



RESEARCH ARTICLE

## Quality changes of European eel (*Anguilla anguilla*) stored under refrigerated conditions at $2\pm 1^\circ\text{C}$

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### ABSTRACT

This study aims to determine microbiological, sensory and color changes of whole European eel (*Anguilla anguilla*) aerobically stored at  $2.00\pm 1.00^\circ\text{C}$  for 19 days. Samples were analyzed with periodical intervals in terms of Total Mesophilic Aerobic Bacteria (TMAB), Total Psychrophilic Aerobic Bacteria (TPAB), *Pseudomonas sp.* and Lactic Acid Bacteria (LAB). For the sensory analysis, samples were evaluated to describe the changes in skin color and mucus, eyes shape and clarity, texture and odor. Description of color changes consisted of  $L^*$ ,  $a^*$ ,  $b^*$ ,  $\Delta E$ , chroma and hue angle during the storage period. The count of TMAB, TPAB, *Pseudomonas sp.* and LAB were found to be  $1.53\pm 0.08$ ;  $1.08\pm 0.12$ ;  $1.15\pm 0.21$  and  $1.15\pm 0.21$  log cfu/g, respectively. Significant differences were not found for the first 2 days of the storage for any microbiological parameters ( $p>0.05$ ). At the end of the storage time, the counts of microorganisms were significantly increased ( $p<0.05$ ) and reached  $8.08\pm 0.65$ ;  $7.56\pm 0.08$ ;  $7.53\pm 0.76$ ;  $2.80\pm 0.14$  log cfu/g, respectively. In terms of sensory changes whole European eel samples were resulted unacceptable for consumption after 13 days of storage with an  $8.20\pm 0.83$  sensory score while  $9.75\pm 0.95$  was the highest score for the samples on day 19<sup>th</sup>. The changes in the color of the samples were significant on the first and 5<sup>th</sup> days of storage ( $p<0.05$ ).  $\Delta E$ ,  $L^*$  and  $b^*$  values were significantly increased ( $p<0.05$ ) while chroma and  $a$  values were decreased on day 5. Overall results for this study are proving that *Pseudomonas sp.* could be the indicator microorganism that could be used to determine the shelf life of European eel together with the sensory analysis, linear correlation with storage time was not obtained for  $\Delta E$  or any other color parameters and whole European eel could be stored at  $2.00^\circ\text{C}$  for 13 days based on sensory and microbiological quality changes.

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## Introduction

Aquatic products have a significant role in human health as they provide a balanced and healthy diet as high-quality nutrient sources. The consumption of aquatic animals is associated with various advantages for health with their nutrient content such as protein, fatty acids, vitamins and minerals (Parian & Mullin, 2016; Hassoun & Karoui, 2017; Giannakourou et al., 2019). European eel (*Anguilla anguilla*) is a commercially important species in Türkiye as it has been worldwide due to its excellent quality attributes, such as white meat due to its abundance of omega-3 lipids, flavor and high-fat content (El-Obeid et al., 2018; Lambrianidi et al., 2019).

Eels are generally classified as warm-water fish, and they migrate long distances to inland waters for feeding and to the ocean for breeding. They store fat by their nature of spawning migration. Eels are classified under the genus *Anguilla* which is distributed worldwide (van Ginneken & Maes, 2005; Righton et al., 2016). There are four commercially valuable species in 19 species of the *Anguilla* genus. *A. anguilla* is distributed in Europe and the relevant Mediterranean coasts. Consumers can purchase eels from the market as fresh products (usually alive or gutted and decapitated). The high-fat content of eels is rich in omega-3 lipids, which expands the potential for eel species, and therefore, eels are attaining worldwide attention (El-Obeid et al., 2018). This attention is important; however, the European eel is listed among the critically endangered species on the International Union for Conservation of Nature (IUCN) red list (Pike et al., 2020). Therefore, eels are under conservation which regulates their fishery, import and export quotes. Efficient use and consumption are also crucial for their conservation, while they are prone to rapid nutritional value losses. The loss of nutritional quality takes place primarily through lipid oxidation, which greatly reduces their estimated shelf life (Küçükgülmez et al., 2013; Yanar et al., 2013; Choulitoudi et al., 2017). Globally, fish losses due to microbiological spoilage account for approximately 10% of the produced fish, whether they are aquaculture or fishery products. In this manner, the expiration date estimates become more important to avoid spoilage and disposal of eels.

The lipid content of European eels is typically higher than most fish species. The nutritional composition of the European eel has been described in many studies. In general, it depends on age, life stage, weight, and time of year (Heinsbroek et al., 2007). The reported ranges for moisture, protein, and lipid

ratios in European eels were 60–73%, 15–20%, and 4–30%, respectively (Schreckenbach et al., 2001; Lupatsch et al., 2003; Özogul et al., 2005; Özogul et al., 2014; Gomez-Limia et al., 2021; Tunçelli et al., 2022). Few research reported that European eels accumulate lipids before spawning and that lipid levels increase as the fish grows (Larsson et al., 1990; Saito et al., 2015; van Ginneken et al., 2018). Larsson et al. (1990) also reported that the lipid content of European eel muscle tissue increases while the yellow eel's metamorphosis into silver eels.

Fish quality parameters are of paramount importance for aquatic animals, as they are directly related to consumer-perceived attributes of appearance, smell, taste, or texture. (Özogul et al., 2005; Hassoun & Karoui, 2017). Freshness is the most important attribute in evaluating fish quality. Consumers can see the organoleptic properties of whole fish. The sensory method is the most satisfying way to assess the quality of freshness, as it best reflects consumer acceptance (Connel, 1995). Quality also depends on internal parameters in addition to external parameters that are important for consumer acceptance. These internal parameters are usually accepted as initial microbial load, microbial activity (Dalgaard, 1995), problematic post-harvest handling, processing conditions (Giannakourou et al., 2023), and microbial spoilage (Ashie et al., 1996), which directly affects the shelf life of fish products (Baklori et al., 2012; Lambrianidi et al., 2019).

The quality of fish becomes progressively worse with post-mortem changes through the chemical attributes, protein and lipid degradations, and microbial spoilage (Ashie et al., 1996). In terms of spoilage, that is the process by which seafood decomposes to the point that it is no longer acceptable for consumption based on the above-mentioned criteria. These events result in reduced sensory quality and nutritional value of fish (Özogul et al., 2006) which makes the product less valuable and buyable by the consumers.

The shelf life of aquatic products is short; therefore, they are often subjected to processing technologies to extend their shelf life. Anyhow, today's consumers prefer high-quality, more "natural" products made with gentle procedures that are microbiologically safe, nutritious, and healthy with minimal additives, seeking fresh or minimally processed foods (Erkmen & Bozoglu, 2016). In this context, quality changes of whole European eel were monitored during the chilled storage under refrigeration conditions and microbiological, sensory and color changes were assessed to determine the shelf life of fresh whole European eels.

## Material and Methods

### Material

Whole European eel was used in this study. Samples were collected from Muğla province and transferred to the laboratory in an insulated box covered with ice. The average weights of the samples were  $355.23 \pm 129.13$  g (WL-2002L) and average lengths were  $566.35 \pm 55.30$  mm. European eel samples were aerobically stored at  $2.0 \pm 1.0^\circ\text{C}$  for 19 days in pouches to prevent drying of the samples and periodically analyzed in terms of the changes in microbiological, color and sensory quality.

### Microbiological Analysis

In each sampling day, 10 g of sample was aseptically removed and homogenized in 90 ml buffered peptone water (Merck, Darmstadt, Germany) in stomacher for 2 min. Serial dilutions were prepared and the counts of TMAB and TPAB were determined on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) with pour plate method, LAB was determined on De Man Rogosa and Sharp (MRS) agar (Merck, Darmstadt, Germany) with double layer pour plate method and *Pseudomonas sp.* was on Cephaloridin-Fucidin-Cetrimide (CFC) agar with CFC supplement (Merck, Darmstadt, Germany) with spread plate method. Incubation time for the microorganisms was 30, 5, 25 and  $25^\circ\text{C}$  for 3, 10, 5 and 3 days, respectively. Analyses were performed in 2 replicates and results were represented in log cfu/g (Sallam, 2008).

### Color Analysis

The color changes of *A. anguilla* were measured with portable 3NH color meter (Shenzen ThreeNH Tech. China). Calibration was performed with white and black samples and measurements were conducted with 10 replicates of each sample from the head to the tail of the skin of the fish. From the measurements  $L^*$ ,  $a^*$ ,  $b^*$ , hue angle and chroma values were obtained. The parameter  $L^*$  represents the lightness between 0 and 100 which indicates white and black, respectively. For other parameters  $-/+ a^*$  for red/green,  $-/+ b^*$  for yellow/blue, hue is the angle between  $0^\circ$  and  $360^\circ$  dividing the color space into four place and chroma is the saturation of the color (Cavaco et al., 2021; Karki et al., 2023). Hue and chroma was calculated based on the Eqs. 1 and 2, respectively. From the obtained values of  $L^*$ ,  $a^*$  and  $b^*$ ,  $\Delta E$  which represents the total color changes in the sample was calculated in accordance with the Eq. 3 (Oliveira & Balaban, 2006).

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$\text{Hue} = \text{Arctan}(b^*/a^*) \quad (2)$$

$$\Delta E = \sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2} \quad (3)$$

### Sensory Analysis

Sensory changes of whole eel were evaluated in accordance with the method described by Arkoudelos et al. (2007) with slight modifications. Specimens were tested in terms of the changes in skin color and mucus, eyes shape and clarity, texture firmness and odor. Each parameter ranges between 0 and 2 which is representing good quality and inappropriate for consumption, respectively. Samples were assumed to be spoiled when the total sensory score reached the scores of 8 out of 10. During 19 days of storage 2 to 5 trained panelists were attended for sensory session and results were recorded as mean  $\pm$  standard deviation (SD).

### Statistical Analysis

Results obtained from the duplicate analysis were subjected to variance analysis (ANOVA). Tukey's test was used to compare the mean values at the significance level of  $p < 0.05$ . Statistical tests were performed by using statistical package program IBM SPSS (SPSS Inc., Chicago, 2008). Polynomial quadratic function was used in order to estimate the effect of storage time in color values (El-Gendy et al., 2014; Gunathilake et al., 2019).

## Results and Discussion

### Microbiological Changes

During 19 days of storage of whole European eel, microbiological changes such as TMAB, TPAB, *Pseudomonas sp.* and LAB were assessed and results were shown in Table 1. The initial counts of TMAB, TPAB, *Pseudomonas sp.* and LAB were found to be  $1.53 \pm 0.08$ ,  $1.08 \pm 0.12$ ,  $1.15 \pm 0.21$  and  $1.15 \pm 0.21$  log cfu/g which indicates a good quality for fish as indicated by Scherer et al. (2006). During the storage the counts of bacteria were progressively increased and reach  $8.08 \pm 0.65$ ,  $7.56 \pm 0.08$ ,  $7.53 \pm 0.76$  and  $2.80 \pm 0.14$  log cfu/g, respectively at the end of the storage time ( $p < 0.05$ ). Statistical differences were not significant ( $p > 0.05$ ) for the first two days of the storage for TPAB, *Pseudomonas sp.* and LAB. Among the bacterial groups TMAB has reached the highest count at the end of the storage time followed by TPAB, *Pseudomonas sp.* and LAB. For the acceptable limit of indicated by ICMSF (1986) 7.00 log cfu/g

was exceeded on day 13 for TMAB and TPAB and on day 19 for *Pseudomonas* sp. in contrast with the bacteria group tested in here LAB were not exceed the acceptable limit of 7.00 log cfu/g. Moreover, the highest count was obtained on day 13 for LAB. Similar results were reported by Arkoudelos et al. (2007) for TMAB Researchers were reported the initial counts of Total Viable Count (TVC) for farmed eel (*A. anguilla*) stored under different packaging types (air, vacuum and modified atmosphere (MAP)) at 0°C as 2.8 log cfu/g. The counts of TVC were exceeded the acceptable limit of 7.00 log cfu/g after 18 days for air packed samples. For the initial counts of *Pseudomonas* sp. were reported to be less than 10 cfu/g however exceeded the 7.00 log cfu/g after 5 days of storage for air packed samples. In terms of the counts of *Pseudomonas* sp. 5 days of storage at 0°C until the acceptable limit is exceeded is not in agreement with our findings which were 19 days. The differences between the same bacteria could be due to the differences between the strains that naturally occurred in the fish and metabolic activity of the strains (Illikoud et al., 2019).

The growth of LAB in whole chilled European eel is indicating that the LAB is not dominant at the time of spoilage. The highest count was reached 3.21±0.05 log cfu/g on day 13 and slightly decreased to 2.80±0.14 log cfu/g ( $p>0.05$ ). The results were comparable to those reported for European eel (*A. anguilla*) fillets stored at 4°C (Giannakourou et al., 2023). Slower growth of LAB in European eel fillets was reported as the initial count was started 2 log cfu/g and reached 4.6 log cfu/g at day 8. However, in another study the growth of LAB was reported faster in fresh eel fillets stored at 2°C compared to the findings for whole European eel as the numbers of LAB was found to be 6 to 7 log cfu/g on 10<sup>th</sup> day of the storage (Lambrianidi et. al., 2019). The differences in LAB counts

between whole and filleted eel could be the results of filleting process which causes higher exposure of the muscle and thus resulting the higher bacterial growth quality loss during storage (Islami et al., 2015).

### Color Changes

The changes in color namely  $L^*$ ,  $a^*$ ,  $b^*$  and  $\Delta E$  that are indicating Lightness/darkness (100/0), green/red (-/+), blue/yellow (-/+ and total color changes, respectively (Ünal Şengör et. al., 2018) are shown in Figure 1. Linear correlation was not found between storage time and representative color parameters. But instead, polynomial function was able to explain the regression with the values of  $R^2 = 0.89, 0.87, 0.89$  and  $0.88$ . During 19 days of storage  $L^*$ ,  $b^*$  and  $\Delta E$  values were increased on day 5 and highest values were found to be 6.12, -4.80 and 3.87 ( $p<0.05$ ) and decreased in accordance with the storage time and reported to be 5.21, -6.75 and 0.93. Significant differences ( $p<0.05$ ) were found for  $b^*$  values on the last day of the storage. In contrast to  $L^*$ ,  $b^*$   $\Delta E$  values,  $a^*$  values were decreased on day 5 ( $p<0.05$ ) and lowest and highest values were found to be 6.44 and 8.44, respectively. Samples were darker and had yellowish and reddish color on day 5. Significant differences were not found between the initial and last day of experiment ( $p>0.05$ ). However, fluctuations in the color values indicates high enzymatic activity. Darker color on the skin of the samples could be the results of the activity of calpains and cathepsins (Haard, 2002). Similar results for  $L^*$ ,  $a^*$  and  $b^*$  values were reported by Küçükgülmez et al. (2013) for European eel fillets during refrigerated storage. In the study, no significant differences were reported for chitosan coated and non-coated European eel fillets.

**Table 1.** Microbiological changes (mean±SD) in whole European eel stored at 2.00°C\*

Storage time (days)	TMAB	TPAB	<i>Pseudomonas</i> sp.	LAB
0	1.53±0.08 <sup>a</sup>	1.08±0.12 <sup>a</sup>	1.15±0.21 <sup>a</sup>	1.15±0.21 <sup>a</sup>
2	1.38±0.12 <sup>b</sup>	1.45±0.21 <sup>a</sup>	1.00±0.00 <sup>a</sup>	1.00±0.00 <sup>a</sup>
5	2.15±0.21 <sup>c</sup>	2.16±0.12 <sup>b</sup>	2.58±0.15 <sup>b</sup>	2.20±0.03 <sup>b</sup>
8	4.99±0.12 <sup>d</sup>	5.21±0.05 <sup>c</sup>	5.30±0.18 <sup>c</sup>	3.06±0.02 <sup>c</sup>
13	7.41±0.02 <sup>e</sup>	7.52±0.02 <sup>d</sup>	6.23±0.33 <sup>d</sup>	3.21±0.05 <sup>c</sup>
19	8.08±0.65 <sup>f</sup>	7.56±0.08 <sup>e</sup>	7.53±0.76 <sup>e</sup>	2.80±0.14 <sup>c</sup>

**Note:**\*different superscripts representing significant differences ( $p<0.05$ ) in each column. Results were shown in log cfu/g.

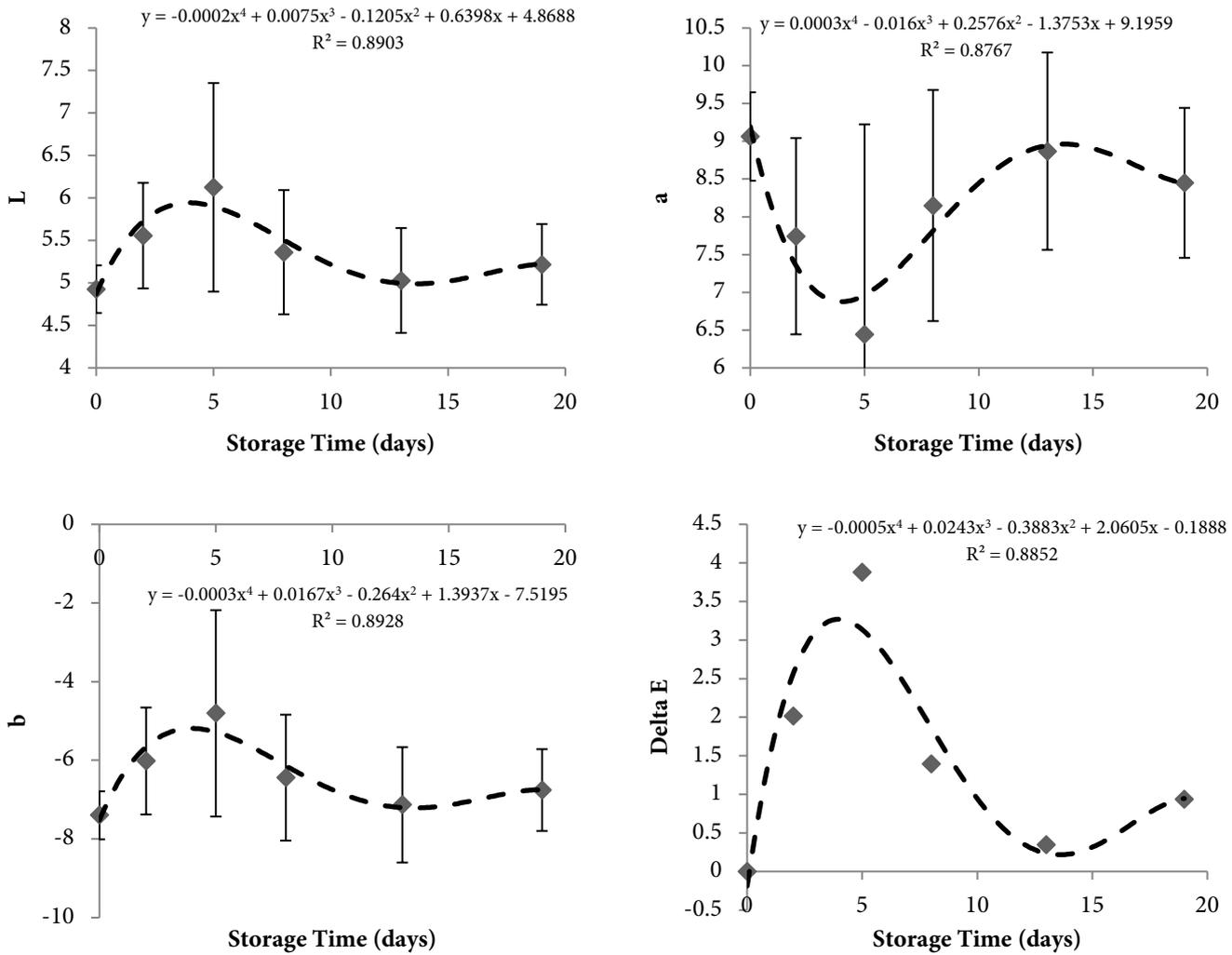


Figure 1. Color changes of whole European eel stored at 2.00°C

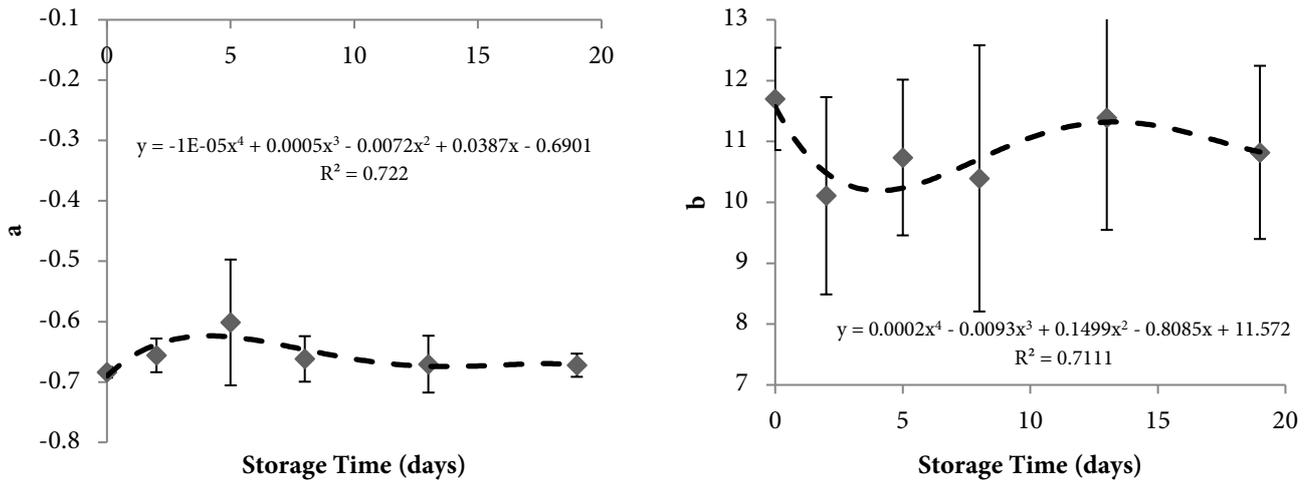


Figure 2. Changes in a. Hue and b. Chroma values for whole European eel stored at 2.00°C

The changes in Hue angle and Chroma in whole European eel stored under refrigerated conditions are shown in Figure 2 a - b. Hue angle is representing the position of the value on the color coordinate system from 0° to 360° in which the positions

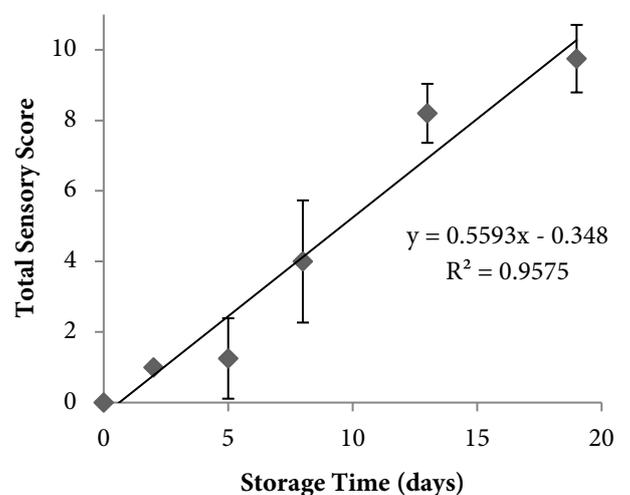
were divided into 4 area in 90° parts. Moreover, the first 90° area represents red, orange and yellow, the second yellow, yellow-green and green, the third green, cyan and blue and finally the last area of the coordinate system is representing red color in

the system (Cavaco et al., 2021). The changes in Hue angle in this study (Figure 2a) was not significantly changed ( $p>0.05$ ) and remain constant in the second quadrant. The color of whole European eel in terms of Hue angle indicating that the color of the samples was slightly from yellow to yellow green. Hue angle values were found to be  $-0.68\pm 0.008$  on the first day of the storage and slightly changes  $-0.67\pm 0.01$  at the end of the storage time ( $p>0.05$ ) ( $R^2=0.72$ ). In terms of the Chroma which defines the intensity of the color, the color of the samples was less saturated at the beginning of the storage and in accordance with the storage time the saturation is increased (Figure 2b). The least saturated values were obtained on 2<sup>nd</sup> day of the storage and found to be  $10.10\pm 1.62$  and on the last day of the storage Chroma values were found to be  $10.82\pm 1.42$  ( $R^2=0.71$ ). In terms of significant changes in Chroma and Hue angle, similar results were reported by Boziaris et al. (2021) for high pressure processed (HPP) Pacific wild pink salmon (*Oncorhynchus gorbuscha*), Alaska pollock (*Gadus chalcogrammus*) and yellow-fin Tuna (*Thunnus albacares*). Researchers reported that no significant ( $p>0.05$ ) changes were found among treated and un-treated samples. In another study the effect of fishing season and storage conditions (vacuum packed or modified atmosphere packed) on the quality of European plaice (*Pleuronectes platessa*) fillets stored at 4°C were investigated. In accordance with the results of the study no significant differences between groups in Chroma and Hue angle were reported (Tsoukalas et al., 2022). As chroma indicates the saturation and hue angle stands for determination of the color itself, these two parameters could give a perspective in terms of quality loss in seafood during storage (Esteves et al., 2021; Setiady et al., 2007; Wade & Glencross 2011). Compared to the results given in the literature it should be noted that during storage time of 19 days no significant effect ( $p>0.05$ ) has been found for *timexchroma* and *timexhue angle* for whole eel stored at  $2.00\pm 1.00^\circ\text{C}$ .

### Sensory Changes

The changes in total sensory quality in whole European eel stored at  $2.00\pm 1.00^\circ\text{C}$  are shown in Figure 3. Total sensory score is obtained by summing the individual parameters namely the score of skin color and slime, eyes shape and clarity, texture firmness and odor of the samples. During each session panelists were recorded the changes in mentioned attributes in terms of their description. In accordance with the records of the panelists skin color, eyes shape and texture firmness were not significantly changed during 19 days of chilled storage. However, the changes in skin slime, eyes clarity and odor were

significant ( $p<0.05$ ). The initial total sensory score was  $0.00\pm 0.00$  which indicates that the samples were in good quality. During 19 days of storage significant increases ( $p<0.05$ ) were found in total sensory score and reached  $9.75\pm 0.95$  at the end of the storage time. Additionally, linear correlation is obtained between storage time and total sensory score for whole European eel ( $r=0.97$ ). In this study samples were stored fresh under chilled conditions. In this context storage time together with the microbiological changes to explain the storage time and spoilage of European eels could be the major factors that affects the quality of the samples (Mchazime & Kapute, 2018; Shabani et al., 2019). The sensory shelf life of whole European eels was found to be 13 days in accordance with the results of sensory analysis in this study. Similar results were reported for gutted European eel (*A. anguilla*) stored with and without ice at  $+3\pm 1^\circ\text{C}$  for 19 days. The shelf life of the samples stored in ice were found to be 12-14 days and for those stored without ice the shelf life was reported for 5-7 days (Özogul et al., 2005). In another study, Özogul et al. (2014) determined the effects of natural antioxidant (laurel and myrtle) on the quality of vacuum packed European eels (*A. anguilla*) stored under refrigerated conditions. Researchers reported the sensory shelf life of the samples as 12 days for control group (vacuum packed and untreated), 16 and 20 days for the group that treated with laurel and myrtle. Küçükgülmez et al. (2013) evaluated the effect of chitosan on quality changes of European eel (*A. anguilla*) fillets under refrigerated temperatures ( $+4\pm 1^\circ\text{C}$ ) and reported that all the groups in the study namely treated samples with different concentrations of extracted and commercial chitosan were rejected in terms of the sensory quality on 15<sup>th</sup> day of storage.



**Figure 3.** Sensory score changes in whole European eel stored at  $2.00^\circ\text{C}$

## Conclusion

The quality attributes of whole European eel stored at  $2.00\pm 1.00^{\circ}\text{C}$  were monitored in terms of the changes in microbiological, color and sensory scores. At the beginning of the storage the TMAB, TPAB, *Pseudomonas* sp. and LAB were slightly higher than  $1.00 \log \text{cfu/g}$  which indicating that European eel samples were in good quality. After 13 days of storage TMAB and TPAB were exceeded the acceptable limit of  $7.00 \log \text{cfu/g}$  while the counts of *Pseudomonas* sp. were  $7.53\pm 0.76 \log \text{cfu/g}$  on day 19. The counts of LAB were not reached the acceptable limit at the end of the storage time and thus decided not suitable for the decision on shelf life of the samples. Color parameters such as  $L^*$ ,  $a^*$ ,  $b^*$  and  $\Delta E$  were not followed a linear increase/decrease during 19 days of storage time. The samples were slightly darker, yellowish and reddish at the end of the storage time. As for the sensory quality changes, the score of total sensory changes were significantly different ( $p < 0.05$ ) at the end of the storage time compared to the initial sensory scores of the samples. Overall results of this study indicating that the shelf life of whole European eel at  $2.00\pm 1.00^{\circ}\text{C}$  were found to be 13 days. For the determination of the shelf life of samples microbiological changes such as TMAB, TPAB and *Pseudomonas* sp. together with total sensory scores should be used. Additionally, it could be suggested that combination of lower storage temperatures (i.e., storage in the ice) and usage of natural antimicrobials would have positive effect on prolonging the shelf life of whole European eel.

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## Compliance With Ethical Standards

### Authors' Contributions

İYG: Laboratory work, design of the study and manuscript, statistical analysis.

EB: Field work, sensory analysis, contributing to manuscript writing

All authors read and approved the final manuscript.

### Conflict of Interest

The authors declare that there is no conflict of interest.

### Ethical Approval

For this type of study, formal consent is not required.

## Data Availability Statements

All data generated or analyzed during this study are included in this published article.

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