The lower FFA values could be due to the reduction of air from the environment as a result of modified atmosphere packaging. Kadem et al. (2020) applied caper bush (*Capparis spinosa*) root extract to trout fillets in MAP during 28 days of storage and they also found significantly lower FFA values.

Table 5 shows the variations in TBA values during cold storage of sea bass fillets packaged in modified atmosphere. Peroxides are formed as the primary product as a result of auto-oxidation, and the determination of the amount of thiobarbituric acid (TBA) produced in the advanced stage of oxidation is a commonly used method to evaluate the degree of lipid oxidation. The rise in TBA is mostly because of auto-oxidation and enzymatic hydrolysis during storage (Wang et al., 2016). It was suggested that TBA value < 4 mg MDA/kg is high-quality quality sea bass with high consumer acceptance (Masniyom et al., 2002). In this study, TBA results did not exceed the above-mentioned value during the storage period. Statistical differences in TBA values were observed between application and the control groups during storage ($p < 0.05$). TBA values at the beginning of the storage ranged from 0.40 to 0.55 mg MA/kg. While the lowest TBA value was 0.35 mg MA/kg for the JE group on the 12th day, the highest TBA value was determined as 0.55 mg MA/kg for the control on the 0th day. On the last day of storage, BE and ME groups (0.39 mg MA/kg) had the lowest TBA values, followed by the JE group (0.40 mg MA/kg). Olatunde et al. (2019) studied the combined use of MAP and ethanolic coconut shell extract and determined that TBA values remained below 4 mg for all groups at the end of storage period (18 days), having lower TBA values for the group with ethanoic coconut shell extract. Zhou et al. (2020) investigated the effects of gelatin active coating with eugenol together with modified atmosphere packaging on Chinese sea bass. It was determined that the TBA values in treatment groups were relatively lower than the control. They indicated that the inhibitory influence of lipid oxidation was also due to the existence of eugenol, which performs as an antioxidant.

In the current study, generally low levels of PV, FFA and TBA were observed in *Padina pavonica* (ME), *Rubus fruticosus* (blackberry) leaves (BE), and *Juniperus oxycedrus* needles (JE). This could be attributed the high level of phenols and antioxidant capacities of *Padina pavonica* (Saidani et al., 2022, 2022agalj et al., 2022), *Rubus fruticosus* leaves (Koczka et al., 2018; Mîrza, 2021) *Juniperus oxycedrus* needles (Ben Mrid et al., 2019; Djellouli et al., 2022). Plant extracts (except JWE) and macroalgae extract used together with modified atmospheric packaging decreased the values of oxidation parameters. It was reported that plant based extracts are good sources of phenolic compounds, such as phenolic acids, tannins, elagitannins, flavonoids and anthocyanins, having antioxidant and antimicrobial effects (Lopez-Corona et al., 2022 .; Tufail et al., 2022). Barbieri et al. (2022) reported that the main phenolic acid in extract of the *J. oxycedrus* needles was vanillic acid (10.51 mg/l) while the abundant flavonoids were apigenin (7.66 mg/l) and rutin (6.95 mg/l). As for blackberry (*R. fruticosus*) leaves, the major phenolic acid was chlorogenic acid (6.22 mg/l) and rutin was the most abundant flavonoid (29.88 mg/l). GeneralićMekinić et al. (2021) detected phenolic acids such as p-hydroxybenzoic acid, protocatechuic acid, t-ferulic acid, p-coumaric acid, and o-coumaric acid while five phenolic compounds (kaempferol, ferulic acid, naringenin, delphinidin-3-oglucoside and ellagic acid) in *P. pavonica* extracts were also reported by Sudha and Balasundaram (2018).

In addition, MAP effectively delayed the spoilage process and extended the shelf life of fresh fish, especially in treatment groups It was indicated that the selection of optimum MAP parameters such as gas composition and gas to product volume ratio is a very important step in the design of MAP for fish products (Tsironi & Taoukis, 2018). MAP,

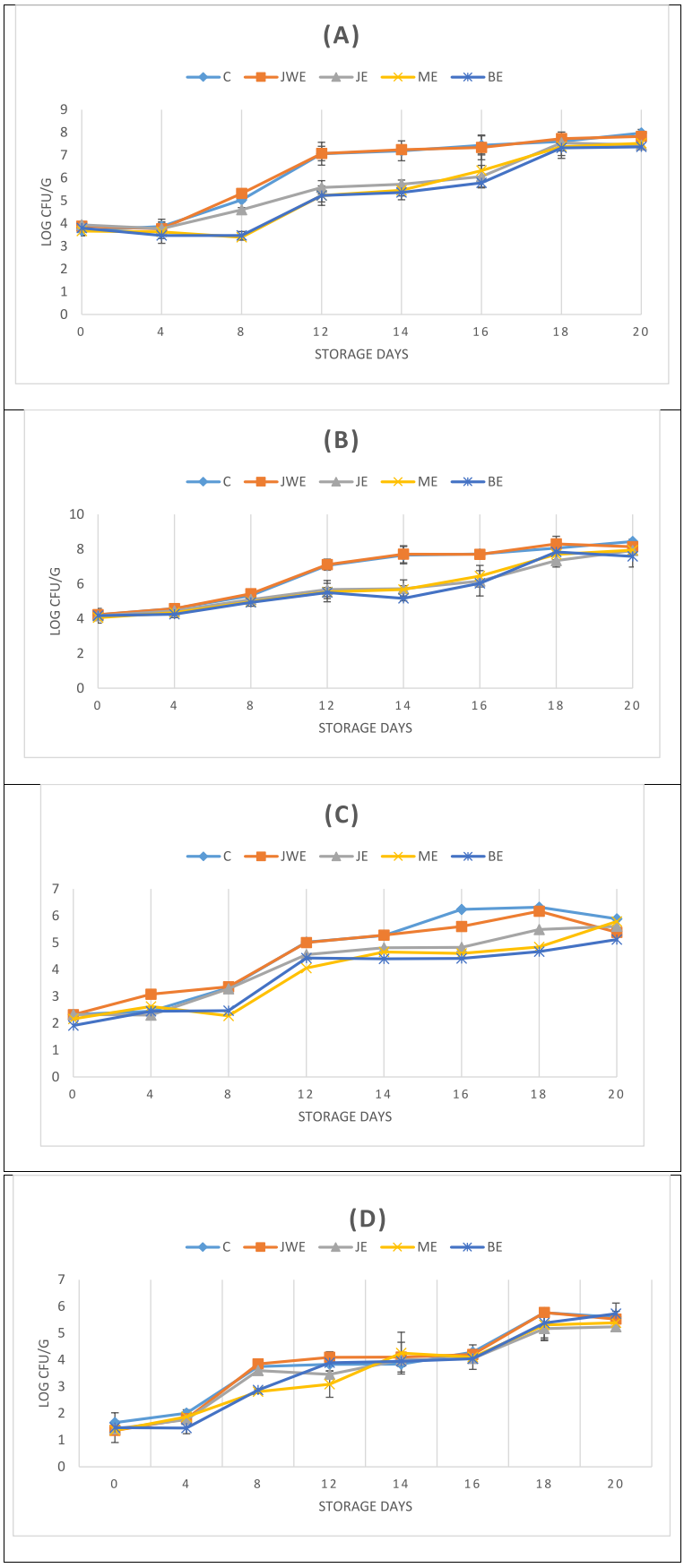


Fig. 2. Total mesophilic (a), psychrophilic (b), coliform (c) and lactic acid bacteria (d) counts of sea bass fillets stored at 2 ± 2 °C under MAP.

with the design of an appropriate atmosphere, can inhibit the oxidation of lipids and the growth of aerobic bacteria, both of which will extend the shelf-life of fish products as found in this study. Mixtures of O_2, CO_2, and N_2 are commonly used in different combinations and varying concentrations in commercial applications of MAP technology (Nie et al., 2022).

3.3. Microbiological changes

Fig. 2 displays microbiological indices of sea bass fillets stored in MAP. The initial mesophilic and psychrophilic bacterial load in the control group were 3.68 and 4.22 log cfu/g, respectively. Higher initial microbial load has been reported for sea bass fillets (Kritikos et al., 2020; Paleologos et al., 2004; Tsironi et al., 2019). The growth of aerobic and Gram-negative bacteria slows down due to the increase in CO_2 and decrease in O_2 in the packaging system (Tsironi et al., 2019). The numbers of mesophiles and psychrophiles in the extract-treated groups were below 3.94 and 4.23 log cfu/g, respectively. The increase in psychrophilic bacteria in fish meat was higher than in mesophiles. Thomas et al. (2021) found that the initial flora in sea bream was dominated by the families *Comamonadaceae, Enterobacteriaceae, Moraxellaceae, Comamonadaceae, Anaplasmataceae* and *Bacillaceae*, but at the end of the storage period in MAP, the fish microflora was determined by psychrotrophic and psychrophilic members (*Pseudoalteromonadaceae, Pseudoalteromonadaceae,* and *Pseudoalteromonadaceae*). Although microbial growth showed a rapid increase in the control and JWE groups during storage, the groups treated with extracts mainly BE, ME and JE significantly inhibited the bacterial growth in fish meat ($p < 0.05$). It has been observed that juniper leaf extract is rich in monoterpenoids, proanthocyanidins and flavonols and exhibits good antimicrobial activity against bacteria (Martz et al., 2009). Moreover, terpens, diterpenoids, aldehydes, alcanes and hydrocarbons with antimicrobial properties could be detected in extracts and volatile oil of *Padina pavonica* (Rushdi et al., 2021). Various extracts of blackberry leaves showed strong antimicrobial activity against many tested microorganisms (Krzepiłko et al., 2021). In this study, the reason for the lower bacterial growth in the extract applied groups may be because of the presence of bioactive compounds having antimicrobial properties.

The microbiological limit of 7 log cfu/g (ICMSF, 1986) for fresh and frozen fish was reached on the 12th day for the control and JWE, and on the 18th day for JE, ME and BE groups. Microbiological results were in consistent with the results of sensory assessment. Sea bass stored under MAP at 0, 5, and 10 °C reached 7 log cfu/g levels on days 18, 10 and 6, respectively (Tsironi et al., 2019). MAP notably delayed the growth of mesophilic and, more significantly, of psychrotrophic bacteria in triggerfish fillets at refrigerated storage (Esteves et al., 2021). Poli et al. (2006) reported a lower total number of organisms (<6 log cfu/g) at day 15 in sea bass fillets stored in MAP at 2 ± 1 °C. Similarly, Olatunde et al. (2019) found total viable count of Asian sea bass slices applied with extract of ethanolic coconut husk (200-ppm) and packed in MAP (80% CO_2/10% N_2/10% O_2 and 60% CO_2/30% N_2/10% O_2) less than 6.0 log cfu/g at day 15. In the current study, the highest microbial load was observed in the control and JWE group at the end of storage, with value of 7.97 and 7.83 log cfu/g for mesophiles, and 8.14 and 8.44 log cfu/g for psychrophiles, respectively.

Total coliform counts (Fig. 2) varied between 1.91 log cfu/g for BE group and 2.33 log cfu/g for the control group at the beginning of the storage. Coliform bacteria showed gradual increases during storage. Chen and Xiong (2008) reported that the coliform count in vacuum packed crayfish reached 8.5 log cfu/g on the 21st storage day, but the MAP samples had the lowest coliform count even at the end of storage (<3 log cfu/g). In the current study, the highest increases in coliform counts were noticed in the control and JWE groups. At the day of sensory rejection, total coliform counts were 5.02, 5.00, 5.49, 4.84, and 4.67 log cfu/g for the control, JWE, JE, ME and BE groups, respectively. The highest coliform load was found on the 18th day of storage in the control (6.32 log cfu/g) and JWE group (6.17 log cfu/g). Parlapani et al. (2015) reported a lower number of *Enterobacteriaceae* count (4.6 log cfu/g) in sea bass meat under MAP at the end of storage.

Anaerobic modified atmosphere conditions create a suitable environment for the growth of lactic acid bacteria (Duthoo et al., 2021). The initial LAB count in fish meat varied from 1.36 (JE and ME groups) to 1.65 (the control group) log cfu/g (Fig. 2). LAB in sea bass fillets under MAP condition increased slightly during storage and reached the highest levels in the control and JWE groups, respectively, with value of 5.60 and 5.52 log cfu/g at the end of storage. Slightly higher number of lactic acid bacteria (6 log cfu/g) was noticed at the end of chilled storage of sea bass meat stored under MAP (Kritikos et al., 2020; Masniyom et al., 2002; Parlapani et al., 2015). LAB are tolerant to CO_2 and thus, often present as the dominant organisms in MAP fishery products.

4. Conclusion

The sensory analyses showed that fish fillets treated with BE, JE and ME were preferred more by the panellists, and rejected at 18 days of storage compared to the control (12 days). All extracts (except JWE) together with MAP decreased the values of biochemical parameters of sea bass fillets. The antioxidant and antimicrobial activity of the tested extracts can be related to the presence of phenolic compounds. It can be concluded that the plants-based extracts except JWE (without essential oil) were able to improve the microbiological and oxidative stability of treated sea bass fillets when compared to the control. Thus, they can be used as antioxidant and antimicrobial agents in seafood industry.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Fatih Ozogul reports financial support was provided by Scientific and Technological Research Council of Turkey. Fatih OZOGUL also reports financial support was provided by PRIMA programme.

Data availability

Data will be made available on request.

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