

# The Effects of Pomegranate Peel Extract (PPE) Added to Ice on the Quality and Shelf Life of Horse Mackerel (*Trachurus trachurus*) Under Cold Storage Conditions (4±1°C)

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## Abstract

In this study, the effects of pomegranate peel extract (PPE) incorporated into ice on the quality and shelf life of horse mackerel (*Trachurus trachurus*) under cold storage conditions (4±1°C) were investigated. The primary objective was to assess the impact of PPE on various quality parameters throughout the storage period. Two experimental groups were established: a control group (C) and a PPE-treated group. The control group was stored with conventional ice, whereas the PPE group was stored with ice enriched with pomegranate peel extract. To evaluate the differences between the groups, a comprehensive set of physicochemical (proximate composition, pH, water activity, color, TBA, TVB-N), microbiological (TAMB, TAPB), and sensory quality analyses were conducted. By the end of the storage period, the TVB-N value reached 19.00±0.43 mg N/100g in the control group, while it remained significantly lower at 12.67±0.3 mg N/100g in the PPE-treated group. Similarly, the initial TBA value of 0.39±0.04 mg MA/kg increased to 5.84±0.19 mg MA/kg in the control group, whereas it only reached 1.64±0.20 mg MA/kg in the PPE group, indicating a substantial reduction in lipid oxidation. From a microbiological perspective, both groups remained below the critical threshold of 7 log cfu/g for total psychrophilic and mesophilic aerobic bacteria throughout the storage period. However, the PPE-treated group exhibited a notably lower bacterial load compared to the control, suggesting an antimicrobial effect of PPE. Color measurements (L\*, a\*, and b\* values) were also monitored, revealing an increase in the b\* value over time. However, this shift did not negatively impact sensory evaluations; on the contrary, the resulting yellowish hue was found to be visually appealing by the panelists. In the final sensory assessment, the control group was rated close to degraded fish quality, whereas the PPE-treated group was evaluated as being much fresher, underscoring the positive influence of PPE on fish quality. In conclusion, the application of PPE-enriched ice significantly extended the shelf life of horse mackerel by an additional five days under cold storage conditions. The strong antioxidant potential of pomegranate peel extract, functioning as a natural preservative, highlights its promising role in the long-term preservation of fishery products in similar applications.

## Introduction

Fish, which is included in aquatic products, has an important place in human nutrition with its richness in unsaturated fatty acids and protein, mineral substance and vitamin content, and easy digestion. Fish, which have a limited shelf life due to their high water content and weak connective tissue, appear as sensitive food products where chemical and microbiological deterioration occurs rapidly (Hosseini et al., 2016;

Maghami et al., 2019; Ceylan et al., 2019; Aubourg, 2023). The deterioration of fish is microbial and chemically based and results from biological reactions such as lipid oxidation, microbial growth, and their metabolic activities. Fishery products should be consumed within a short time after hunting, or in cases where this is not possible, they should be preserved by processing in various ways to slow down the deterioration (Can and Kaşıkçı, 2018). In this context, cooling technology is traditionally preferred on a global

scale. In this technology, seafood is kept cold at  $-1/+4^{\circ}\text{C}$ . Ice cooling, crushed ice cooling, flake ice cooling, tube ice cooling, and eutectic ice cooling are the main cooling techniques and materials used in cold storage conditions for seafood products. However, although the development of microorganisms and biochemical events is slowed down in all cooling techniques, they cannot be completely stopped, so the storage life of fish, which is an aquatic product, is limited (Gökoğlu et al., 2004; Binici and Kurtkaya, 2014; Baptista et al., 2020). In this context, antioxidant food additives with high protective activity can be used to increase the effect of cooling technology on fish shelf life. Such food additives are used to slow down or stop the chemical and microbiological reactions that cause changes in unpleasant taste and odor, sticky structure, gas formation, color, and texture, which are the first and main signs of spoilage in fish. Antioxidant substances contain molecules that have phenolic functions and prevent the formation of free radicals that damage cells, while antimicrobial substances inhibit the growth of microorganisms by slowing down the growth rate of microorganisms in food (Sezer and Bozkurt; 2021; Çağlak et al., 2021; Esertaş et al., 2023; Kara vd., 2024). Antioxidants can be found as natural antioxidants as well as synthetic antioxidants and are used in nutritional supplements. Natural antioxidant food additives are naturally present in fruits, vegetables, and plants. Various studies have reported that these substances prevent food spoilage and increase shelf life (Halliwell, 1990; Celep et al., 2012; Shen et al., 2022; Çağlak and Karşlı, 2016; Kara and Çağlak, 2021; Zemzemoğlu et al., 2022).

The pomegranate (*Punica granatum* L.) is the root fruit of a shrub, and its cultivation is common in Western Asia and the surrounding Mediterranean regions. The parts of the fruit consist of 50% shell, 10% seeds, and 40% grains. Known as a valuable nutrient, all parts of the pomegranate contain phenolic acids, flavonoids, and hydrolyzable tannins, which are highly bioactive compounds. However, the fact that the peel of the pomegranate has a higher antioxidant capacity than the seeds of the fruit revealed that it is a potent source of bioactive compounds (Chen et al., 2020; Soltanzadeh et al., 2022). Pomegranate peels contain significant amounts of phenolics, including flavonoids (anthocyanins, catechins, and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic, and ellagic acid). There are studies in which pomegranate peel is used as a natural food additive to improve food quality (El-Nashi et al., 2015; Martínez et al., 2019; Kaderides et al., 2020). Some in vivo studies have reported that pomegranate peel extracts have antidiabetic, anti-inflammatory, and antidiarrheal functions (Lee et al., 2010; Parmar and Kar, 2007; Qnais et al., 2007; Smaoui et al., 2019; Giri et al., 2023). In this study, the shelf life was determined by changes in physical (pH, water activity, color), chemical (proximate, TBA, TVB-N), microbiological (TAMB, TAPB ),

and sensory quality parameters in cold storage ( $+4\pm 1^{\circ}\text{C}$ ) of horse mackerel treated with special ice containing pomegranate peel extract, which has natural antioxidant potential.

## Materials and Methods

### Collection and Preparation of Fish Sample

In the study, 240 horse mackerel (*Trachurus trachurus*) fished in Rize (Türkiye), with an average length of  $12.11\pm 0.57$  cm and an average weight of  $14.93\pm 1.78$  g, were used. The fish were brought to the Fisheries Faculty Fishing and Processing Technology Laboratory (Rize/Türkiye) on ice on the day they were hunting, and the studies were started.

### Preparation of Pomegranate Peel Extract and Ice Groups

In order to prepare pomegranate (*Punica granatum* L.) peel extract, attention was paid to the selection of the pomegranate fruit, which was not mixed with chemical contaminants, when obtaining the peel. For this purpose, pomegranates were collected from the orchard that was not exposed to any spraying process belonging to a private person who does not have a commercial purpose, and sampling was carried out (Figure 1).

After the collected pomegranates were washed, the fruit part was removed so that only the peel layer remained. Under hygienic conditions, pomegranate peels were dried in a pol-eko-aparatura brand oven at  $60^{\circ}\text{C}/ 24$  hour and then ground into powder with the help of a laboratory grinder (Waring Commercial Blender, USA). From the powdered samples, 50 g of powder was taken at the concentration ratio of 5% whose antioxidant value was found to be high in previous studies (Çağlak et al., 2022), and dissolved in distilled water so that the total volume would be 1000 ml. Solvent and raw material mixtures were mixed in a shaking water bath at  $40^{\circ}\text{C}$  (Nüve ST-402, Ankara, Turkey) at 100 rpm for 24 hours without any light. The mixtures were filtered using Whatman (No. 1) filter paper. The extract solution, which was sterilized under ultraviolet light, was kept in the flow cabinet for 5 minutes to cool. The solution (90 mL) was distributed in an even, thin layer on styrofoam plates disinfected with 70% alcohol in the cabinet. The freezing process of the aqueous extracts in the prepared dishes was carried out at  $-80^{\circ}\text{C}$  for 15 minutes. Normal ice production was carried out with the help of the LAB312 snow-type ice machine.

### Treating Fish with Ice and Storage Conditions

In the study, 240 horse mackerel were divided into pomegranate peel extract (PPE) and C groups to be used as a whole. In the study, the groups were prepared in

the form of ice/fish/ice (separately for the C and PPE groups) by placing 10 horse mackerel on each plate in styrofoam plates, the dimensions of which are 12×20 cm, previously sterilized with 70% ethyl alcohol. The prepared dishes were covered with cling film and stored in a Vestfrost VF 1268 brand refrigerator at 4±1°C. During the storage, the water from the melted ice was removed, and ice additions were made according to the group. The proximate composition (moisture, crude protein, crude fat, and crude ash (%)) analyses of the products were performed in groups and at the 0<sup>th</sup>, 2<sup>nd</sup> and 10<sup>th</sup> days of storage. Physicochemical and sensory analyses were performed on the 0<sup>th</sup>, 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup> days. The analyses performed to determine the sensory and textural changes during the shelf life of the products were done on raw products. Microbiology analyses were performed on the 0<sup>st</sup>, 4<sup>th</sup>, and 10<sup>th</sup> days. One package was randomly taken from each treatment and homogenized, and all analyses were performed in triplicate.

### Analytical Methods

For the moisture content of the products, 5 g of samples were dried in an oven at 105°C until a constant weight was obtained (AOAC, 1995). Crude fat analysis was carried out in a soxhlet extractor device (Velp SER 148/6, Milano, Italy) using petroleum ether as the solvent. Crude protein analysis was carried out by the Kjeldahl method (AOAC, 1980), and crude ash analysis was carried out by the burning method in a muffle furnace at 550°C (AOAC, 1980), and the pH of the samples was measured by a pH meter (Hanna, HI 3220, Germany) using a mixture of 10 g of samples in 20 ml of distilled water (Curran et al., 1980). The water activity

(*a<sub>w</sub>*) of samples was measured using an Aqualab 4TE instrument (Decagon, Pullman, WA, USA). Instrumental color analysis of the samples was performed using a colorimeter (CR-10, Konica Minolta, Japan). The L\*, a\*, and b\* values were determined according to the CIE color chart. The L\* value indicates the transmission/opacity (the darkest black at L\* = 0, and the brightest white at L\* = 100) of the sample. The a\* value indicates the redness (+)/greenness (-) of the sample. The b\* value indicates the yellowness (+)/blueness (-) of the sample. Total volatile basic nitrogen (TVB-N) was determined by the Lücke and Geide method (İnal, 1992; Varlık et al., 1993). Thiobarbituric acid (TBA) was measured by the method of Tarladgis et al. (1960). Sensory analysis was carried out based on the Organoleptic (Freshness) Inspection and Parasite Control Chart of Fresh and Chilled Fish in the Fisheries Quality Control Handbook of the Ministry of Agriculture and Rural Affairs, Turkey (2000). Parameters of eyes, skin, tissue, flesh, and abdomen, kidney and blood, gill appearance, and gill odor were taken into consideration in the evaluation (Table 1). The parameter indicated 4 points of very fresh fish meat, while 1 point indicated the quality of degraded fish. In order to determine the microbiological changes of the samples, total aerobic mesophilic (TAMB) and psychrophilic (TAPB) bacteria counts were performed according to the method proposed by Halkman (2005). All tests were performed in triplicate. Data were expressed as mean±SD. Statistical analyses were conducted using JMP 5.0.1 (SAS Institute, Inc., Cary, NC, USA) software. Significant differences among samples were determined using analysis of variance (ANOVA) and the TUKEY test at a significance level of 0.05.



Figure 1. Dried pomegranate peel and powder used in the study (original).

## Results and Discussion

### Proximate Composition

Seafood has 66–84% water, 15–24% protein, 0.1–22% fat, and 0.8–2% inorganic materials (Huss 1988). The crude protein, crude lipid, ash, and dry matter content of raw (control group) horse mackerel were found to be 18.39±0.27, 8.04±0.14, 1.66±0.10, and 28.68±1.05% on day 0th, respectively (Table 2). The proximate composition of the horse mackerel showed similarities to the findings of Tokur and Aksun (2018) and Simeonidou et al. (1997), with few differences. The proximate composition of horse mackerel reported in different studies (Boran ve Karaçam, 2011; Fernandez-Jover et al., 2007) indicated some differences, especially for the lipid content and protein. Such differences in the composition of fish are strongly related to their nutrition, catching season (spawning cycles), environment, fish size, and gender (Sallam, 2007).

The crude protein, crude lipid, ash and dry matter content of horse mackerel on the 10th day after being treated with pomegranate peel extract added ice were found to be 16.95±1.26, 9.42±0.12, 0.61±0.20 and 28.34±1.12 %, respectively. There was an apparent increase in protein, lipid, and dry matter content of horse mackerel treated with pomegranate peel extract

added to ice compared to the control group ones, but a decrease in crude ash. According to these findings, the addition of antioxidants did not cause a considerable change in the overall chemical composition of horse mackerel.

### pH and Water Activity Value

Changes in the pH values of the control and pomegranate peel extract groups during storage are shown in Figure 2. The initial pH value of the raw fish was found to be 6.68±0.01. The initial values (6.63–6.68) were similar to values reported by other authors in horse mackerel (Meksika et al., 2009; Albertos et al., 2015). When the pH value was analyzed statistically during the storage period, a significant difference (P<0.05) was found between the groups (C and PPE), except for the 0th and 2nd days. The pH values of the PPE group samples were consistently lower than those of the control samples during storage (P<0.05); this change was reported by Selahvarzi et al. (2021). It may be due to the antimicrobial properties of the pomegranate peel extract expressed by If the water activity, which is the supporter of bacterial growth and represents the water in the food, is below 0.9, growth activity is not observed in the majority of spoiling bacteria (Abbas et al., 2009; Duyar et al., 2020).The

**Table 1.** Sensory evaluation form

EXAMINATION SUBJECT	CRITERIA			
	FRESHNESS CATEGORY			
	4	3	2	1
	APPEARANCE			
SKIN	Vivid and bright, no color loss, clear difference between abdomen and back	Vivid but slightly yellowish color, Abdominal and back color difference less	Yellowish faded color, Skin does not fold when you tilt the fish	Pale, dull color Skin separates from meat
MUCOSA	Transparent and flowing (watery) mucosa (slimy substance)	slightly turbid mucous membrane	slimy mucous membrane	Milky mucous, opaque (not transparent)
EYE	convex(bulging) transparent cornea,black shiny pupil	Transparent layer with convex slightly sunken reflections, Black pale pupil	Flat opaque cornea, opaque pupil, blood smear around the eye	Concave (*) milky in the center Cornea, gray pupil
GILLS	Bright color, no mucous	less colorful slightly clear mucous membrane	discolored, opaque mucosa	Yellowish(*) milky mucosa (slime)
MEAT	Tight and flexible smooth surface	Decreased flexibility	slightly soft (loose), Low flexibility, polished (velvety) and pale surface	Soft and loose(*) easily separated scales(*) rough surface
SMELL	The smell of seaweed	Neither bad, nor the smell of seaweed	Slightly sour	Sour
PARASİTE	It will be examined with organoleptic examination (in the digestive system and muscles) and never will not be found.			

**Table 2.** The proximate composition of control and pomegranate peel extract groups

Days	Groups	Crude Protein (%)	Crude Lipid (%)	Ash (%)	Dry matter (%)
0 <sup>th</sup> . day	Control (C)	18.39±0.27A <sup>a</sup>	8.04±0.14 B <sup>a</sup>	1.66±0.10 A <sup>a</sup>	28.68±1.05 A <sup>a</sup>
	Pomegranate Peel Extract (PPE)	18.39±0.27A <sup>a</sup>	8.04±0.14 B <sup>a</sup>	1.66±0.10 A <sup>a</sup>	28.68±1.05 A <sup>a</sup>
2 <sup>nd</sup> . day	Control (C)	17.31±0.53A <sup>a</sup>	8.57±0.06 A <sup>a</sup>	1.16±0.23 B <sup>a</sup>	24.91±2.68 AB <sup>a</sup>
	Pomegranate Peel Extract (PPE)	17.52±0.41A <sup>a</sup>	8.93±0.12 A <sup>a</sup>	1.37±0.42 A <sup>a</sup>	25.17±1.07 B <sup>a</sup>
10 <sup>th</sup> . day	Control (C)	15.71±0.02A <sup>a</sup>	8.75±0.00 A <sup>b</sup>	0.71±0.17 C <sup>a</sup>	22.66±1.98 B <sup>b</sup>
	Pomegranate Peel Extract (PPE)	16.95±1.26A <sup>a</sup>	9.42±0.12 A <sup>a</sup>	0.61±0.20 B <sup>a</sup>	28.34±1.12 A <sup>a</sup>

C: Kontrol, PPE: Pomegranate Peel Extract. Different capital letters (A, B, C...) in the same column indicate the difference within the same groups on different days (P<0.05). Different lowercase letters (a, b, c...) on the same line indicate the difference between groups on the same day (P<0.05).

water activity ( $a_w$ ) measurement results on the 2nd, 4th and 6th days of the study were determined  $0.9903 \pm 0.01$ ,  $0.9953 \pm 0.01$  and  $0.9970 \pm 0.02$  in the C group, and  $0.9898 \pm 0.01$ ,  $0.9917 \pm 0.01$  and  $0.9920 \pm 0.02$  in the PPE group, respectively. When analyzed statistically, there was no significant difference in water activity values between days and between groups ( $P > 0.05$ ). Water activity ( $a_w$ ) values ranged from 0.9903 to 0.9970. There was a clear trend toward storage. The increase in water activity is due to the ice treatment process.

### Color Changes

The most important parameter in determining quality for the consumer is color (Ginés et al., 2004). According to the color measurement results of the study, the L value for the C and PPE groups was measured as  $23.08 \pm 3.31$  on day 0. On the last day of storage, this value reached  $62.99 \pm 2.44$  in the C group and  $37.75 \pm 2.12$  in the PPE group (Figure 3). The  $a^*$  value of horse mackerel at the beginning of the study was determined as  $4.78 \pm 0.79$ . This value did not change much on the last day of storage in the PPE group with the effect of PPE added ice, and it was observed to have a low value of  $4.28 \pm 1.41$  and  $2.27 \pm 0.16$  in the Control group. The  $b^*$  value, which was determined as  $11.02 \pm 0.62$  on the first day of storage, increased to  $14.74 \pm 2.55$  in the C group and  $45.03 \pm 6.29$  in the PPE group on the last day ( $P < 0.05$ ). While no significant changes were observed in L (brightness) and  $a^*$  values, there was a significant increase in the  $b^*$  value, especially in the PPE group. It is thought that this significant increase is due to the pomegranate peel, which has been proven in other studies to also be used as a natural dye (Anandhan and Prabakaran, 2018). In addition, the data in the literature suggesting that yellowishness may increase in foods as a result of lipid oxidation supports the result of our study (Shi et al., 2014; Khazaei et al., 2016; Vital et al., 2016).

### TVB-N Changes

The analysis of volatile nitrogenous compounds such as trimethylamine, dimethylamine, and ammonia, known as the TVB-N index, has been accepted as an indicator of spoilage in meat and meat products. These compounds come from the degradation of nitrogenous compounds due to microbial and enzymatic activities in meat (Ruiz-Capillas and Moral, 2001; Jiang et al., 2022). Changes in TVB-N values in fish samples during chilled storage are shown in Figure 4. According to the chemical analysis results of the study, TVB-N values for all groups were measured as  $14.78 \pm 0.8$  mg N/100g on day 0. On days 6, 8 and 10, were determined that  $17.6 \pm 0.61$ ,  $16.9 \pm 0.41$  and  $19.00 \pm 0.43$  mg N/100 g were in group C,  $9.15 \pm 0.35$ ,  $11.96 \pm 0.26$ , and  $12.67 \pm 0.3$  mg N/100 g in the PPE group, respectively ( $P < 0.05$ ). The TVB-N value level was significantly lower for the pomegranate peel extract samples than for the control samples. Similarly, some researchers reported that plant extract additives have a positive effect on TVB-N content in seafood (Karslı et al., 2021; Çağlak and Karslı, 2016; Pezeshk et al., 2015). It was clearly observed as a result of the TVB-N changes that the PPE group had a protective effect.

### TBA Changes

According to the chemical analysis results of the study, the initial TBA values were measured as  $0.39 \pm 0.04$  mg MA/kg. While it increased to  $5.72 \pm 0.19$  mg MA/kg on the 8th day in the C group, it was  $1.19 \pm 0.40$  mg MA/kg in the PPE group on the same day ( $P < 0.05$ ). On the last day of storage, with the effect of ice with pomegranate peel extract, the TBA value was  $1.64 \pm 0.20$  mg MA/kg in the PPE group, while this value reached  $5.84 \pm 0.19$  mg MA/kg in the Control group (Figure 4). It is known that the antioxidant activity of PPE depends on the phenolic components it contains (Turgut et al., 2016). Phenolic-containing antioxidants both prevent the formation of free oil radicals and have the

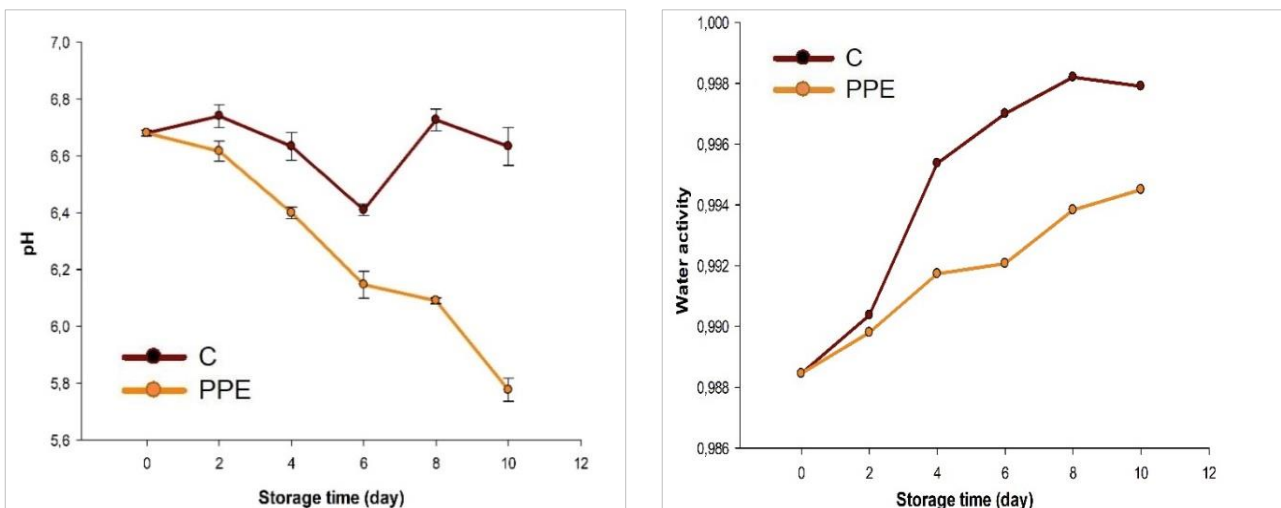


Figure 2. Changes in pH and  $a_w$  of control and pomegranate peel extract groups C: Control, PPE: Pomegranate Peel Extract.

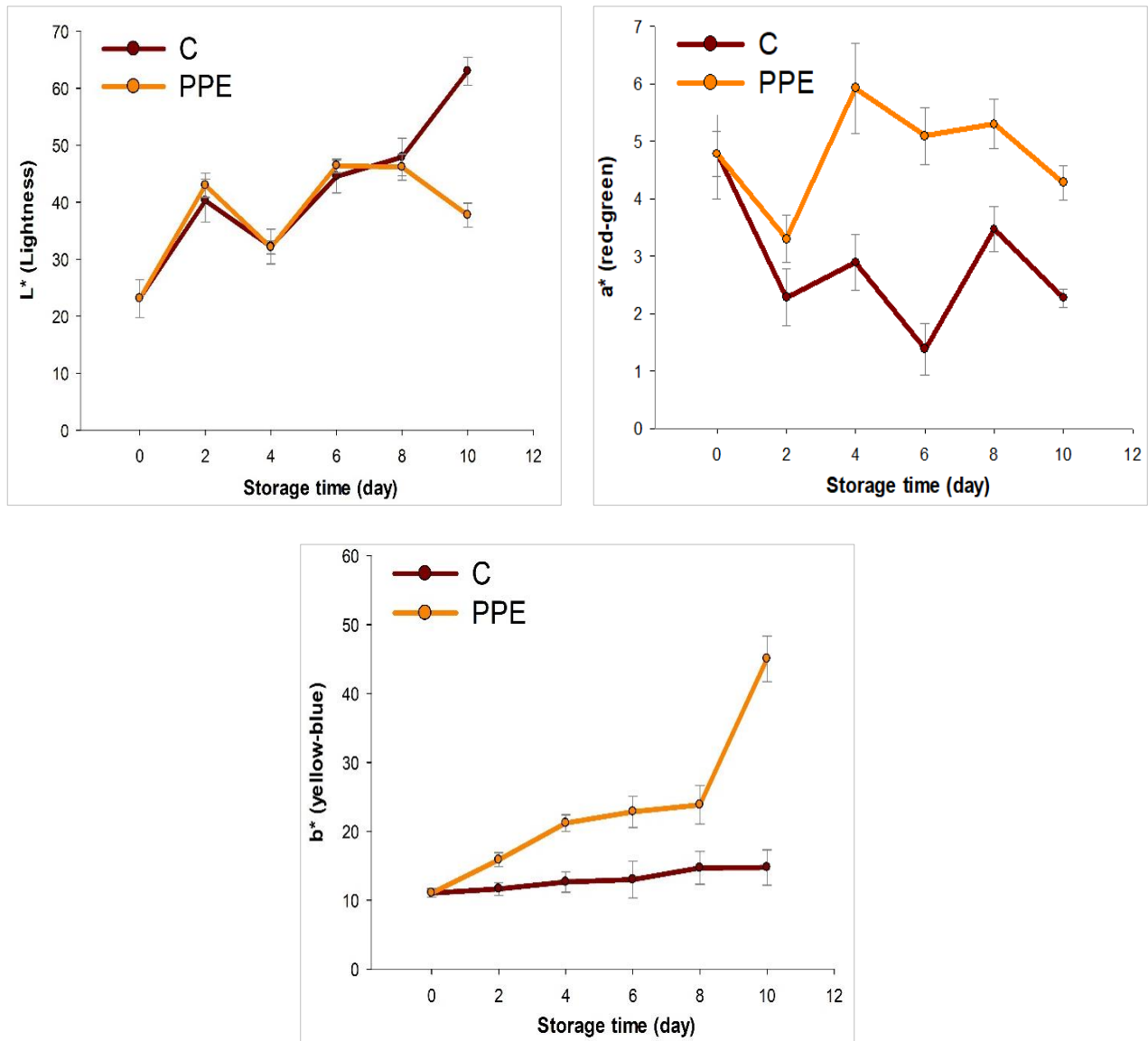


Figure 3. Changes in L\*, a\* and b\* values of control and pomegranate peel extract groups C: Control, PPE: Pomegranate Peel Extract.

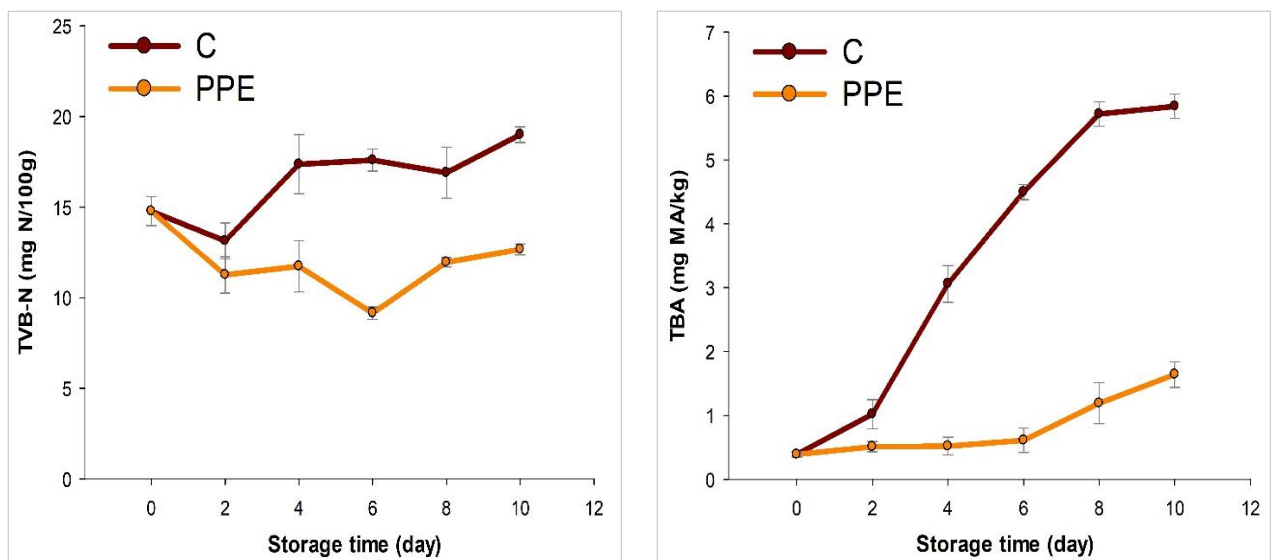


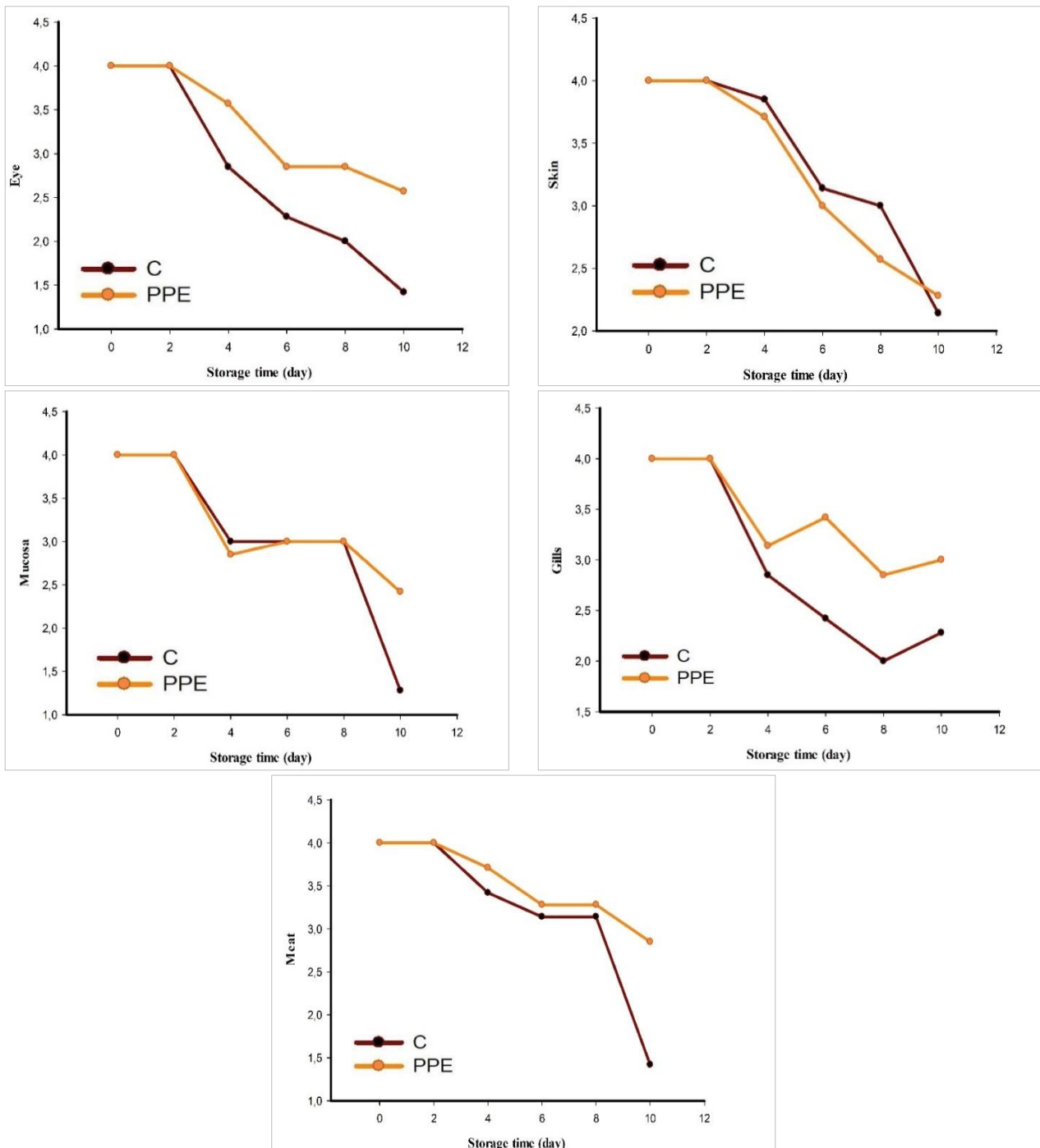
Figure 4. Changes in TVB-N (mg/100g) ve TBA mg MA/kg values of control and pomegranate peel extract groups C: Control, PPE: Pomegranate Peel Extract.

ability to chelate metal ions. Radicals are responsible for reacting with or absorbing oxygen in the process of autooxidation. Thus, phenolics can prevent free radical formation and propagation by chelating metal ions, especially iron and copper (Saldamli, 2007; Kanatt et al., 2010). It was determined that the high phenolic content of PPE in ice-cooling showed antioxidant activity and significantly increased its protective effect.

**Sensory Changes**

Sensory evaluation is an appropriate and universally accepted assessment of the quality and

freshness of food. It is an easy and simple method for determining food acceptability (Lim et al., 2011). Eye, gill, skin, meat, and mucosa values of horse mackerel showed a statistically significant decrease in all groups during storage ( $P < 0.05$ ) (Figure 5). When the groups were compared on the 10th day of storage, the PPE group remained at 3 points, while the control group remained below 2 points. According to these data, it was determined that pomegranate peel extract contributed to the improvement of sensory quality. In other studies in the literature, it has been revealed that some extracts used in preservation techniques (like ginger, clove, garlic extract, grape seed extract, and *Origanum vulgare*



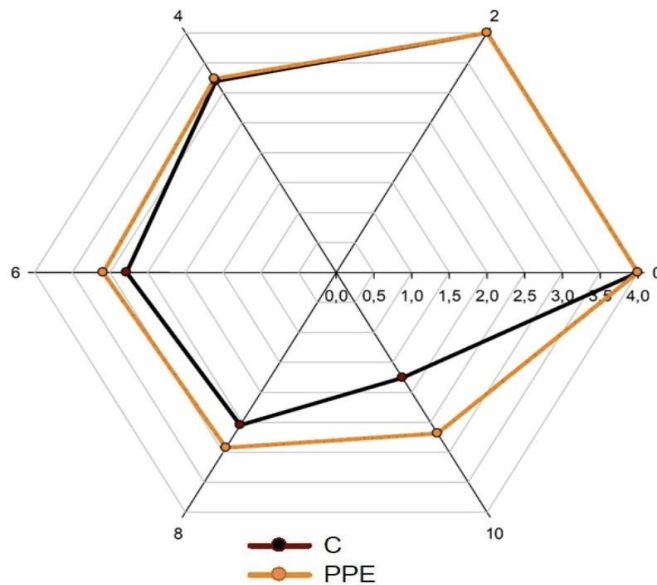
**Figure 5.** Sensory scores (eye, gills, skin, meat, mucosa) of control and pomegranate peel extract groups. C: Control, PPE: Pomegranate Peel Extract.

essential oil) have a positive effect on the sensory quality of seafood (Iheagwara, 2013; Tairu et al., 2017; Islam et al., 2022; Mojaddar Langroodi et al., 2021). In the mean sensory evaluation, there was no change between 0 and 2 days in both groups. Compared to group C, the PPE group was evaluated with higher scores by the panelists on the 8th and 10th days when the shelf life ended sensibly (Figure 6). According to the results of our study and the literature, it has been observed that the practices that affect the quality positively also have a positive effect as a result of the sensory analysis (Di Turi et al., 2009; Cao et al., 2020; Dulal et al., 2023).

**Microbial Counts**

Table 3 shows the change in the number of total mesophilic aerobic and total psychrophilic aerobic bacteria during the storage of horse mackerel samples. The baseline value (day 0) of both groups for total mesophilic aerobic bacteria was 2.57 log cfu/g. Rodríguez et al. (2005) reported that the total aerobic mesophilic bacteria load in newly caught horse mackerel was 3 log cfu/g, and this result is consistent with our study. From a statistical point of view, there was a significant difference between the groups on the 10th day (P<0.05). When the PPE group and the C group were

compared on the 4th day of storage, the PPE group preserved its initial value, while an increase was observed in the C group. Psychrotrophic bacteria are the main group of microorganisms responsible for the spoilage of fresh fish stored at low temperatures (4°C). Therefore, the enumeration of these bacteria is an indicator of the quality of cold-stored fish meat (Mol et al., 2007). The total aerobic psychrophilic initial value (day 0) was determined to be 3.81 log cfu/g in the study. It was determined that the number of psychrophilic aerobic bacteria increased in all groups, and the storage reached 5.93 log cfu/g in the Control group and 4.98 log cfu/g in the PPE group on the 10th day. Significant differences were found between the group to which pomegranate peel extract was added and the control group (P<0.05). It was determined that both bacterial species and study groups did not exceed the recommended maximum limit of 7 log cfu/g in raw fish on the 10th day (ICMSF, 1986; Ehsani and Jasour, 2012). In the PPE group, this situation is related to the antimicrobial effect of pomegranate peel extract, which contains components such as caffeic, syringic, sinapic, p-coumaric, ferulic, ellagic, gallic, and cinnamic acids, as well as being stored with ice (Singh et al., 2018). In the control group, it is due to the effect of cold storage only with ice. Uçak (2020) investigated the total mesophilic



**Figure 6.** Overall sensory scores of control and pomegranate peel extract groups. C: Control, PPE: Pomegranate Peel Extract.

**Table 3.** Changes in total aerobic mesophilic and psychrophilic bacteria counts of control ve pomegranate extract groups

Days	Groups	Total Aerobic Mesophilic (TMAB (log cfu/g))	Total Aerobic Psychrophilic (TPAB (log cfu/g))
0 <sup>th</sup> . day	Control (C)	2.57±1.76 A <sup>a</sup>	3.81±0.24 C <sup>a</sup>
	Pomegranate Peel Extract (PPE)	2.57±1.76 A <sup>a</sup>	3.81±0.24 B <sup>a</sup>
4 <sup>th</sup> . day	Control (C)	3.05±2.03 A <sup>a</sup>	4.98±0.53 A <sup>a</sup>
	Pomegranate Peel Extract (PPE)	2.58±1.77 A <sup>a</sup>	4.71±0.30 A <sup>b</sup>
10 <sup>th</sup> . day	Control (C)	3.85±0.26 A <sup>a</sup>	5.93±0.58 B <sup>a</sup>
	Pomegranate Peel Extract (PPE)	3.28±1.26 A <sup>b</sup>	4.98±0.58 A <sup>a</sup>

C: Kontrol, PPE: Pomegranate Peel Extract. Different capital letters (A, B, C...) in the same column indicate the difference within the same groups on different days (P<0.05). Different lowercase letters (a, b, c...) on the same line indicate the difference between groups on the same day (P<0.05).



and total psychrophilic bacterial loads of trout burgers to which pomegranate peel extract was added during cold storage as a quality parameter and reported that the extract had a suppressive effect on bacterial growth. Research data demonstrating the antimicrobial effect of pomegranate peel extract supports our findings (Özdemir et al., 2014; Akarca and Başpınar, 2019; Chen et al., 2020; Gull et al., 2021).

## Conclusion

In recent years, the food industry has been seeking antioxidants from natural sources, mainly due to negative toxicological reports on many synthetic-based compounds. In this context, it is known that pomegranate peel extract is a good alternative to synthetic antioxidants for the food industry. PPE-added ice application has a high potential to improve the general taste and therefore shelf life of seafood products under cold storage conditions. In addition, in this study, it will be an important advantage to be able to develop PPE as a natural antioxidant in a commercial formulation with the widespread availability of pomegranate peel, which is the waste product of processing factories. More research is needed to determine the results of different extract ratios in the use of PP extract-added ices. In addition, while rejecting the use of synthetic antioxidants (in vitro or in vivo), nutritional and toxicological studies should be conducted to stabilize the health-safe edible dosage of this natural food additive.

## Ethical Statement

Ethical approval was not required for this study.

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## Author Contribution

A.K. Data Curation; Formal Analysis, Investigation, Visualization and Writing -original draft, Conceptualization, Writing -review and editing, E.Ç. Conceptualization, Methodology, Writing -review and editing, O.K. Formal Analysis, Investigation, Ö.Ö. Formal Analysis, Investigation, E.H.T. Formal Analysis, Investigation.

## Conflict of Interest

The authors declare no conflict of interest.

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