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Research Article **Araştırma Makalesi**

Effect of Drying Medium on Proximate and Mineral Composition of Croaker Fish peculiar to the Coastline of Akwa Ibom State, Nigeria

Kurutma Ortamının Nijerya'nın Akwa Ibom Eyaleti Kıyı Şeridine Özgü Çiroz Balığının Organik Madde ve Mineral Bileşimi Üzerine Etkisi

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Abstract: The present study investigated the nutritional composition of dried croaker (*Pseudotolithus typus*) fish from the coastline of Eastern Obolo, Ibeno, Ikot Abasi, and Mkpat Enin Local Government Areas of Akwa Ibom State, in Southern Nigeria. The quality of the products dried using a designed active solar dryer was compared to that of the same products dried using a traditional roadside dryer commonly used by local fish vendors. The result revealed a significant difference ($p < 0.05$) in the proximate properties of the products from the two drying mediums. The ash content, crude fiber, and lipid were higher in products from the conventional dryer than products from the solar dryer. The protein content was slightly higher in the products from the active solar dryer (47.67±0.17 %) than in those from the conventional dryer (43.33±0.19 %). These findings suggest that the products from the active solar dryer were more suitable for consumption. Carbohydrate content was higher in the products from the conventional roadside dryer (4.13±0.02 %) than in the products from the solar dryer (3.74±0.03 %). The energy level was also higher in the solar-dried products (467.17±0.39 Kcal) as compared to those from the conventional dryer (430.33±0.34 Kcal). These differences were attributed to factors such as exposure of the product to impurities and the nature of the drying medium. The study confirmed the presence of higher concentration of macro elements (Sodium, Potassium, Calcium, Magnesium, and Sulphur) in products from the conventional dryer than in those from the active solar dryer. The mineral composition of the products from the two methods also exhibited significant differences ($p < 0.05$). The study recommends the consumption of products dried from the active solar dryer based on low levels of arsenic, chlorine and iodine, which were less than the 0.010 Mg/kg prescribed by the World Health Organization.

Keywords
- Solar drying
- Fish processing
- Proximate properties
- Mineral elements
- Southern Nigeria

Özet: Bu çalışmada, Güney Nijerya'daki Akwa Ibom Eyaleti'nin Doğu Obolo, Ibeno, İkot Abasi ve Mkpat Enin Yerel Yönetim Bölgelerinin kıyı şeridinden kurutulmuş kraker (Pseudotolithus typus) balığının besin bileşimi araştırılmıştır. Tasarlanan aktif solar kurutucu kullanılarak kurutulan ürünlerin kalitesi, yerel balık satıcıları tarafından yaygın olarak kullanılan geleneksel yol kenarı kurutucusu kullanılarak kurutulan aynı ürünlerin kalitesiyle karşılaştırılmıştır. Sonuçlar, iki kurutma ortamından elde edilen ürünlerin yakın özelliklerinde önemli farklılıklar ($p < 0.05$) olduğunu ortaya koymuştur. Kül içeriği, ham lif ve lipid, geleneksel kurutucudan elde edilen ürünlerde güneş enerjili kurutucudan elde edilen ürünlerden daha yüksekti. Protein içeriği aktif solar kurutucudan elde edilen ürünlerde (%47,67±0,17) konvansiyonel kurutucudan

Anahtar kelimeler
- Güneşte kurutma
- Balık işleme
- Organik özellikler
- Mineral elementler
- Güney Nijerya

elde edilenlere göre (%43,33±0,19) biraz daha yüksekti. Bu bulgular, aktif solar kurutucudan elde edilen ürünlerin daha sağlıklı olduğunu göstermektedir. Karbonhidrat içeriği geleneksel yol kenarı kurutucusundan elde edilen ürünlerde (%4,13±0,02) solar kurutucudan elde edilen ürünlere (%3,74±0,03) kıyasla daha yüksekti. Enerji seviyesi de güneşte kurutulan ürünlerde (467,17±0,39 Kcal) geleneksel kurutucudan elde edilenlere (430,33±0,34 Kcal) kıyasla daha yüksekti. Bu farklılıklar, ürünün yabancı maddelere maruz kalması ve kurutma ortamının niteliği gibi faktörlere bağlanmıştır. Çalışma, geleneksel kurutucudan elde edilen ürünlerde aktif solar kurutucudan elde edilenlere kıyasla yüksek konsantrasyonda makro elementlerin (Sodyum, Potasyum, Kalsiyum, Magnezyum ve Kükürt) varlığını doğrulamıştır. İki yöntemle elde edilen ürünlerin mineral bileşimi de önemli farklılıklar göstermiştir ($p < 0.05$). Çalışma, Dünya Sağlık Örgütü tarafından öngörülen 0.010 Mg/kg değerinden daha düşük olan düşük arsenik, klor ve iyot seviyelerine dayanarak aktif solar kurutucudan kurutulan ürünlerin tüketimini önermektedir.

1.INTRODUCTION

Drying fish and preserving the dried product for an extended period is a significant challenge faced by fish processors in Akwa Ibom State, Southern Nigeria. Some of the methods used for drying these products are outdated and the hygienic state of some are questionable. Roadside fish vendors in Southern Nigeria are grappling with the difficulties of maintaining the product's quality and understanding the changes in the nutritional and chemical properties of the products (Edet et al, 2024). These challenges have led to a decline in interest for their services. According to Ahmed et al. (2022), the nutritional content of dried fish products could influence consumer preference for these products. Drying technique could influence the physical, chemical, nutritional and sensory properties of the product. These properties are relevant in defining the general acceptability of such products by consumers. This assertion was further corroborated by Alahmad et al. (2021) in their study of the influence of drying techniques on the physicochemical, nutritional and morphological properties of bighead carp (*Hypophthalmichthys nobilis*) fillets. Rusal et al. (2021) also shared a similar view when they studied the effect of drying methods on the physico-chemical, microbiological and sensory properties of torpedo scad (*Megalaspis cordyla*).

The proximate composition of dried fish products has been thoroughly examined by multiple authors to determine their shelf life and quality (Siddiky et al., 2017; Avila et al., 2022; Balavinayagamani et al., 2020; Moshood et al., 2014; Bolowa et al., 2011; Fitri et al., 2022; Rasul et al., 2021). In a study by Ezugwu (2021), the proximate composition of four different fish species (croaker, mackerel, catfish, and tilapia) showed significant variation in their properties. Protein content across all species ranged from 46.09 to 67.71%, with croaker having the highest lipid content at 32.11%, compared to 8.32% for catfish and 8.68% for tilapia. Kim et al. (2020) studied the effect of various drying methods on the physicochemical characteristics and textural features of croaker. They reported that crude protein increased from 19.03 to 31.32 % after drying of the product in a hot air oven. Abraham-Olukayode (2013) investigated the changes in the proximate composition of certain fish species subjected to various processing methods in Southern Nigeria. The study found that processed croaker fish samples had higher nutritional value than the fresh samples based on experimental data, with moisture, ash, lipid, and protein content differing significantly between the fresh and oven-dried samples.

Abimbola (2016) conducted a study on the proximate and mineral composition of two species of croaker fish from a Lagoon in Lagos. The study found that the *Pseudotolithus senegalensis species* had higher moisture and ash content when compared to the *P. typus species*. However, fresh samples of *P. typus species* had higher fat (0.86 %), fiber (2.01 %), and carbohydrate content (5.05 %). A similar study by Metha & Nayak (2017) revealed that the crude protein content in *Johnius dussumieri* species of croaker fish was slightly lower (15.40 %) than what was reported by Metha et al. (2014) for fresh *P. typus*. The high moisture content and low-fat content classified the fish as lean. Njinkoue et al. (2016) also analyzed the proximate composition and mineral elements of two marine fish samples from the Cameroon coastline that were subjected to drying. They described the products as rich in protein and low-fat content of less than 0.5 %.

Ezugwu (2021) confirmed the presence of both macro and micro mineral elements, including calcium, potassium, sodium, phosphorus, manganese, iron, zinc, and magnesium, in croaker fish. He underscored the significance of these elements in promoting healthy nutrition. Chukwu (2009) reported that drying methods influence the nutritional properties of fish and aquatic related products. The study revealed that potassium content in Tilapia increased from 0.00012 (in its raw state) to 0.00058 % (after drying). Phosphorus content also increased from 0.00012 to 0.00042 % after drying the product. Abimbola (2016) also verified the existence of these mineral elements while examining two species of croaker fish, with the *P. typus* variety containing 22.64 mg/kg of calcium and 22.04 mg/kg for *P. senegalensis*. Manganese had the lowest composition of less than 2.00 mg/100g for both varieties. Moreover, various authors have highlighted the presence of macro and micro elements in different fish species, emphasizing their critical role in defining the edibility of such products (Ahmed et al., 2017; Gong et al., 2019; Simpson & Uche, 2019; Moses, 2019; Dou et al., 2020; Namaga et al., 2020; Ayebusi, 2021; Umamageshawari et al., 2022).

There is limited information available regarding studies on the proximate and mineral composition of croaker fish dried using an active solar drying system, despite the increasing demand for the product in Southern Nigeria. Availability of such information could help in safeguarding the health of consumers and the choices they make with respect to medium of drying the product. This study aimed to investigate how drying mediums affect the proximate and mineral content of croaker fish peculiar to the long coastline of Akwa Ibom State, South South Nigeria.

The present article is structured in four sections. The first section gives an overview of the research, highlights research gaps and captures the objectives of the study. The second section highlights the materials and methods used to achieve the objectives of the research, while the results from the experiments are discussed in the third section. The fourth section concludes the research and highlights recommendations which could help in optimizing drying and preservation techniques for croaker fish and related products in the coastal zone of Akwa Ibom State.

2.MATERIALS AND METHODS

2.1.Material for Experiment

Fourty samples of net weight of 10 kg were bought from a local fish vendor along the coastline connecting Eastern Obolo, Ibeno, and Ikot Abasi as well as Mkpat Enin Local Government Areas of Akwa Ibom State, Nigeria, in February 2024. The average weight of each individual product was 255 g, while the average length was 30.4 cm. These values were consistent with the typical measurements obtainable for the products in the study area. The samples were promptly placed in an iced container and transported to the Agricultural Products Processing and Storage Laboratory of the Department of Agricultural Engineering, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin.

2.2.Sample Preparation and Experimental Set Up

The products were carefully washed, cleaned, and sliced with a sterilized steel knife. Removal of the gills ensured that the bitter taste of the section did not affect the edible part while also eliminating bacterial agents in the process. The samples were subjected to drying in an active indirect solar dryer, while the conventional roadside dryer served as a control experiment. The conventional dryer was a regular charcoal fired dryer used by fish vendors, whose drying tray takes between five to ten products depending on the size of the products. The product had direct contact with the fuel source (Charcoal). The solar powered dryer was an enclosed dryer which had a 5-blade axial blower which was powered by the solar PV system. The blower was placed in the basement of the dryer to ensure air was trapped efficiently to the drying chamber to burn the charcoal at a high intensity for drying of the product. A heat exchange medium was used as an intermediary between the fuel source and the drying chamber. This wasn't the case for the conventional dryer as the drying was dependent on ambient factors such as temperature, wind speed and relative humidity. The wind speed for an average drying day ranged from 0.5 to 2.5 m/s. The relative humidity ranged from 40 to 85 %, while the air temperature was between 27 and 36 °C. The factors contributed immensely to the performance of the conventional dryer.

Compressed air into the dryer averaged 2.0 m/s in terms of speed during a typical drying experiment. The temperature inside the chamber of the solar enclosed dryer ranged between 40 to 60 ℃ during a drying experiment. This high temperature range and speed of the blower aided faster drying of the product in the solar enclosed system.

2.3.Drying Time and Temperature

The products were arranged in the drying chamber of the respective mediums equidistant from each other. It took 85 minutes for samples in the conventional dryer to attain a constant weight, while samples in the active solar dryer reached an equilibrium weight in 135 minutes. The temperature range for the active solar dryer was between 35 to 60 ℃. After the drying experiment, the samples from the two mediums were allowed to cool before being subjected to proximate and mineral analyses. The dried products from the experiments were not similar in appearance as shown in Figure 1 (Conventional Dryer) and Figure 2 (Active Solar Dryer) respectively, as observed after the experiment.

Figure 1: Dried Products in the Conventional roadside dryer.

Figure 2: Dried Products in the Active solar dryer.

2.4.Proximate Properties

The chemical composition of the dried samples was determined using the standard set by the Association of Official Chemists (AOAC, 2020). The properties analyzed included crude protein, crude lipid, carbohydrate, moisture, and ash contents. After the drying experiment, the samples were carefully wrapped in a foil to prevent rewetting and contamination. The moisture content was determined using a hot air oven (Model DHG-9101-2SA) manufactured by Jiangsu Instruments Technology Limited, China. The temperature of the oven was set at 105 °C. The crude protein was determined using an automatic Kjeldahl Protein Analyzer (Model ZDDN-11) manufactured by WINCOM Company Limited, China. For lipid content, 2 g of the sample was placed in an extraction thimble and refluxed in an extraction flask filled with petroleum ether for six hours. A rotary evaporator (Model F250 - manufactured by Julabo Instruments, United Kingdom), was used to evaporate ether, and the weight loss was recorded as the crude lipid. The crude fiber was obtained by infusing 200 ml of N-hexane for 2 hours in a 250 ml conical flask. 5 ml of sulfuric acid was added to the flask, and the mixture was boiled and refluxed for half an hour. The hot solution was then filtered through suction. The total ash was obtained by igniting a previously dried sample (2.00 g) in a muffle furnace at 500 °C for 4 hours. The carbohydrate content was obtained by subtracting the total lipid, protein, fiber, ash, and moisture from 100. Each experiment was replicated three times for samples from each medium.

2.5.Mineral Composition

The mineral composition of the dried samples was determined using standard procedures. The minerals considered included Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Copper (Cu), Zinc (Zn), Iron (Fe), Manganese (Mn), Silicon (Si), Arsenic (As), Phosphorus (P), Sulphur (S), Chlorine (Cl_2), and Iodine (I_2). The dried products were crushed and sieved before digestion. An Atomic Absorption Spectrophotometer (Spectrum Lab 752 Pro Model) manufactured by Shenyang Ebetter Optics Company Limited, China, was used to analyze the samples for metal concentration. The principle of Beer and Lambert's law was applied. All standards were prepared in 1000 mg/L, which was equivalent to 1000 PPM stock standard,

except for Sodium and Potassium. Five grams (5 g) of the crushed sample were placed into a digestion bottle. 50 ml of HNO_3 was added to the sample and dried for 2 hours at 110 °C. After two hours, the samples were removed from the oven and allowed to cool for an hour before they were filtered and analyzed using the Spectrophotometer. The results of the experiments were obtained in mg/L and multiplied by the Digestion Factor to obtain solid sample values in mg/kg.

The samples were analyzed for nitrogen and potassium using a flame photometer (FP640NC model) manufactured by WINCOM Company Limited, China. Before being introduced to the flame, the samples were nebulized to ensure a fine spray for efficient atomization. As the samples entered the flame, the solvent evaporated, leaving behind dissolved elements in the form of atoms. The high temperature of the flame excited the atoms, causing them to emit light at specific wavelengths characteristic of the elements in the sample. A monochromator was used to select the desired wavelength of light emitted by the sample while blocking undesired wavelengths. The detector captured the appropriate wavelength and converted it into an electrical signal. The signal processor then measured the intensity of the signal, providing insight into the concentration of the element in the sample. The measured signal was further compared to calibration curves (standards) to quantitatively determine the concentration of the elements. This method was also used by Njikoue et al. (2016) for a similar product.

2.6.Analysis of Data

The experiments for the proximate properties and mineral composition were replicated five times, and the mean value ± standard deviation was used as a reference. To determine significant differences between the data for the dried products from the two mediums, a one-way ANOVA was performed at a 5 % level of significance ($p < 0.05$). Statistical analysis was done using Minitab statistical software version 20.0.

3.RESULTS AND DISCUSSION

3.1.Proximate Properties

Table 1 highlights the proximate properties of the dried products from the active solar dryer and the conventional roadside dryer. The initial moisture content of the fresh samples was 74.50±0.32 % (dry basis) in the active indirect solar dryer and 74.74±0.18 % (dry basis) for fresh samples in the conventional dryer. The final moisture content was higher for samples in the active solar dryer (7.75±0.12 % - dry basis) than for samples dried using the conventional roadside dryer (7.43±0.12 % - dry basis). The difference between the products from the two methods was found statistically significant ($p < 0.05$) as shown in Table 2. The t-value of 5.13 obtained from the experimental data indicates the distinction between the pooled standard error and the difference in the value of moisture content of the products from the two mediums. The moisture content values for the products from the two methods align with the study by Ezugwu (2021), who reported 5.56 % (dry basis). The moisture level of the product is responsible for limiting microbial activities in the dried product and enhancing the shelf life of the product after drying. Most seafood vendors in the study area have abandoned the trade due to the consistent challenge of breaking even, due to the high rate of losses.

Table 1: Proximate properties of the dried products from the different mediums.

Proximate Property	Solar Dryer	Conventional Dryer
Moisture Content (%)	7.75±0.12	7.43±0.07
Ash (%)	4.14±0.03	4.19±0.01
Crude Fiber (%)	30.73±0.28	34.53±0.26
Lipid (%)	6.25±0.03	6.30±0.01
Protein (%)	47.67±0.17	43.33±0.19
Carbohydrate (%)	3.74±0.03	4.13±0.02
Energy Level (Kcal)	467.17±0.39	430.33±0.34

* n, number of replications = 5.

The ash content in the product from the conventional roadside dryer was higher (4.19±0.01 %) than the ash content in products dried using the active solar dryer (4.14±0.03 %). There was significant difference ($p<0.05$) in ash content for products dried using the different methods. This result contrasts with the study of Njikoune et al. (2016), which reported 7.28 ± 0.25 % ash content for the fresh edible part of *P. typus* and 1.28 % for smoked products (Amusan et al., 2018). These values were closer to the 5.90 % reported by Bolawa et al. (2011) for a similar product obtained in the riverine area of Lagos, Nigeria. These values were also higher than those obtained by Tang et al. (2009) for the

Pseudosciaena crocea R. (2.5±0.12 %) species. The ash content signifies low toxicity level for the product, good texture, taste and nutritional properties of the dried product.

Crude fiber content of samples dried in the conventional dryer (34.53±0.26 %) was higher than that of samples dried using the active solar dryer (30.73±0.28 %). This attribute is crucial for ensuring better absorption and digestion of the dried product. Additionally, there was significant difference ($p < 0.05$) in the values obtained from the different drying methods, which contradicted the findings of Bolawa et al. (2011) and Ibe (2021).

The lipid content was observed to be higher in the samples dried in the conventional dryer (6.30 ± 0.01 %) compared to those dried in the active solar dryer (6.25±0.03 %). There was significant difference ($p < 0.05$) in the lipid content between the samples from the two different mediums. These values closely align with the 6.6 % obtained by Abraham-Olukayode (2013) for the same product dried in the open sun, but were higher than 1.48 % obtained by Amusan et al. (2018). This property is essential for defining the energy characteristics of the dried products and for preventing cardiovascular and heart diseases.

The protein content was found to be higher in products dried in the active solar dryer (47.67±0.17 %) compared to those dried in the conventional dryer (43.33±0.19 %). Significant difference ($p < 0.05$) was observed in the values obtained for the products in the two mediums. The recorded result surpassed the 39.22% obtained by Amusan et al. (2018) for smoked croaker from retail outlets in the Makoko environs of Lagos, Nigeria. However, it differed from the findings of Abraham-Olukayode et al. (2013) for the same product. The higher value obtained for the products dried using the active solar dryer could potentially enhance the amino acid content in the product, thereby helping to reduce the risk of high blood pressure and stroke.

The carbohydrate content was observed to be higher in samples dried in the conventional dryer (4.13±0.02 %) compared to those dried in the active solar dryer (3.74±0.03 %). These values were lower than the 2.49 % reported by Anene et al. (2013) for the *Pseudotolithus elongatu*s species of croaker and the range of 0.20 to 0.49 % reported for fresh products by Ondo-Azi et al. (2013). However, they were closer to the 5.05 % obtained for the *P. senegalensis* species of the fish (Abimbola, 2016). There was a significant difference ($p < 0.05$) between the values obtained from the two drying methods as shown in Table 2. The low carbohydrate content is an indicator that the dried product will have a significant impact on blood sugar levels for persons that consume them.

The energy level was higher in products from the solar dryer (467.17±0.39 Kcal) than those from the conventional dryer (430.33±0.34 Kcal). The variation in the values of the proximate properties of the products from the different drying mediums was due to the airflow into the respective dryers, exposure of the products to the atmosphere, the interaction between the products and the fuel source (charcoal), and the quantity of heat and air circulation in the drying chamber.

Table 2: Tukey Simultaneous Tests for Differences of Means of Proximate Properties.

Proximate Property	Difference of Dryer	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	P-Value
Moisture Content	Solar Dryer - Conventional	0.3220	0.063	(0.177, 0.467)	5.13	0.001*
Ash	Solar Dryer - Conventional	-0.050	0.015	(-0.084, -0.016)	-3.43	0.009*
Crude Fiber	Solar Dryer - Conventional	-3.802	0.171	(-4.196, -3.408)	-22.27	0.001*
Lipid	Solar Dryer - Conventional	-0.058	0.014	(-0.091, -0.025)	-4.08	0.004*
Protein	Solar Dryer - Conventional	4.344	0.113	(4.083, 4.605)	38.31	0.001*
Carbohydrate	Solar Dryer - Conventional	-0.396	0.016	(-0.433, -0.359)	-25.05	0.001*
Energy Level	Solar Dryer - Conventional	36.846	0.226	(36.325, 37.367)	163.14	0.001*

*Significant difference between data from the two mediums; CI – Confidence Interval.

3.2.Mineral Composition

3.2.1.Main Elements

Microelements such as sodium, potassium, calcium, and sulphur were higher in samples dried using the conventional dryer than in samples subjected to drying in the active solar dryer. Phosphorus was higher in the samples dried in the active solar dryer than in the conventional as shown in Table 3. There were significant differences in the values obtained for samples in both mediums ($p < 0.05$) represented in Table 4. The high sodium content (68.16 ± 0.26 mg/kg) in the conventional dryer means the product could subject consumers to threats of high blood pressure and cardiovascular diseases. The result was higher than the value of 64.27 ± 0.25 mg/kg obtained for products from the active solar dryer. These values obtained for the dried samples from both mediums were higher than 11.20 ± 0.09 mg/kg obtained by Adejonwo (2016), who studied the mineral composition *P. senegalensis* and *P. typus species*, and obtained an average of 9.89 ± 1.65 mg/kg.

The potassium content (33.05 ± 0.05 mg/kg) in products dried using the active solar dryer was lower than that in products dried using the conventional dryer (35.15 ± 0.04 mg/kg). This suggest that consuming products dried using the solar dryer may reduce the risk of kidney disease in humans. These values were closer to what was obtained by Abimbola (2016) in a similar study (30.39 mg/kg) for croaker.

The calcium content from samples in the active solar dryer (32.18 ± 0.13 mg/kg) was lower than what was obtained for products dried in the conventional dryer (34.45 ± 0.33 mg/kg). The high calcium content indicates the presence of vitamin D in the product, which can contribute to building strong bones and teeth. The calcium content aligns with the findings of Chen et al. (2022). They opined that calcium content less than 40 mg/kg promotes suitability of croaker fish for consumption by humans.

The samples from both drying mediums exhibited high magnesium content, indicating that the products were nutritious. The low phosphorus content in the solar-dried samples (2.65 ± 0.03 mg/kg) and the conventional dryer samples (2.42 ± 0.03 mg/kg) suggested that the products possessed high antioxidant properties. These results slightly exceeded the values obtained by Adejonwo (2016), which were 1.84 ± 0.16 and 1.68 ± 0.23 mg/kg, respectively. The flavour of the dried products from both methods was appealing, implying low sulphur content, similar to the description of Petricorena (2014).

Table 3: Mineral composition of the dried products from the different mediums.

Type	Mineral Component (Mg/Kg)	Solar Dryer	Conventional Dryer
Macro Elements	Sodium	64.27 ± 0.25	68.16 ± 0.26
	Potassium	33.05 ± 0.05	35.15 ± 0.04
	Calcium	32.18 ± 0.13	34.45 ± 0.33
	Magnesium	14.32 ± 0.35	16.15 ± 0.33
	Phosphorus	2.65 ± 0.03	2.42 ± 0.03
	Sulphur	0.02 ± 0.00	0.04 ± 0.01
Micro Elements	Copper	3.13 ± 0.03	2.82 ± 0.02
	Zinc	18.57 ± 0.08	23.22 ± 0.27
	Manganese	2.16 ± 0.02	2.46 ± 0.02
	Silicon	0.82 ± 0.02	0.67 ± 0.01

* n, number of replications = 5.

3.2.2.Micro Elements

The levels of copper and silicon were higher in the samples dried in the active solar dryer than the conventional dryer as shown in Table 3. The concentration of Zinc and Manganese were higher in samples from the conventional dryer than the products from the solar dryer. These findings did not agree with the studies conducted by Ogundira et al. (2012) and Njikuoe et al. (2016). The duo obtained separate values for the edible part and bone for the product. Manganese content was higher than what Adejonwo (2016) obtained in his study of similar product from a Lagoon in Lagos, Southern Nigeria.

In comparison with the standards of the Food and Agricultural Organization and the World Health Organization (FAO, 2001), the trace elements were in range and not higher in concentration, making the products from both mediums fit for consumption. Similarly, the levels of Arsenic, Iodine and Chlorine in samples from both drying mediums were less than 0.01 mg/kg, indicating that the samples were less toxic, but also with enhanced shelf life and

reduced risk of poisoning and obesity for persons that consume the dried product. These values were lower than the WHO standards for acceptable levels of arsenic, iodine and chlorine (0.01 mg/kg), posing the product as fit for consumption (FAO, 2001).

Table 4: Tukey Simultaneous Tests for Differences of Means of Mineral components.

Mineral Component (Mg/Kg)	Difference of Dryer	Difference of Means	SE of Difference	Simultaneous 95 % CI	T-Value	P-Value
Sodium	Solar Dryer - Conventional	-3.893	0.210	(-4.475, -3.312)	-18.58	0.001*
Potassium	Solar Dryer - Conventional	-2.107	0.038	(-2.211, -2.002)	-55.86	0.001*
Calcium	Solar Dryer - Conventional	-2.267	0.203	(-2.831, -1.703)	-11.16	0.001*
Magnesium	Solar Dryer - Conventional	-1.830	0.277	(-2.599, -1.061)	-6.60	0.003*
Copper	Solar Dryer - Conventional	-0.313	0.021	(-0.372, -0.255)	-14.86	0.001*
Zinc	Solar Dryer - Conventional	-3.750	0.159	(-4.192, -3.308)	-23.53	0.001*
Magnesium	Solar Dryer - Conventional	-0.297	0.013	(-0.334, -0.259)	-22.25	0.001*
Silicon	Solar Dryer - Conventional	0.157	0.011	(0.127, 0.186)	14.86	0.001*
Phosphorus	Solar Dryer - Conventional	0.230	0.023	(0.167, 0.293)	10.06	0.001*
Sulphur	Solar Dryer - Conventional	-0.017	0.003	(-0.026, -0.007)	-5.00	0.007*

* Significant difference between data from the two mediums; CI – Confidence Interval.

4.CONCLUSIONS

This research examined croaker fish from the coastal area of Akwa Ibom State based on their chemical composition and mineral content after undergoing drying experiments. The study concluded that the appearance of dried *P. typus* is a function of the medium used for drying the product. Results of the proximate analysis showed significant differences in the proximate properties (moisture content, ash content, crude fiber, protein and lipid). Moisture content was higher in the solar dried samples, than the conventional dryer, but the reverse was the case for ash content. Protein content was slightly higher in products from the active solar dryer (47.67±0.17 %) as against the conventional dryer (43.33±0.19 %). The result suggests that products from the active solar dryer could help reduce the risk of high blood pressure and promote healthy dieting. Carbohydrate level was higher in products from the conventional dryer (4.13±0.02 %) than in products from the solar dryer (3.74±0.03 %). The energy level was higher in solar-dried samples (3.74±0.03 %) than in those from the conventional dryer (430.33±0.34 Kcal). The difference in the values of the chemical properties of the products from the drying mediums was attributed to factors such as airflow into the respective dryers, exposure of the products to the atmosphere, and interaction between the products and the fuel source (charcoal), and the quantity of heat and air circulation in the drying chamber. Air flow into the dryer was through a force convective means. A blower linked to a solar PV cell was attached at the basement of the dryer which enhanced circulation of air to burn the charcoal with much intensity and further produced the much-desired hot air to heat up the drying chamber for faster drying of the product. However, this wasn't the case for the conventional dryer as no blower was attached to the dryer to accelerate flow of hot air in the drying chamber. The drying process was dependent on ambient conditions and time of the day. The macro elements found in samples from both dryers were Sodium, Potassium, Calcium, Sulphur, Magnesium, and Phosphorus. The results showed that Sodium, Potassium, Calcium, Magnesium, and Sulphur were of higher concentration in products dried using the conventional dryer than in products from the active solar dryer, with Phosphorus higher in the

solar-dried samples. However, there was a significant difference for the samples in both mediums. The concentration of trace elements identified in the samples from both drying methods was within the standards prescribed by the World Health Organization, confirming the fitness of the products for consumption and low toxicity level (< 0.01 mg/kg). The study recommends an active solar dryer for drying the products to promote healthy dieting based on reduced interaction with impurities and environmental factors.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Conceptualization: PE; Literature: PE; Methodology: PE, BE, UU, AU; Experiment: PE, BE, UU, AU; Data analysis: PE; Manuscript writing: PE, UU; Supervision: PE

All authors approved the final draft.

ETHICAL APPROVAL:

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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Research Article **Araştırma Makalesi**

Genetic Diversity of Cultured Rainbow Trout (*Oncorhynchus mykiss*) Populations in Türkiye Based on Mitochondrial DNA Cytochrome b (cyt-b) Sequence Analysis

Türkiye'deki Kültür Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Popülasyonlarının Mitokondriyal DNA Sitokrom b (cyt-b) Dizi Analizine Dayalı Genetik Çeşitliliği

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Abstract: This study aimed to investigate the genetic diversity and structure of rainbow trout (*Oncorhynchus mykiss*) cultured populations in different provinces of Türkiye, based on the cytochrome b (cyt-b) gene region of mitochondrial DNA (mtDNA). Tissue samples were collected from a total of 98 fish (seven fish per province) across 14 provinces, followed by DNA isolation. The mtDNA cyt-b gene region (754 bp) was amplified using PCR. Genetic diversity indices, genetic structure, and phylogenetic analyses were calculated from the cyt-b gene sequence data. The average nucleotide frequencies of the four nucleotides cytosine (C), thymine (T), adenine (A), and guanine (G) were found to be 26.2%, 24.9%, 26.2%, and 22.8%, respectively, with a higher A + T content (51%) compared to G + C content (49%). A total of 645 polymorphic nucleotides were identified across the 14 populations, and 50 distinct haplotypes were defined. Haplotypic diversity ranged from 0.91 to 0.48, while nucleotide diversity (π) varied between 0.26 and 0.00. The highest genetic diversity was observed in the Tokat and Van populations, whereas the lowest was recorded in the Elazığ and Kahramanmaraş populations. AMOVA analyses revealed that 71.1% of the genetic diversity was found between populations, while 28.9% was found within populations. Pairwise F_{ST} values ranged from 0.38 to 0.99, with an average F_{ST} of 0.71. Phylogenetic analyses indicated that the populations clustered into two main groups, with further sub-groupings within these clusters. Notably, the Kahramanmaraş and Elazığ populations were found to be significantly differentiated from other populations. In conclusion, despite rainbow trout not being a native species in Türkiye, the findings of this study indicate that the genetic diversity of the populations is at a good level. However, in order to preserve the genetic diversity of cultured rainbow trout populations and to increase effective population size, it is recommended that breeders engage in fry exchanges and import new rainbow trout specimens to enhance genetic diversity.

Keywords
- Cytochrome b
- Genetic diversity
- Genetic structure
- mtDNA
- Phylogenetic analysis

Özet: Bu çalışmada, Türkiye'deki farklı illerdeki gökkuşağı alabalığı (*Oncorhynchus mykiss*) kültür popülasyonlarının mitokondriyal DNA (mtDNA) sitokrom b (cyt-b) gen bölgesine dayalı genetik çeşitliliğinin ve yapısının incelenmesi amaçlanmıştır. Çalışma kapsamında 14 ildeki toplam 98 balıktan (il başına yedi balık) alınan doku örnekleri ile DNA izolasyonu yapılmış ve mtDNA cyt-b gen bölgesi (754 bp) PCR ile çoğaltılmıştır. Cyt-b gen bölgesi sekans verilerinden genetik çeşitlilik indeksleri, genetik yapı ve filogenetik analizlerle ilgili hesaplamalar yapılmıştır. Çalışmada dört nükleotidin (sitozin (C), timin (T), adenin (A) ve guanin (G)) ortalama frekansları sırasıyla %26.2, %24.9, %26.2 ve %22.8 olarak bulunmuş; A + T içeriğinin (%51) G + C içeriğinden (%49) daha yüksek olduğu belirlenmiştir. 14 popülasyondan toplam 645 polimorfik nükleotid tespit edilmiş ve 50 farklı haplotip tanımlanmıştır. Haplotip

Anahtar kelimeler
- Sitokrom b
- Genetik çeşitlilik
- Genetik yapı
- mtDNA
- Filogenetik analiz

çeşitliliği 0.91 ile 0.48 arasında, nükleotid çeşitliliği ise 0.26 ile 0.00 arasında değişmiştir. En yüksek genetik çeşitlilik Tokat ve Van popülasyonlarında, en düşük ise Elazığ ve Kahramanmaraş popülasyonlarında gözlenmiştir. AMOVA analizleri, genetik çeşitliliğin %71.1'inin popülasyonlar arasında, %28.9'unun ise popülasyonlar içinde olduğunu göstermiştir. İkili F_{ST} değerleri 0.38 ile 0.99 arasında değişmiş ve ortalama F_{ST} değeri 0.71 olarak bulunmuştur. Filogenetik analizler, popülasyonların iki ana gruba ayrıldığını ve bu grupların kendi içinde farklı alt gruplar oluşturduğunu göstermiştir. Özellikle Kahramanmaraş ve Elazığ popülasyonlarının diğer popülasyonlardan belirgin şekilde farklılaştığı gözlenmiştir. Sonuç olarak gökkuşağı alabalıklarının gen kaynağı ülkemizde olmamasına rağmen çalışmada elde edilen bulgular popülasyonların genetik çeşitliliğinin iyi düzeyde olduğunu göstermektedir. Ancak gökkuşağı alabalığı kültür popülasyonlarının genetik çeşitliliğini korumak ve etkili popülasyon büyüklüğünü artırmak için yetiştiricilere yavru balıkları takas etmeleri ve genetik çeşitliliği artırmak için yeni gökkuşağı alabalıkları ithal etmeleri önerilebilir.

1.INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed aquaculture species globally, with a long history of domestication (Longo et al., 2024). In recent years, Türkiye has emerged as one of the leading producers of this species, alongside Chile and Iran, driven by increasing global demand for rainbow trout (FAO, 2020). The extensive farming of rainbow trout has led to the development of numerous genetically distinct strains due to selective breeding practices aimed at enhancing desirable traits (Stanković et al., 2015; Wiens et al., 2018; D'Ambrosio et al., 2019; Kause et al., 2022). More than 75 rainbow trout subspecies have been identified worldwide, highlighting the genetic diversity within the species (Glover, 2008; Abdullah et al., 2019). In Türkiye, while some aquaculture companies import rainbow trout eggs to renew their genetic stocks, many rely on locally sourced broodstock, which may give rise to unique, region-specific genetic strains (Karataş, 2019). However, the lack of comprehensive records regarding the origins and sizes of the initial rainbow trout culture populations raises concerns about the genetic management of these stocks (Ağdamar, 2010; Oral, 2011).

The high fecundity of fish species, including rainbow trout, allows the establishment of populations from a limited number of breeding pairs (Saura et al., 2021; Kurta et al., 2023). However, prolonged reliance on a narrow genetic base can lead to inbreeding, which reduces genetic diversity and increases the likelihood of genetic problems within populations (Martsikalis et al., 2014; Paul et al., 2022). Inbreeding depression can decrease heterozygosity, result in the loss of important alleles, and reduce adaptability to environmental changes, ultimately impacting production efficiency and increasing the risk of deformities (Lind et al., 2012; Grant et al., 2017; Karataş, 2019). Therefore, understanding the genetic diversity in aquaculture populations is crucial for effective breeding programs and the long-term sustainability of the species (Lhorente et al., 2019; Houston et al., 2020). Genetic diversity is a critical factor influencing populations' resilience to environmental fluctuations and overall survival (Leitwein et al., 2017; Cossu et al., 2021). Higher genetic diversity increases the likelihood that some individuals within a population possess alleles that confer an advantage under changing conditions, facilitating the transmission of favorable traits to future generations (Wiens et al., 2018; D'Ambrosio et al., 2019). As a result, assessing the existing genetic diversity in target populations is essential before initiating any selective breeding program (Akhan and Canyurt, 2005; Chavanne et al., 2016; Grant et al., 2017). This evaluation is particularly important in rainbow trout aquaculture, where the potential for inbreeding depression requires careful genetic management to preserve diversity and adaptability (D'Ambrosio et al., 2019; Lhorente et al., 2019; Chu et al., 2020).

Despite the importance of genetic diversity in aquaculture, there is a notable lack of comprehensive studies focusing on the genetic structure of rainbow trout culture populations in Türkiye. Previous research has employed various markers, such as RFLP (Togan et al., 2002), RAPD (Akhan and Canyurt, 2005), microsatellites (Aksakal, 2009; Ağdamar, 2010; Oral, 2011), and mitochondrial DNA (mtDNA) (Sarmaşık et al., 2008; Karataş, 2019), to assess genetic diversity. However, these studies have often focused on limited geographic areas or

specific genetic markers, and most have not utilized sequence analysis to elucidate genetic differences. Consequently, while some research has been conducted to evaluate the genetic diversity of rainbow trout culture populations in Türkiye, no published studies have used sequence analysis to assess population structure using any region of the mitochondrial genome. Mitochondrial DNA is a powerful tool for analyzing population structure and genetic diversity due to its haploid nature and maternal inheritance (DeSalle et al., 2017; Baisvar et al., 2019; Sun et al., 2019; Peng et al., 2021). Numerous studies have successfully employed mtDNA sequence data to investigate the genetic diversity of various fish species, emphasizing the usefulness of the cytochrome b (cyt-b) gene region in understanding population structures (Ha et al., 2020; Sultana et al., 2022; Zhang et al., 2023).

Given the importance of genetic diversity for the sustainability of aquaculture, it is imperative to comprehensively evaluate the genetic diversity of rainbow trout culture populations in Türkiye using advanced molecular techniques. This study aims to address this knowledge gap by comparing the genetic diversity of rainbow trout culture populations in different provinces of Türkiye through sequence analysis of the mtDNA cyt-b gene region. By elucidating the genetic structure and diversity of these populations, this research will provide valuable insights for future breeding programs and conservation strategies, ultimately contributing to the sustainable management of rainbow trout aquaculture in Türkiye.

2.MATERIALS AND METHODS

2.1.Study Area, Sample Collection, and DNA Extraction

In this study, *Oncorhynchus mykiss* specimens were collected between October 1, 2023, and March 1, 2024, from 14 randomly selected provinces across Türkiye, covering all geographical regions, with two provinces from each region (Figure 1).

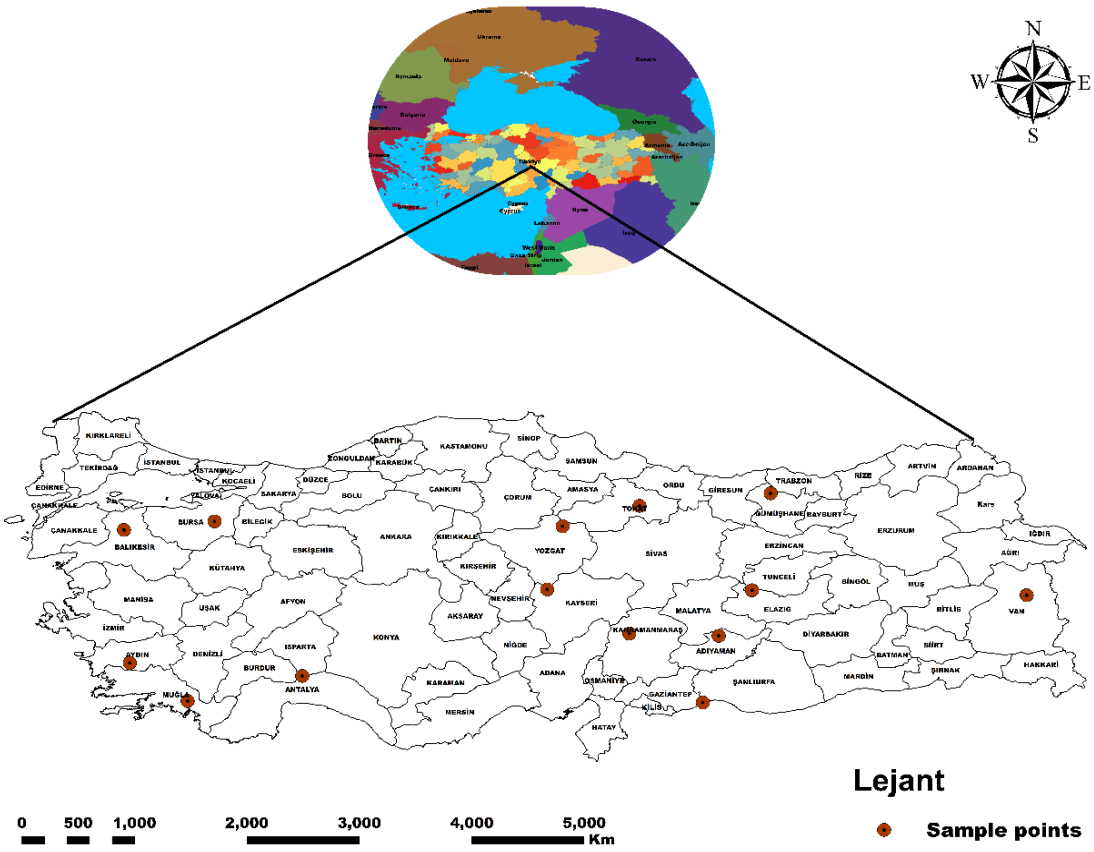

Figure 1. Sample points of rainbow trout culture populations.

A total of 98 rainbow trout (seven fish per province) were anesthetized using clove powder at a concentration of 200 mg/L (Naderi et al., 2017). The fish samples were transported under a cold chain to the Aquaculture Department Laboratory of Van Yüzüncü Yıl University's Faculty of Fisheries. Muscle tissue samples (25–50 mg) were taken from each fish, fixed in 96% ethanol, and stored until DNA extraction. Tissue samples were first homogenized using a tissue lyser, and DNA was extracted using the SuSpin Tissue DNA Isolation Kit (Sugenomics) according to the manufacturer's protocol, with modifications per Karataş (2019). The concentration and purity of the extracted DNA were determined using a NanoDrop 2000/2000c (Thermo Scientific) spectrophotometer at 230/260/280 nm. The DNA samples were stored at -20°C until further processing.

2.2.PCR Amplification

PCR amplification of the mtDNA cyt-b gene region was performed using a RotorGene 6000 (Qiagen) thermal cycler. The reaction used specific primers designed for the partial cyt-b gene region of rainbow trout mtDNA (GenBank accession no: NC_001717.1): forward primer 5'-AGAAACCTGGAATATCGGAGTTGTA-3' and reverse primer 5'-GATGGTGAAGTAAATTACAGAGGC-3'. The PCR mixture consisted of a total volume of 25 µL, containing 10 µL of PCR Master Mix (Sugenomics), 3 µL of forward and reverse primers, 4 µL of H2O, and 5 µL of template DNA. The PCR protocol began with an initial denaturation at 94°C for 10 minutes, followed by 45 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 45 seconds. A final extension step at 72°C for 10 minutes completed the PCR process.

2.3.Sequencing and Data Analysis of the Cyt-b Gene Region of mtDNA

Genetic diversity analyses of the mtDNA cyt-b gene region were performed to assess the genetic variation and population structure of rainbow trout populations from different provinces. PCR amplicons were sequenced using the Sanger sequencing method on an Applied Biosystems 3500/3500 xL Genetic Analyzer. The raw sequence data were obtained in Ab1 format for further analysis. Genetic diversity indices, including base composition, nucleotide diversity (π), haplotype diversity (Hd), haplotype number (H), polymorphic nucleotides count (Ps), average number of nucleotide differences (k), allele number (Na), and expected heterozygosity, were calculated for the cyt-b gene region using standard procedures (Tajima, 1983). Calculations were performed using Arlequin v3.5.2.2 (Excoffier et al., 2005) and DnaSP v6.12.03 (Rozas et al., 2017) software, with a 95% confidence interval and 1000 bootstrap iterations. Population differentiation (F_{ST}) was calculated pairwise using Arlequin v3.5.2.2 (Weir and Cockerham, 1984) with 1000 random permutations for significance testing. Additionally, molecular variance (AMOVA) was computed to test the significance of population structure and analyze differentiation within and between populations using Arlequin. Principal Coordinate Analysis (PCoA) based on F_{ST} values was analyzed using GenAlEx ver. 6.5 (Peakall and Smouse 2006). Evolutionary distance analyses were performed using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods in MEGA X, following established methodologies (Kimura, 1980; Felsenstein, 1985; Saitou and Nei, 1987; Tamura, 1992; Kumar et al., 2018). To identify the optimal model, the MEGA X program was utilized, and the Tamura 3-Parameter (T92-G) model, which assumes a higher rate of transitions compared to transversions, was selected as the most suitable (Tamura, 1992; Tamura et al., 2021). The gamma distribution was applied to account for variation in substitution rates among sites. Bootstrap analyses were performed with 1,000 replicates for each method to assess branch support. A single phylogenetic tree was constructed using the ML method, with bootstrap values from both ML and NJ methods annotated at the respective branches to indicate consistency across methods.

3.RESULTS

3.1.Genetic Diversity Indices

A total of 754 base pairs (bp) of the mtDNA cyt-b gene region were amplified, and after correction and alignment, 726 bp fragments were analyzed to determine the genetic diversity among rainbow trout culture populations. The average frequencies of the four nucleotides cytosine (C), thymine (T), adenine (A), and guanine (G) across the 98 samples collected from 14 different provinces were found to be 26.2%, 24.9%, 26.2%, and 22.8%, respectively. The A + T content (51%) was higher than the G + C content (49%).

As shown in Table 1, a total of 645 polymorphic nucleotides were identified among

98 individuals from 14 different rainbow trout culture populations. The highest number of polymorphic nucleotides was observed in the Tokat population, while the lowest number was found in the Kahramanmaraş population. Based on these polymorphic regions, a total of 50 haplotypes were identified. The highest number of haplotypes was found in the Tokat and Van populations. The haplotype diversity (Hd) ranged from 0.91 ± 0.10 to 0.48 ± 0.17, and nucleotide diversity (π) ranged from 0.26 ± 0.05 to 0.00 ± 0.00. Both haplotype and nucleotide diversity were highest in the Tokat and Van populations and lowest in the Elazığ and Kahramanmaraş populations. Additionally, the average number of nucleotide differences (k) was highest in the Tokat population (185.86) and lowest in the Kahramanmaraş population (0.95).

Table 1. Number of polymorphic nucleotides (Ps), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k) in rainbow trout culture populations.

Populations	n	Ps	h	Hd	π	k
Adıyaman	7	56	4	0.81±0.13	0.04±0.00	29.52
Aydın	7	198	4	0.71±0.18	0.13± 0.05	94.19
Balıkesir	7	240	2	0.57±0.12	0.19±0.04	137.14
Bursa	7	216	3	0.67± 0.16	0.17±0.03	119.52
Elazığ	7	12	3	0.52±0.20	0.01±0.00	3.81
Gümüşhane	7	174	3	0.67±0.16	0.14±0.03	98.19
Isparta	7	27	3	0.76±0.12	0.02±0.01	13.24
Kahramanmaraş	7	2	2	0.48±0.17	0.00±0.00	0.95
Kayseri	7	49	4	0.71±0.18	0.03±0.01	23.33
Muğla	7	94	4	0.86±0.10	0.07±0.01	52.76
Şanlıurfa	7	275	4	0.81±0.13	0.18±0.06	131.33
Tokat	7	328	5	0.91±0.10	0.26±0.05	185.86
Van	7	269	5	0.86±0.14	0.17± 0.06	122.14
Yozgat	7	75	4	0.81± 0.13	0.04±0.01	31.71

3.2.Genetic Structure

AMOVA analysis of the mtDNA cyt-b gene sequences of rainbow trout showed that genetic variation among populations accounted for 71.1% of the total variation, while genetic variation within populations accounted for 28.9% of the total variation (Table 3). As shown in Table 4, pairwise F_{ST} values, which indicate the degree of genetic differentiation between populations based on the mtDNA cyt-b gene region, ranged from 0.38 to 0.99, with an average F_{ST} value of 0.71. The PCoA results revealed that PC1 (11.62%) distinguished the Kahramanmaraş, Gümüşhane, Tokat, Balıkesir, Şanlıurfa, Aydın, and Muğla populations from those in Kayseri, Bursa, Van, Yozgat, Adıyaman, Elazığ, and Isparta. Meanwhile, PC2 (10.69%) further separated the populations into two groups: Kahramanmaraş, Gümüşhane, Kayseri, and Bursa, and Tokat, Balıkesir, Şanlıurfa, Aydın, Muğla, Van, Yozgat, Adıyaman, Elazığ, and Isparta (Figure 2).

Table 3. AMOVA analysis based on mtDNA cyt-b gene sequences.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
Among populations	13	8716.122	90.52403 Va	71.10	0.0000±0.0000
Within populations	84	3091.429	36.80272 Vb	28.90	
Total	97	11807.551	127.32675		
Fixation index (F_{ST})			0.71096		

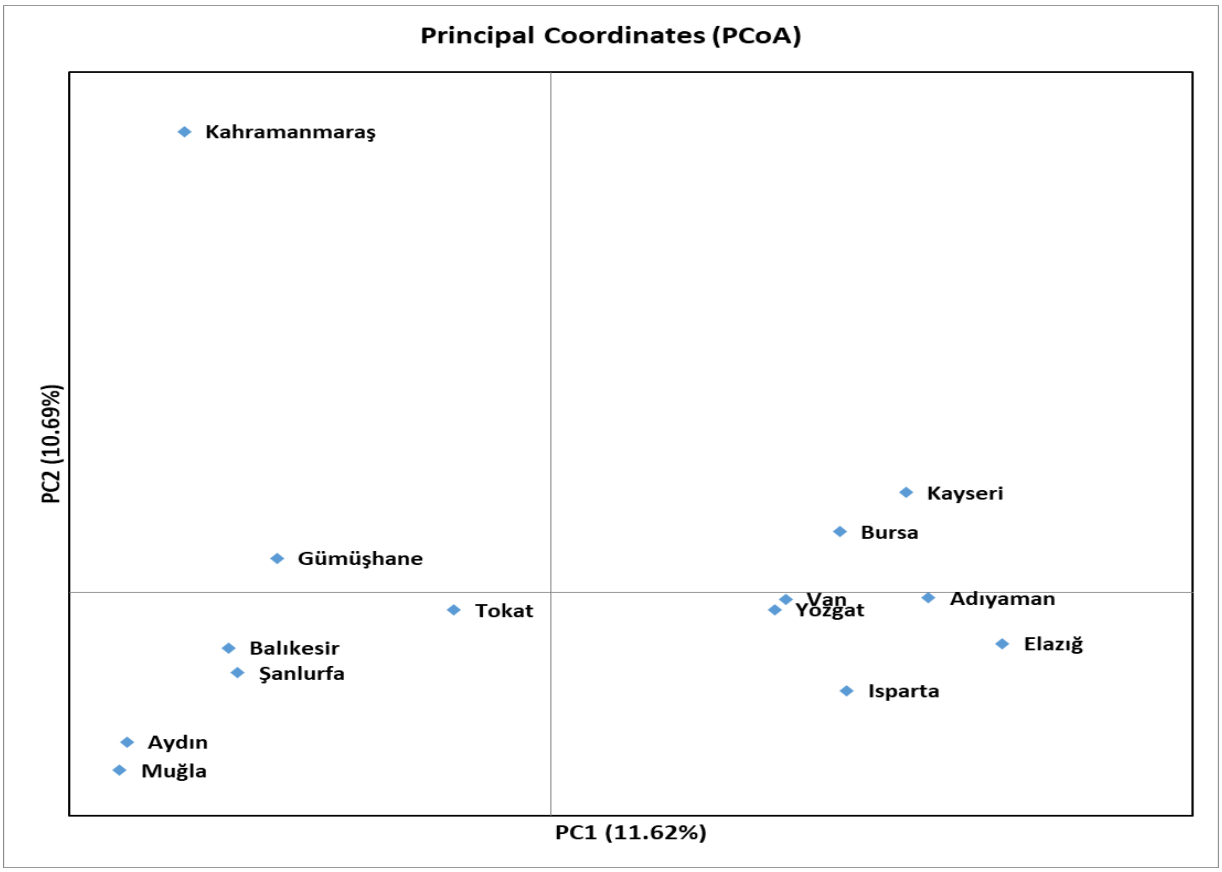

Figure 2. PCoA results are based on F_{ST} values.

Table 4. Pairwise F_{ST} (below diagonal) and p-values (above diagonal) based on the mtDNA cyt-b gene region.

	Adıyaman	Aydın	Balıkesir	Bursa	Elazığ	Gümüşhane	Isparta	Kahramanmaraş	Kayseri	Muğla	Şanlurfa	Tokat	Van	Yozgat
Adıyaman		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Aydın	0.78		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Balikesir	0.70	0.47		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Bursa	0.64	0.65	0.55		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Elazığ	0.92	0.83	0.74	0.70		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Gümüşhane	0.75	0.60	0.41	0.58	0.79		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Isparta	0.87	0.80	0.71	0.71	0.95	0.76		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Kahramanmaraş	0.94	0.83	0.72	0.74	0.99	0.72	0.97		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Kayseri	0.87	0.80	0.71	0.65	0.93	0.75	0.91	0.94		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Muğla	0.84	0.58	0.58	0.69	0.89	0.70	0.87	0.89	0.86		0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
Şanlurfa	0.75	0.53	0.44	0.63	0.78	0.52	0.75	0.78	0.76	0.64		0.00±0.00*	0.00±0.00*	0.00±0.00*
Tokat	0.60	0.44	0.38	0.46	0.65	0.47	0.62	0.63	0.63	0.52	0.46		0.00±0.00*	0.00±0.00*
Van	0.66	0.62	0.55	0.49	0.72	0.61	0.67	0.77	0.70	0.67	0.60	0.44		0.00±0.00*
Yozgat	0.89	0.79	0.73	0.73	0.94	0.80	0.92	0.95	0.90	0.86	0.75	0.64	0.71	

* P <0.05

3.3.Phylogenetic Analysis

The Neighbor-Joining (NJ) dendrogram based on the mtDNA cyt-b gene region revealed that the rainbow trout culture populations clustered into distinct groups. According to the NJ tree, the 14 populations formed two major groups, each further divided into subgroups. In the first group, the Kahramanmaraş population formed its distinct subgroup. Another subgroup consisted of the Tokat, Balıkesir, and Gümüşhane populations, with the Tokat and Balıkesir populations being closely related. In the subgroup containing the Şanlıurfa, Aydın, and Muğla populations, the Aydın and Muğla populations were more closely related to each other. In the second major group, the Elazığ population separated from all other populations to form its group. Another subgroup included the Isparta and Van populations, while a third subgroup consisted of the Bursa, Kayseri, Yozgat, and Adıyaman populations, with Bursa and Kayseri showing a closer relationship. The Maximum Likelihood (ML) dendrogram based on the mtDNA cyt-b gene region demonstrated that the cultured rainbow trout populations clustered into distinct groups. The ML tree revealed that the 14 populations formed two main groups, each further subdivided into subgroups. In the first group, the Kahramanmaraş population was distinctly separated from the others, while Muğla-Aydın and Balıkesir-Tokat populations formed closely related binary subgroups. Şanlıurfa and Gümüşhane populations were also included in this group, though their similarity to the other populations was lower. The second group comprised seven populations divided into two subgroups. The Elazığ population was distinct from all other populations, forming its subgroup. The Bursa-Kayseri populations, which exhibited a very close relationship, were grouped and shared similarities with Yozgat, Adıyaman, Van, and Isparta populations, collectively forming the second subgroup.

The phylogenetic tree, displayed in Figure 3, illustrates the genetic relationships among the studied populations. Bootstrap values from both the NJ and ML methods are shown at each branch as ML/NJ, highlighting the high consistency and reliability of the two methods.

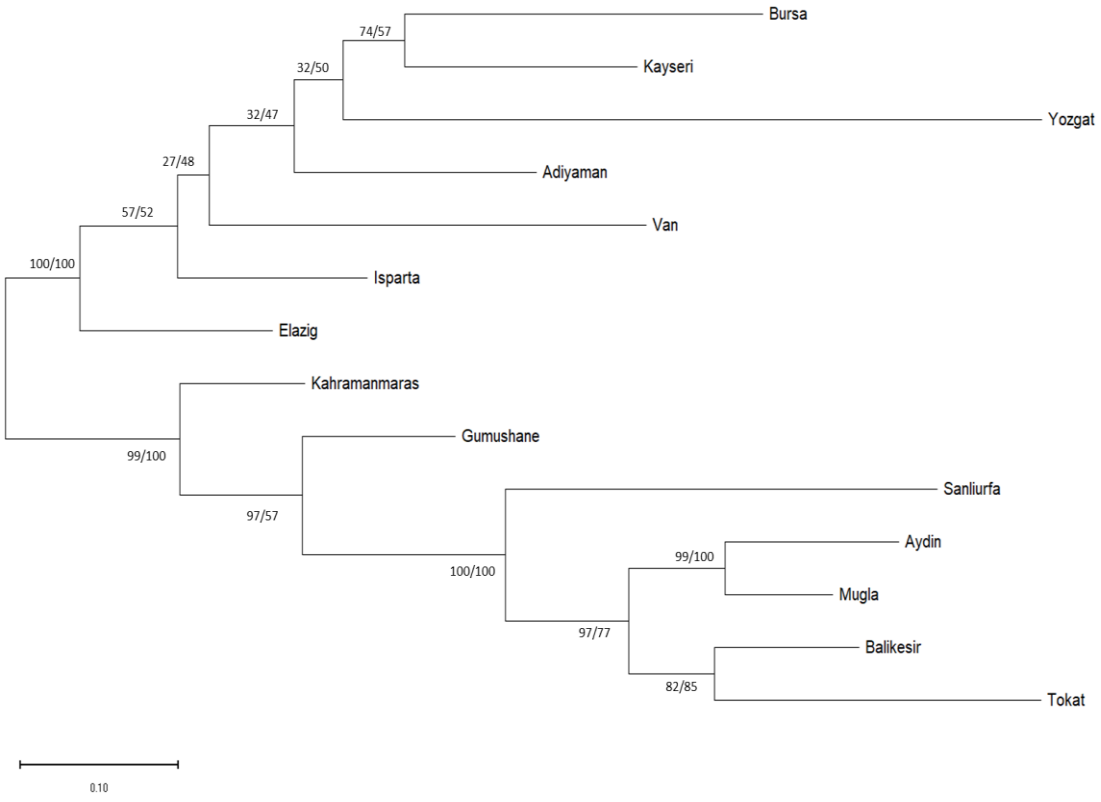

Figure 3. Phylogenetic tree constructed using the Maximum Likelihood (ML) method. Bootstrap values derived from both the ML and Neighbor-Joining (NJ) methods are indicated at each branch as ML/NJ. Branch lengths are proportional to genetic distance, and the scale bar represents substitutions per site.

4.DISCUSSION AND CONCLUSION

Genetic studies are of great importance as they enhance our understanding of genetic diversity and evolution, thereby providing valuable guidance for the conservation and management of genetic resources and the development of breeding programs (Zhang et al., 2018; Fang et al., 2022). The genetic structure of populations essentially defines the total genetic diversity and its distribution within and among a set of populations (Sultana et al., 2022). Molecular markers serve as effective tools for investigating and monitoring the genetic status of populations, including both differentiation and variation (Kumar et al., 2017). In this context, mtDNA is widely used to assess genetic diversity and structure in fish species (Zhang et al., 2018). This study presents a comprehensive analysis of the genetic diversity and structure of rainbow trout (*Oncorhynchus mykiss*) cultured populations from different provinces in Türkiye, using the mtDNA cyt-b gene region. The findings underscore substantial genotypic differences among populations, along with notable variations in nucleotide diversity, haplotype diversity, and the frequency of polymorphic nucleotides. When examining the sequence composition of the mtDNA cyt-b gene region in rainbow trout populations, a higher observed A+T content (51%) compared to G+C content (49%) is consistent with the typical base composition of mtDNA in many fish species, including rainbow trout. The genetic differences among the rainbow trout culture populations, as presented in Table 1, reveal a total of 645 polymorphic nucleotides across 14 different populations. Additionally, the presence of various haplotypes in rainbow trout culture populations was observed. Based on these polymorphic regions, a total of 50 haplotypes were identified. The occurrence of distinct haplotypes within the studied populations indicates the existence of genetic diversity. Moreover, the large number of polymorphic nucleotides serves as a strong indicator of genetic diversity within these populations (Nei, 1987; Whitmore, 1990). Therefore, the presence of distinct haplotypes and a high number of polymorphic nucleotides among rainbow trout culture populations from different provinces in Türkiye suggests the existence of significant genetic diversity.

The study found that haplotype diversity ranged from 0.91±0.10 to 0.48±0.17, while nucleotide diversity varied between 0.26±0.05 and 0.00±0.00 (Table 1). Indeed, previous studies on the genetic diversity of rainbow trout have reported similar findings regarding haplotype and nucleotide diversity. For instance, a study on the genetic structure of rainbow trout farmed in Greece reported haplotype diversity ranging from 0.87±0.04 to 0.59±0.06 and nucleotide diversity from 0.36±0.19 to 0.18±0.10 (Martsikalis et al., 2014). Similarly, Colihueque et al. (2019) estimated the genetic diversity of rainbow trout stocks in Chile, reporting haplotype diversity between 1.00±0.50 and 0.00±0.00, and nucleotide diversity ranging from 0.011±0.00 to 0.00±0.00.

The AMOVA analysis for the mtDNA cyt-b gene sequences in rainbow trout revealed that 71.1% of the total genetic diversity was attributed to variation between populations, while 28.9% of the total diversity was due to within-population variation (Table 3). These results indicate that the primary source of diversity lies between populations. In previous studies, Martsikalis et al. (2014) reported that 5.65% of the genetic diversity in rainbow trout populations farmed in Greece was attributable to between-population differences, while 94.35% was due to within-population variation. Similarly, Colihueque et al. (2019) found that 38.35% of the genetic diversity in rainbow trout stocks in Chile was due to between-population variation, with 61.65% attributed to within-population diversity. Another study examined the population structure and genetic diversity of rainbow trout broodstock in Brazil using SNP markers, revealing that 17.29% of the genetic diversity was attributed to differences between populations, while 86.51% was attributed to variation within populations (de Araújo Júnior et al., 2023). Devaa et al. (2024) reported that 44.30% of the genetic diversity in trout populations in India was due to between-population variation, while 55.70% was due to within-population diversity.

An examination of the F_{ST} values for the 14 rainbow trout culture populations revealed high genetic differentiation between populations (Table 4). The pairwise F_{ST} values based on the mtDNA cyt-b gene region ranged from 0.38 to 0.99, with an average F_{ST} value of 0.71 (Tables 3 and 4). These results suggest a high level of genetic diversity among the studied populations. When compared to previous studies, the F_{ST} values observed in this study differ significantly. In studies on rainbow trout culture populations in Türkiye, Ağdamar (2010) and Oral (2011)

reported F_{ST} values of 0.06±0.18 and 0.06±0.04, respectively. Karataş (2019), in a study of rainbow trout hatcheries in Van, Türkiye, based on 10 mtDNA primers, reported an F_{ST} value of 0.21. In another study, Carcamo et al. (2015) reported an F_{ST} value of 0.222 in rainbow trout populations in Chile. Faccenda et al. (2018) reported F_{ST} values ranging from 0.12 to 0.06 in rainbow trout stocks from Trentino, Italy. Colihueque et al. (2019) reported an F_{ST} value of 0.38 in rainbow trout stocks in Chile. Devaa et al. (2024) found F_{ST} values ranging from 0.59 to 0.22 in trout populations in India. In a study analyzing the genetic differentiation and assignment of commercial rainbow trout strains using a SNP panel, Liu et al. (2017) reported Fst values ranging from 0.056 to 0.195. Another study assessed the population structure and genetic diversity of rainbow trout broodstock in Brazil using SNP markers, reporting an Fst value of 0.172 (de Araújo Júnior et al., 2023). The differences in F_{ST} values observed between this and other studies may be attributed to variations in the molecular markers used or differences in the geographic regions studied. As Glover (2008) noted, it is challenging to directly compare genetic variation across studies that use different marker sets. In comparison to other studies, the pairwise F_{ST} values obtained in this study are significantly higher. However, when considering studies on genetic diversity in other fish species based on the mtDNA cyt-b gene region, similar F_{ST} values have been reported (Habib et al., 2011; Zhu et al., 2016; Kumar et al., 2017; Ha et al., 2020). Thus, it is plausible that the mtDNA cyt-b gene region is conserved in rainbow trout populations.

The Neighbor-Joining (NJ) and Maximum Likelihood (ML) dendrograms based on the mtDNA cyt-b gene region revealed that the rainbow trout populations were grouped into distinct clusters (Figures 3). These dendrograms, constructed using genetic distance data, demonstrated that the populations formed two primary groups, each further subdivided into subgroups, reflecting the genetic relationships among the populations. Similar results were observed in previous studies on rainbow trout culture populations in Türkiye, where Ağdamar (2010) and Oral (2011) also reported the division of populations into two major groups. Additionally, a study conducted in Norway, which compared the genetic characterization of rainbow trout populations, found that the populations were split into two main groups based on the dendrogram (Glover, 2008). Another study by Devaa et al. (2024) on trout populations in India also reported the division of populations into two major groups.

The PCoA results revealed that PC1 (11.62%) and PC2 (10.69%) together explain only 22.31% of the total variation. Although these results account for only a small portion of the genetic variation, it is evident that the Kahramanmaraş population is genetically distinct from the others, forming an independent group. This finding, supported by high F_{ST} values and phylogenetic analyses, indicates that Kahramanmaraş should be considered a separate genetic group.

When the 14 rainbow trout culture populations sampled from different provinces in Türkiye were evaluated according to the aforementioned criteria, the findings indicated that, despite rainbow trout not being a native species in the country, the genetic diversity of these populations is at a good level. In particular, the high levels of haplotype and nucleotide diversity observed in the Van and Tokat populations suggest that these populations may have exchanged fish with other populations or imported new rainbow trout specimens, leading to higher genetic diversity compared to others. The high level of genetic diversity and differentiation among rainbow trout culture populations in Türkiye suggests that these populations have adapted to their local environments due to geographic isolation and varying ecological conditions. The distinct genetic structures observed in populations such as those from Kahramanmaraş and Elazığ highlight the importance of considering local adaptations in conservation and management strategies. The F_{ST} ratio of rainbow trout culture populations suggests that there is genetic differentiation among the populations. However, it is not sufficient to assess rainbow trout culture populations in Türkiye solely based on the high F_{ST} value. A high F_{ST} value does not guarantee that inbreeding issues will not arise in these populations over time. Therefore, to fully understand the situation, all genetic parameters should be evaluated together.

In conclusion, this study, which compared the genetic diversity of 14 rainbow trout culture populations in Türkiye, provided valuable insights into the genetic structure of these populations. One of the challenges in rainbow trout farming is estimating genetic diversity.

Since populations in confined environments like hatcheries are generally smaller, they are more susceptible to genetic variations compared to wild populations. As a result, reductions in genetic variation due to inbreeding and genetic drift are common in cultured populations. The loss of genetic variation corresponds to a loss of genetic potential for stock improvement and adaptation to environmental changes. Therefore, monitoring changes in the genetic structure of cultured populations, relative to wild populations, is essential. In general, the impact of reduced genetic diversity in cultured populations is the loss of adaptive capacity to environmental changes. The results of this study are expected to contribute to better management of production capacities in rainbow trout farms. Indeed, applying genetic diversity models in breeding programs is highly beneficial, providing useful information for improved management practices. It is recommended that rainbow trout breeders exchange fry or import new specimens to enhance genetic diversity and increase the effective population size. Furthermore, future studies covering a broader range of distribution areas with more samples will undoubtedly contribute to rainbow trout aquaculture in Türkiye. In this context, future research should prioritize expanding genetic analyses to incorporate nuclear DNA markers, RAD-seq, SNP analyses, and a broader range of populations, offering a more comprehensive understanding of the genetic diversity and structure of rainbow trout in Türkiye. Long-term monitoring and conservation programs should be implemented to preserve the genetic diversity and integrity of these populations and ensure the sustainability of rainbow trout culture in the region.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Fiction: İB, BK; Literature: İB, BK; Methodology: İB, BK; Performing the experiment: İB, BK; Data analysis: İB, BK; Manuscript writing: İB, BK, Supervision: İB, BK. All authors approved the final draft.

ETHICAL STATEMENTS

This study was conducted with the approval of Animal Experiments Local Ethics Committee of Van Yuzuncu Yıl University (protocol no: 2023/05–07).

DATA AVAILABILITY STATEMENT

The data used in the present study are available upon request from the corresponding author. Data is not available to the public due to privacy or ethical restrictions.

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Research Article **Araştırma Makalesi**

Determination of the Shelf-Life of Cooked Twaite Shad (*Alosa fallax nilotica*) Marinated with Rose, Hawthorn, Pomegranate Vinegars

Gül, Alıç ve Nar Sirkesiyle Marine Edilen Pişirilmiş Tirsi Balığının (*Alosa fallax nilotica)* Raf Ömrünün Belirlenmesi

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Abstract: In this study, it was aimed to evaluate twaite shad (*Alosa fallax nilotica*) as a cooked marinade in order to increase its consumption. The fish were placed in heat and water-resistant bags and boiled in a water bath by immersion method (65 ± 1°C for 35 min) and then 3 different fruit vinegars (pomegranate, rose and hawthorn) were used for marinating. The ripening process was carried out in the refrigerator at 4°C for 24 hours. At the end of the ripening process, the fish were packaged by adding red pepper, black peppercorns and bay leaves in the glass jars filled with olive oil. The prepared cooked shad marinades were stored in the refrigerator at 4°C for microbiological analysis, pH, water activity, sensory changes (odor, taste, color, texture and general evaluation) and identification of microorganisms were performed using API test kits. Lactic acid bacteria, *Lactobacillus plantarum Lactococcus lactis*, and *Pediococcus pentosaceus, and yeast species Rhodotorula mucilaginosa* and *Candida albicans* were detected in cooked fish marinades. *Staphylococcus aureus*, fecal coliform bacteria and *E. coli*, coliform bacteria were not detected in any of the marinade groups during storage. According to the general evaluation of sensory analyses, on the 60th day, the groups prepared with hawthorn, pomegranate and rose vinegars decreased to a value of 4.03±0.54, 4.21±0.53 and 4.68±0.96, respectively. The most acceptable marinade group on this day was the rose vinegar marinade group, while the group prepared with hawthorn vinegar was the least acceptable marinade group. The results of sensory and microbiological analyses were in parallel. According to the statistical analysis results, it was observed that there was a significant difference in microbiological and sensory values depending on storage ($p<0.05$). The shelf-life of cooked twaite shad marinades was determined as 60 days.

Keywords
- Twaite shad
- Cooked marinades
- Different vinegars
- Shelf-life
- Quality control

Özet: Bu çalışmada, tirsi balığının (*Alosa fallax nilotica*) tüketiminin arttırılması amacıyla pişirilmiş marinat olarak değerlendirilmesi hedeflenmiştir. Tirsi balıkları, ısıya ve suya dayanıklı poşetler içerisine konularak su banyosunda daldırma yöntemiyle haşlandıktan (65 ± 1°C'de 35 dk) sonra 3 farklı meyve sirkesi (nar sirkesi, gül sirkesi ve alıç sirkesi) uygulanmıştır. Olgunlaştırma işlemi buzdolabında 4°C'de 24 saat yapılmıştır. Olgunlaştırma işlemi sonunda balıklar cam kavanozlar içerisinde kırmızı pul biber, karabiber ve defne yaprağı ilave edilerek zeytinyağı ile paketlenmiştir. Hazırlanan pişirilmiş tirsi balığı marinatları mikrobiyolojik analiz (Koliform Bakteri Sayımı, Laktik Asit Bakteri Sayımı, Toplam Mezofilik Aerobik Bakteri Sayımı, *Staphylococcus aureus* Sayımı, Küf-Maya Sayımı, Toplam Psikrofilik Bakteri Sayımı, Fekal Koliform Bakteri ve *E. coli* Sayımı), pH, su aktivitesi, duyusal değişimler (koku, tat, renk, doku ve genel değerlendirilmesi) dışında bozulmaya neden olan bakterilerin tanımlanması amacıyla 4°C'de buzdolabında depolanmıştır. Bakteri

Anahtar kelimeler
- Tirsi balığı
- Pişirilmiş marinatlar
- Farklı sirkeler
- Raf ömrü
- Kalite kontrol

tanımlamaları API test kitleri kullanılarak gerçekleştirilmiştir. Pişirilmiş balık marinatlarında *Lactobacillus plantarum, Lactococcus lactis* ve *Pediococcus pentosaceus* laktik asit bakterileri ile *Rhodotorula mucilaginosa* and *Candida albicans* maya türleri saptanmıştır. Depolama boyunca hiçbir marinat grubunda *Staphylococcus aureus,* fekal koliform bakteri ve *E. coli,* koliform bakteri saptanmamıştır. Duyusal analizlerin genel değerlendirmesine göre 60.günde alıç sirkesi ile hazırlanan grup 4,03±0,54, nar sirkesi ile hazırlanan grup 4,21±0,53, gül sirkesi ile hazırlanan grup 4,68±0,96 değerine düşmüştür. Genel değerlendirme sonuçlarına göre, 60. gün en kabul edilebilir gül sirkesi ile hazırlanan marinat grubu iken alıç sirkesi ile hazırlanan grup en düşük kabul edilebilen marinat grubu olmuştur. Ürünler duyusal açıdan ve mikrobiyolojik olarak 60. günde reddedilmiştir. Duyusal analizler ve mikrobiyolojik analiz sonuçları paralellik göstermektedir. Mikrobiyolojik ve duyusal değerler istatistiksel analiz sonuçlarına göre depolamaya bağlı olarak önemli farklılık olduğu gözlemlenmiştir ($p<0,05$). Pişirilmiş tirsi balıkları marinatlarının raf ömrü 60 gün olarak belirlenmiştir.

1.INTRODUCTION

Fish are essential food sources of functional lipids and protein, and heat treatment can provide various benefits, including better sensory properties, nutritional quality, improved preservation, flavor, and the ability to digest. Nonetheless preparing foods at high temperatures may also adversely impact proteins and lipids regarding thermal degradation reactions and nutritional values (Manful et al., 2020). For this reason, improving the sensory quality of fish meat is critical for increasing fish intake (Öz and Uçak, 2023). With the increasing consumer awareness and desire for healthy food and natural ingredients, there is an urgent need to develop and supply novel products in the market (Boutheina et al., 2023). In recent years, growing consumer awareness about health has encouraged the food industry to adopt healthier cooking methods, especially those that minimize the formation of heat-induced harmful chemicals, such as air frying and boiling (Khan et al., 2024). In addition to this, ripening, smoking, and/or marinating are examples of technologies that can increase food quality and enhance sensory properties. Marinating is an old culinary method that involves soaking, rubbing, or injecting items, mainly meat and fish, with a marinating solution or liquid such as vinegar, wine, organic acids, lemon juice, brine, soy sauce, essential oils, herbs, and spices (Kılınç, 2003, Gargi and Sengun, 2021). The quantity of marinade accumulated over time and the consistency of its distribution in meat are major elements influencing the quality of marinated meat products (Shi et al., 2023). The word "marinated fish" or "pickles" refers to fish items manufactured from fresh, frozen, or salted fish or fish parts that have been processed with an edible organic acid, typically acetic acid and salt, before being placed in brine, sauces or oil (Sallam et al., 2007). Fish are marinated in salt and acetic acid. During the marinating process, distinct nitrogen components diffuse from the fish meat into the brine (Szymczak and Lepczynski, 2016). Nitrogen losses from meat to brine not only deteriorate the quality, yield, and nutritional value of a food product, but also represent a source of pollution for the liquid environment (Szymczak et al., 2015). On the other hand, this technique not only adds value to the marinated items, but also enhances or amplifies flavor, aroma, and/or color, improves texture, and helps in (bio) preservation (Zhang et al., 2022). While the marinating procedure lowers the pH, it also increases the quality, safety, and stability of food products (Cavalcanti et al., 2023). Marinades and pickles are frequently preferred to extend the shelf-life of fish products. In addition to this, marinating is known to have a significant impact on the microbiome composition of the meats. It is reported that marinating the fish has raised the concentration of lactic acid bacteria and effectively concealed spoilage odors, although freshness scores fell (Jaaskelainen et al., 2023). Typically, two types of marinades (made from raw or lightly boiled/cooked fish) are manufactured and preferred for consumption (Armani et al., 2012). Acid, salt, sugar, and other ingredients are used for cold marinades. They are semi-preserved products that include solutes and are not heat treated. It regulates the activity of microorganisms and adds to the organoleptic qualities of the finished product (Boziaris et al., 2013). However, marinades have limited shelf-life (Kılınç and Çaklı, 2005). In the seafood sector, marinating has been usually used for a large population of pelagic fish such as sardines, herring, mackerel, anchovies, and bonito (Fuselli et al., 1994). Clupeidae is one of the most

commercially important fish families in the world. Within Clupeidae, the genus Alosa (subfamily Alosinae), the type of *Alosa fallax* has been found in Europe and twaite shad *Alosa fallax nilotica,* (Geoffroy Saint-Hilaire, 1808) in Mediterranean and Aegean Sea (Balık, 1995; Faria et al., 2006; Altinelataman et al., 2009). Twaite shad from the family Clupeidae is a pelagic species and although they live in the seas, some forms of streams and they have adapted to freshwater lakes. While the production of twaite shad (*Alosa fallax nilotica*) was reported to be 1,642 tons in Türkiye in 2017, it reached 3,065 tons in 2021 with an increase rate of 86.66%. Protection of a shad by the following such a large amount of production is becoming very important (Anonymous, 2022). The catching amount of shad was indicated as 1928,9 tons in 2023 in Türkiye (TUİK, 2024).

Although there have been a large number of studies on marinades (Kılınç and Çaklı, 2004; Kılınç and Çaklı, 2005, Bilgin et al., 2011; Moon et al., 2017; Testa et al., 2019; Babikova et al., 2020; Kaminski et al., 2022; Boutheina et al., 2023; Kılınç et al., 2023; Wang et al., 2024), there have been a limited number of studies about twaite shad (Balık, 1995; Faria et al., 2006; Taşovo et al., 2022) as well as twaite shad marinades (Erdem et al., 2015). Additionally, there is no study about the identification of the microbiological flora of the cooked marinated twaite shad. Therefore, the purpose of this study was to investigate the shelf-life of cooked twaite shad marinated with different vinegars (rose, hawthorn, pomegranate) as well as to define the microbiological flora of cooked twaite shad marinades.

2.MATERIAL AND METHODS

Raw material consisted of a 25 kg fillet-shaped twaite shad fish (*Alosa fallax nilotica*, Geoffroy Saint-Hilaire,1808) from the Clupeidae family, weighing 36.09 ± 1.0 g and measuring 20 ± 1.0 cm. Figure 1 depicts a sample of cleaned filleted twaite shad fish utilized in this study.

Figure 1. Filleted Twaite Shad Fish (*Alosa fallax nilotica*, Geoffroy Saint-Hilaire,1808).

2.1.Preparation and Storage of Samples

Fish fillets were transported to the Processing Laboratory of Ege University, Faculty of Fisheries, within 30-40 minutes under cold chain conditions in a styrofoam box filled with ice (fish: ice ratio 1:1), provided by Sevda Balık. The fillets were rinsed in ice water for approximately 1-2 minutes to remove blood residues. After rinsing, the fillets were immersed in a water bath (Memmert, Germany) and cooked at 65°C ± 1 for 35 minutes. Once cooked, they were placed in heat-resistant and water-resistant bags, each containing 1 kilogram of fillets. The boiled fish fillets were divided into three groups and marinated with different types of vinegar: pomegranate(P), rose (R), and hawthorn (H) vinegars (Ozem'le Yaşam, Türkiye). A ripening solution containing 5% vinegar and 10% salt was prepared to fully submerge the fish (fish: solution ratio 1:2). The fillets were marinated in this solution for 24 hours in a refrigerator at 4 ± 1°C in plastic containers.

Figure 2. Types of vinegar used for the marination.

After marination, the fish were drained and placed in 250 ml sterile jars prepared under aseptic conditions. Each jar was coated with olive oil to prevent air contact (fish: olive oil ratio 1:1) and included two bay leaves, three black peppercorns, and 10 grams of red pepper flakes. The jars were stored at 4°C, and the samples were analyzed for pH, water activity, microbiological characteristics, and sensory properties to assess changes during storage. Figures 2, 3, and 4 illustrate the vinegars used, the aseptic jar preparation process, and the processing stages, respectively.

Figure 3. Cooked twaite shad marinades.

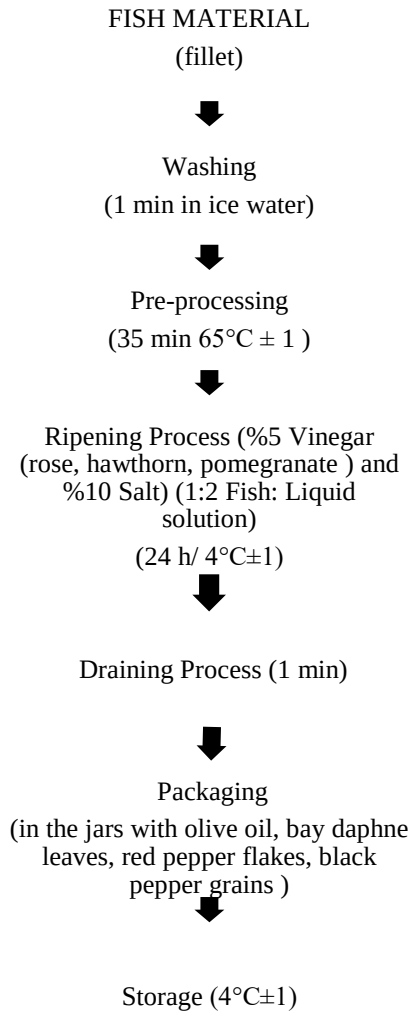

Figure 4. The Processing Stages of Cooked Twaite Shad Marinades.

2.2.The Methods of Analysis

Water activity, pH, microbiological (total mesophilic aerobic bacteria: TMAB, total psychrotrophic aerobic bacteria: TPAB, lactic acid bacteria: LAB, *Staphylococcus aureus*: SA, mold-yeast: MY, coliform bacteria: CB, fecal coliform bacteria: FCB, and *Escherichia coli*: EC) counts in the raw material and marinated groups, as well as sensory analyses, were performed on days 0, 1, 15, 30, 60, and 90. API Test Kits were used to identify microorganisms responsible for deterioration during storage.

2.2.1.pH Analyses

A HANNA model pH meter (HI 2211 pH/ORP Meter) was used to measure pH in raw material, boiled, and marinated product groups. The measurement was carried out after 10 g of samples were homogenized with 10 mL of distilled water. The measurements were repeated three times. The pH analysis was performed using the Bongiorno et al. (2018) method.

2.2.2.Water Activity

A device (Testo, Germany) was used to measure the water activity of all raw, boiled, and marinated product categories. 5g of the separated samples were placed in a measuring cup and measured for 1 minute. The measurements were repeated three times (Anonymous 2, 2004).

2.2.3.Microbiological Analysis

Microbiological analysis of raw materials, boiling products, and marinated products were performed under aseptic circumstances. In each group, 10g of fish meat were placed in sterile bags under aseptic conditions. 90 ml of peptone water was added to the sterile bags and homogenized using the Stomacher equipment (IUL, Barcelona, Spain). 1 ml of homogenous solutions were combined with 9 ml of peptone water. Other dilutions were prepared using a (10^{-1}) stock dilution. The prepared dilution tubes were homogenized using a vortex tube mixer. After that, other decimal dilutions were generated from the prepared dilutions, and appropriate media was utilized for each microorganism's growth following inoculation. The inoculated 3M petrifilms were incubated at appropriate temperatures to allow microorganisms to develop. Each group had three microbiological analyses. The data obtained from the analysis counts were reported as log cfu/g (Harrigan and McCance, 1976).

2.2.3.1.Total Mesophilic Aerobic Bacteria Count

The prepared dilutions were inoculated on 3M Petrifilm Aerobic Counting Plates (1 ml) and incubated at 30˚C for 24-48 hours. The total mesophilic aerobic bacteria count of the samples was then quantified using the method described by Anonymous 1(2022). The red colonies developed following incubation were identified and counted. The data were computed using log cfu/g.

2.2.3.2.Total Psychrotrophic Aerobic Bacteria Count

The total psychrotrophic aerobic bacteria count was conducted using the method of (Anonymous 1, 2022). 1 ml of inoculated petrifilms were cultured for 10 days at 7˚C (Anonymous 1, 2022). The total number of TPAB was calculated as log cfu/g using red colony counts on 3M petrifilm plates (Harrigan and McCance, 1976).

2.2.3.3. Coliform Bacteria Count

3M Petrifilm Coliform Counting Plates were used to count coliform bacteria using the method of (Anonymous 3, 2022). Inoculated (1 ml) 3M petrifilm coliform counting plates were incubated for 24 hours at 30°C (ICMSF, 1986a). Coliform bacteria counts were determined by counting the red-colored gaseous and non-gaseous colonies that formed following incubation. The results were expressed as log cfu/g.

2.2.3.4. Mold- Yeast Counts

3M Petrifilm Mold (Yeast) Mold-yeast counting was performed using counting plates, as specified in the technique (Anonymous 4, 2022). 1 ml of the produced dilutions were added to the 3M petrifilm plates. The inoculated 3M petrifilms were incubated in an incubator at 25°C for 3-5 days to determine the mold-yeast count (Anonymous 8, 2000). After incubation, the blue-green colonies were identified as MYC. The results were expressed as log cfu/g.

2.2.3.5. Fecal Coliform Bacteria and *E. coli* Counts

The fecal coliform bacteria (FCB) and *E. coli* (EC) counts were determined using 3M Petrifilm *E. coli*/Coliform. The analysis was conducted using the method of (Anonymous 5, 2022). 1 ml of the produced dilutions were added to the 3M petrifilm plates. The inoculated 3M petrifilm plates were incubated for 48 hours at 44-45°C, following the procedure of (Mossel and Moreno, 1985). After incubation, colonies containing blue and gas were identified as EC, while red colonies were identified as FCB. The data were computed using log cfu/g.

2.2.3.6. *Staphylococcus aureus* Bacteria Count

The *Staphylococcus aureus* count (SA) was conducted using 3M Petrifilm plates following the method of (Anonymous 7, 2022). Each prepared dilution (1 ml) was then inoculated onto 3M petrifilm plates. Following that, the inoculated 3M petrifilm plates were incubated for 30 hours at 37°C in an incubator (EN500, Nevu, Ankara, Türkiye). SA colonies were defined as those that were red-purple following incubation. The data were computed as log cfu/g using the approach of (Mossel and Moreno, 1985).

2.2.3.7. Lactic Acid Bacterial Count

Lactic acid bacteria counts (LABC) were determined using 3M Petrifilm Lactic Acid Bacteria Counting Plates, as described by Anonymous (6, 2022). 1 ml of produced dilutions were seeded onto 3M petrifilm plates and incubated at 28-37°C for 48 hours ± 3 hours (Baumgart et al., 1986; Kılınç et al.,2022). LABC were defined as colonies that formed after incubation and contained gas or were red. The data were computed using log cfu/g.

2.2.4.Sensory Analysis

The sensory evaluation of the marinades was evaluated by 8 panelists ranging in age from 25 to 50 from Ege University's Faculty of Fisheries who are familiar with fish marinades and marinade goods for sensory analysis. Products

were presented to panelists once a month to assess sensory features such as texture, taste, odour, color rating, and overall acceptance. The sensory analysis form supplied to the panelists was scored on a scale of 1 to 9, with 9 being 'very good', 6.9 to 4.1 being 'good', 4 being 'tolerable' (4 being the rejection line), and 1 to 3.9 being 'unacceptable'. The sensory analysis form is given in Table 1. The sensory analysis forms were modified to follow the procedures of Varlık et al. (1993) and Erdem et al. (2015).

Table 1. The Sensory Analysis Form Used for Determining The Sensory Characteristics of Cooked Marinades.

Name:	**Analysis Day:**		**Date:**
Sensory	**Group code:**	**Group code:**	**Group code:**
Odour			
Taste			
Colour			
Texture			
General Evaluation			

9-7: very good 6,9- 4,1: good 4: 'tolerable' (4 being the rejection line) 3,9 – 1: unacceptability.

2.2.5.The Identification of Microorganisms

2.2.5.1.The Identification of Yeast (API 20 C AUX)

Yeast identification was conducted using API C AUX test kits (Biomérieux, 20 210, France). These kits include 20 cubes of dehydrated substrates that illustrate the results of 19 assimilation tests. The first identified yeast colony was placed in API Suspension Medium (2ml), and a suspension with turbidity equal to 2 McFarland was created. The suspension was put to the API C Medium ampoule with 100 µl. The resultant suspension was injected into the cubes following the user instructions. The kits were incubated at 29°C ± 2°C for 48-72 hours (± 6 hours). The cubes that generated turbidity after incubation were rated positively. The variations in the strip caused by the presence or absence of turbidity in the cubes were determined using a computer-based identification algorithm (Biomérieux, 2005).

2.2.5.2.Identification of lactic acid bacteria (API 50 CHL, API 50 CH)

API CHL Medium (Biomérieux, 50410, France) and the API 50 CH (Biomérieux, 50 300, France) strip were used to identify lactic acid bacteria. API 50 CH test kits contain 50 microtubules. API 50 CHL Medium is a ready-made medium that aids in the fermentation of 49 carbs found in the strip. First, a dense suspension was created by placing the bacterial colony to be detected in API Suspension Medium (2ml). The prepared dense suspension was mixed with API Suspension Medium (5ml) until it reached a turbidity corresponding to 2 McFarland, and the number of drops was recorded. Up to twice as many drops were transferred to API 50 CHL Medium. The homogeneous suspension was inoculated into the API 50 CH test kit as instructed in the user handbook and incubated at 29°C±2°C for 24-48 hours (±6 hours). The alterations that occur in the strip after incubation were determined using a computer identification software (Biomérieux, 2011).

2.2.6.Statistical Analysis

The variations between sensory alterations and microbiological load changes during preservation of marinades made with three distinct vinegar kinds were statistically assessed. All statistical analyses were conducted using the EXCEL program and the SPSS 27.0.1.0 package program. The Skewness and Kurtosis tests were employed to ensure that the data followed the normal distribution. A one-way analysis of variance (ANOVA) was used to evaluate whether there was a statistically significant difference between the days of storage. To see if there was a difference between the groups, one of the post hoc tests was performed with Tukey. All statistical studies followed the procedures outlined by Akdağ (2021) and Yücel (2022).

3.RESULTS

Table 2 shows the (TMABC) of control, boiled and marinated twaite shad with various fruit vinegars (hawthorn, rose, and pomegranate vinegar). TMABC levels were <1 log cfu/g in all marinade groups during on days 0 and 1 of storage. On day 15, TMABC in marinades with hawthorn vinegar was 3.85±0.05 log cfu/g. By

day 30, it had increased to 4.96±0.01 log cfu/g, and by day 60, it was 7.22±0.03 log cfu/g. TMABC levels, in marinades with rose vinegar, were 3.52±0.33 log cfu/g, 4.90±0.05 log cfu/g, and 7.19±0.06 log cfu/g on days 15, 30, and 60, respectively. TMABC levels, in marinades with pomegranate vinegar, were 3.4±0.04 log cfu/g, 4.44±0.04 log cfu/g, and 7.23±0.13 log cfu/g on days 15, 30, and 60. The study indicated that TMABC in fresh fish was 3.41±0.19 log cfu/g, but boiling fish had a detectable level of less than 1 log cfu/g. However, all marinade groups were determined more than the limit of 1.0 x 10^6 cfu/g (6.0 log cfu/g) according to the ICMSF (1986) on the 60-day storage period.

Table 2. The Total Mesophilic Aerobic Bacteria Counts (TMABC) of All Groups of Twaite Shad (log cfu/g).

	Storage Day	TMABC
Control		3.41±0.19
Boiled		< 1
Marinated Groups		
Hawthorn	0	$< 1^{A,a}$
	1	$< 1^{A,a}$
	15	$3.85\pm0.05^{B,a}$
	30	$4.96\pm0.01^{C,b}$
	60	$7.22\pm0.03^{D,a}$
	90	$9.72\pm0.05^{E,b}$
Rose	0	$< 1^{A,a}$
	1	$< 1^{A,a}$
	15	$3.52\pm0.33^{B,a}$
	30	$4.90\pm0.05^{C,b}$
	60	$7.19\pm0.06^{D,a}$
	90	$8.06\pm0.03^{E,a}$
Pomegranate	0	$< 1^{A,a}$
	1	$< 1^{A,a}$
	15	$3.49\pm0.04^{B,a}$
	30	$4.44\pm0.04^{C,a}$
	60	$7.23\pm0.13^{D,a}$
	90	$8.01\pm0.07^{E,a}$

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 3 shows the TPABC in all groups of fish that were cooked and marinated with various fruit vinegars (hawthorn, rose, and pomegranate). The TPABC level in raw fish was 2.57±0.08 log cfu/g, while in boiling fish it was less than 1 log cfu/g, making it undetectable. On day 90 of storage, the number of TPAB in marinade groups increased to 7.9±0.24 log cfu/g in pomegranate vinegar, 7.66±0.11 log cfu/g in hawthorn vinegar, and 7.60±0.04 log cfu/g in rose vinegar.

Table 3. The Total Psychrotrophilic Aerobic Bacteria Counts (TPABC) of all Groups of Twaite Shad (log cfu/g)

	Storage Day	TPABC
Control		2.57±0.08
Boiled		< 1
Marinated Group		
Hawthorn	0	$< 1^{A,a}$
	1	$< 1^{A,a}$
	15	$3.32\pm0.02^{B,a}$
	30	$4.28\pm0.02^{C,a}$
	60	$7.04\pm0.14^{D,a}$
	90	$7.66\pm0.11^{E,a}$
Rose	0	$< 1^{A,a}$
	1	$< 1^{A,a}$
	15	$3.53\pm0.03^{B,b}$
	30	$3.67\pm0.04^{C,b}$
	60	$6.88\pm0.07^{D,a}$
	90	$7.60\pm0.04^{E,a}$
Pomegranate	0	$<1^{A,a}$
	1	$< 1^{A,a}$
	15	$3.32\pm0.05^{B,a}$
	30	$4.25\pm0.05^{C,a}$
	60	$7.07\pm0.23^{D,a}$
	90	$7.90\pm0.24^{E,a}$

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 4 displays the lactic acid bacteria counts (LABC) of all groups of fish that were cooked and marinated with various fruit vinegars (hawthorn, rose, and pomegranate). On day 0 and 1, the LABC of all marinating groups was below detectable levels (<1 log cfu/g). Marinades prepared with hawthorn vinegar had LABC of 2.18±0.07 log cfu/g, 2.48±0.04 log cfu/g, 2.62±0.04 log cfu/g, and 3.93±0.04 log cfu/g on storage days 15, 30, 60, and 90. Additionally, marinades prepared with rose vinegar had LABC of 2.29±0.13 log cfu/g, 2.48±0.03 log cfu/g, 3.61±0.21 log cfu/g, and 3.91±0.05 log cfu/g on these storage days. Moreover, LABC of marinades with pomegranate vinegar were determined to be 2.60±0.06 log cfu/g, 2.90±0.04 log cfu/g, 4.51±0.08 log cfu/g, and 4.99±0.08 log cfu/g on days 15, 30, 60, and 90, respectively.

Table 4. The Lactic Acid Bacteria Counts (LABC) of All Groups of Twaite Shad (log cfu/g).

	Storage Day	LABC
Control		1.91±0.19
Boiled		< 1
Marinated Group		
Hawthorn	0	< 1 ^{A,a}
	1	< 1 ^{A,a}
	15	2.18±0.07 ^{B,a}
	30	2.48±0.04 ^{C,a}
	60	2.62±0.04 ^{D,a}
	90	3.93±0.04 ^{E,a}
Rose	0	< 1 ^{A,a}
	1	< 1 ^{A,a}
	15	2.29±0.13 ^{B,a}
	30	2.48±0.03 ^{B,a}
	60	3.61±0.21 ^{C,b}
	90	3.91±0.05 ^{C,b}
Pomegranate	0	< 1 ^{A,a}
	1	< 1 ^{A,a}
	15	2.60±0.06 ^{B,b}
	30	2.90±0.04 ^{C,b}
	60	4.51±0.08 ^{D,c}
	90	4.99±0.08 ^{E,c}

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 5 shows Mold-Yeast Counts (MYC) of boiled and marinated fruit vinegars (hawthorn, rose, and pomegranate vinegar). The MYC levels in both fresh and boiling fish were found to be below detectable levels (<1 log cfu/g). After 90 days of storage, MYC levels reached to 3.81±0.06 log cfu/g in hawthorn vinegar marinade, 3.54±0.04 log cfu/g in rose vinegar marinade and 2.81±0.12 log cfu/g in pomegranate vinegar marinade. *S. aureus*, coliform, fecal coliform, and *E. coli* were not found in all marinated groups.

Table 5. The Mold-Yeast Counts (MYC) of all Groups of Twaite Shad (log cfu/g).

	Storage Day	MYC
Control		< 1
Boiled		< 1
Marinated Group		
Hawthorn	0	< 1 ^{A,a}
	1	< 1 ^{A,a}
	15	1.94±0.19 ^{B,b}
	30	2.44±0.04 ^{C,a}
	60	3.66±0.06 ^{D,a}
	90	3.81±0.04 ^{D,a}
Rose	0	< 1 ^{A,a}
	1	< 1 ^{A,a}
	15	< 1 ^{A,a}
	30	2.49±0.06 ^{C,a}
	60	2.80±0.26 ^{C,b}
	90	3.54±0.03 ^{D,b}
Pomegranate	0	< 1 ^{A,a}
	1	< 1 ^{A,a}
	15	< 1 ^{A,a}
	30	< 1 ^{A,b}
	60	2.65±0.18 ^{B,b}
	90	2.83±0.09 ^{B,c}

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 6 shows the variations in pH values of marinades throughout storage. The pH values for raw fish were 6.37±0.10, while boiling fish had a pH of 6.67±0.04. The pH variations in marinades produced with hawthorn vinegar were found to be 5.81±0.03, 4.94±0.02, 4.62±0.05, 4.54±0.03, 5.09±0.08, and 5.47±0.01 on days 0, 1, 15, 30, 60, and 90, respectively. In addition, the pH variations in marinades produced with rose vinegar were determined as 5.70±0.11, 5.54±0.04, 5.03±0.08, 5.12±0.11, 5.36±0.05, and 5.51±0.03 on days 0,1,15,30,60, and 90, respectively. Pomegranate vinegar marinades showed pH variations of 5.42±0.15, 5.26±0.10, 4.10±0.01, 4.56±0.06, 5.21±0.06, and 5.36±0.05 on days 0, 1, 15, 30, 60, and 90, respectively. While the pH lowered with the vinegar employed in the marinating procedure, it increased with the storage period.

Table 6. The pH values of all Groups of Twaite Shad.

	Storage Day	pH
Control		6.37±0.10
Boiled		6.67±0.04
Hawthorn	0	5.81±0.03 ^{A,a}
	1	4.94±0.02 ^{B,a}
	15	4.62±0.05 ^{C,a}
	30	4.54±0.03 ^{C,a}
	60	5.09±0.08 ^{D,a}
	90	5.47±0.01 ^{E,a}
Rose	0	5.70±0.11 ^{A,a}
	1	5.54±0.04 ^{A,C,b}
	15	5.03±0.08 ^{B,b}
	30	5.12±0.11 ^{B,b}
	60	5.36±0.05 ^{C,b}
	90	5.51±0.03 ^{C,A,a}
Pomegranate	0	5.42±0.15 ^{A,b}
	1	5.26±0.10 ^{A,c}
	15	4.10±0.01 ^{B,c}
	30	4.56±0.06 ^{C,a}
	60	5.21±0.06 ^{A,a,b}
	90	5.36±0.05 ^{A,b}

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 7 shows the changes in water activity of marinades throughout storage. Water activity changes in marinades prepared with hawthorn vinegar were determined as 0.703±0.01, 0.677±0.00, 0.681±0.02, 0.714±0.01, 0.734±0.01, and 0.747±0.01, whereas water activity changes in marinades prepared with rose vinegar were found as 0.708±0.01, 0.698±0.01, 0.685±0.01, 0.685±0.02, 0.724±0.01, and 0.740±0.01 on days 0, 1, 15, 30, 60, and 90. Additionally, water activity variations in marinades produced with pomegranate vinegar were observed as 0.700±0.00, 0.697±0.00, 0.694±0.01, 0.706±0.01, 0.719±0.01, and 0.731±0.01 on days 0, 1, 15, 30, 60, and 90.

Table 7. The Changes in Water Activity of all Groups of Twaite Shad.

	Storage Day	Water Activity
Control		0.794±0.02
Boiled		0.779±0.00
Marinated Group		
Hawthorn	0	0.703±0.01^{A,B,a}
	1	0.677±0.00 ^{A,a}
	15	0.681±0.02 ^{A,a}
	30	0.714±0.01 ^{B,a}
	60	0.734±0.01 ^{B,C,a}
	90	0.747±0.01 ^{C,a}
Rose	0	0.708±0.01 ^{A,B,C,a}
	1	0.698±0.01 ^{A,b}
	15	0.685±0.01 ^{A,B,a}
	30	0.685±0.02 ^{B,C,b}
	60	0.724±0.01 ^{C,D,a}
	90	0.740±0.01 ^{D,a}
Pomegranate	0	0.700±0.00 ^{A,a}
	1	0.697±0.00 ^{A,b}
	15	0.694±0.01 ^{A,a}
	30	0.706±0.01 ^{A,B,b,a}
	60	0.719±0.01 ^{B,C,a}
	90	0.731±0.01 ^{C,a}

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 8 shows the odour results from the sensory investigation of fish marinades. A decrease in odor ratings of marinated goods using various vinegars was seen according to the increasing storage period. On day 90, the odour values of the hawthorn vinegar group decreased from 7.87 ± 0.83 to 2.18 ± 0.75, while those of the pomegranate vinegar group decreased from 8.00 +1.07 to 2.60 ± 0.40. In the rose vinegar group, marinade odor levels decreased from 7.62 ±1.68 to 2.55 ±0.68 at the end of storage. Table 9 shows the taste findings for the fish marinades throughout storage throughout the study. According to the findings, the marinade group created with rose vinegar was the most acceptable, while the group prepared with hawthorn vinegar received the least acceptance. On day 60, all groups remained below the acceptability limit value (4.00) when tested for taste. Table 10 shows the colour results of fish marinades during storage, whereas Table 11 shows the texture discoveries. Table 12 also shows the general evaluation findings for fish marinades. The general evaluation findings likewise reduced as the number of days of storage increased. The readings of the group prepared with hawthorn vinegar reduced from 7.62±0.51 to 2.72±0.79, falling below the acceptable level (4.0). Additionally, the group cooked with pomegranate vinegar had a range of 7.37±0.91 to 2.87±0.35. Moreover, the group produced with rose vinegar was reduced from 7.25±1.48 to 2.62±0.94, and all the groups identified as falling below the acceptable value (4.0). Fish marinade groups, which were boiled and marinated with various fruit vinegars, were evaluated based on taste, odor, color, texture, and overall assessment factors. The investigation discovered that the sensory outcomes of marinades marinated with various vinegars were consistent with the microbiological results. The shelf-life of all marinades was confirmed to be 60 days.

Table 8. The Odour Results of Cooked Marinated Twaite Shad.

Odour	Day 0	Day 1	Day 15	Day 30	Day 60	Day 90
H	7.87±0.83^{a,A}	5.75±1.90^{a,B}	6.13±0.35^{b,B}	4.25±0.70^{a,C}	3.81±0.65^{a,C}	2.18±0.75^{a,D}
P	8,00 ± 1.07^{a,A}	7.50±0.75^{b,A}	6.75±0.46^{b,A}	5.18±1.31^{ab,B}	3.77±0.99^{a,C}	2.60±0.40^{a,C}
R	7.62 ±1.68^{a,A}	7.12±1.12^{ab,AB}	5.25±0.92^{b,C}	5.75±1.41^{ab,BC}	4.15±0.83^{a,CD}	2.55±0.68^{a,D}

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 9. The Taste Results of Cooked Marinated Twaite Shad.

Taste	Day 0	Day 1	Day 15	Day 30	Day 60	Day 90
H	$7.00\pm0.75^{a,A}$	$6.68\pm1.62^{a,AB}$	$5.62\pm0.44^{a,B}$	$4.26\pm0.7^{a,C}$	$3.53\pm0.75^{a,C}$	-
P	$7.37\pm0.74^{a,A}$	$7.56\pm0.72^{a,A}$	$6.68\pm0.45^{b,A}$	$5.31\pm0.65^{b,B}$	$3.31\pm0.7^{a,C}$	-
R	$7.50\pm1.30^{a,A}$	$7.00\pm1.51^{a,AB}$	$5.45\pm0.72^{a,B}$	$5.72\pm0.98^{b,B}$	$3.76\pm1.05^{a,C}$	-

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 10. The Colour Results of Cooked Marinated Twaite Shad.

Colour	Day 0	Day 1	Day 15	Day 30	Day 60	Day 90
H	$7.87\pm0.35^{a,A}$	$7.46\pm0.79^{a,AB}$	$6.93\pm0.41^{a,B}$	$5.87\pm0.44^{a,C}$	$4.81\pm0.37^{a,D}$	$3.33\pm0.55^{a,E}$
P	$7.37\pm0.91^{a,A}$	$8.12\pm0.64^{a,A}$	$7.87\pm0.35^{b,A}$	$6.18\pm0.53^{a,B}$	$5.01\pm0.75^{a,C}$	$3.46\pm0.39^{a,D}$
R	$7.37\pm1.50^{a,A}$	$7.37\pm0.91^{a,A}$	$6.75\pm0.46^{a,BC}$	$6.43\pm0.62^{a,BC}$	$5.38\pm0.92^{a,C}$	$3.12\pm0.69^{a,D}$

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 11. The Texture Results of Cooked Marinated Twaite Shad.

Texture	Day 0	Day 1	Day 15	Day 30	Day 60	Day 90
H	$7.87\pm0.35^{a,A}$	$7.18\pm1,36^{a,A}$	$6.00\pm0.53^{a,B}$	$5.25\pm0.46^{a,BC}$	$4.56\pm0.56^{a,C}$	$3.01\pm0.94^{a,D}$
P	$7.37\pm0.51^{a,A}$	$7.87\pm0.64^{a,A}$	$8.37\pm0.74^{b,A}$	$5.93\pm0.72^{ab,B}$	$4.83\pm0.79^{a,C}$	$3.23\pm0.60^{a,D}$
R	$7.12\pm1.12^{a,A}$	$7.62\pm0.74^{a,A}$	$7.65\pm0.54^{b,A}$	$6.50\pm0.75^{b,A}$	$5.07\pm0.84^{a,B}$	$3.12\pm1.15^{a,C}$

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

Table 12. The General Evaluation Results of Cooked Marinated Twaite Shad.

General Evaluation	Day 0	Day 1	Day 15	Day 30	Day 60	Day 90
H	$7.62\pm0.51^{a,A}$	$6.56\pm1.34^{a,AB}$	$6.01\pm0.75^{a,BC}$	$5.18\pm0.37^{a,CD}$	$4.03\pm0.54^{a,D}$	$2.72\pm0.79^{a,E}$
P	$7.37\pm0.91^{a,A}$	$7.60\pm0.50^{a,A}$	$7.43\pm0.49^{b,A}$	$5.82\pm0.73^{ab,B}$	$4.21\pm0.53^{a,C}$	$2.87\pm0.35^{a,D}$
R	$7.25\pm1.48^{a,A}$	$7.56\pm0.97^{a,A}$	$6.10\pm0.65^{a,AB}$	$6.37\pm0.69^{b,A}$	$4.68\pm0.96^{a,B}$	$2.62\pm0.94^{a,C}$

N=3; Analyses were performed in three replicates. Microbiological table results are given as Average Value (Xaverage) ± Standard deviation (SD). Capital letters (A, B, C, D, E); show the statistical change difference ($p<0.05$) due to storage within the same group. Lower case letters (a, b); show the statistical change difference ($p<0.05$) between groups on the same days.

In the present study, *Lactobacillus plantarum*, *Lactococcus lactis*, and *Pediococcus pentosaceus* lactic acid bacteria were found in cooked fish marinades, as well as *Rhodotorula mucilaginosa* and *Candida albicans* yeast species. No coliform, fecal coliform, *Escherichia coli*, or *Staphylococcus aureus* were found in any of the marinade groups.

4.DISCUSSION

The quality of the raw product, especially its freshness, has a considerable impact on the quality of the end products (Sampels, 2015). The study found that fish (*Alosa tanaica* Grimm, 1901) maintained in refrigerators (4 ± 0.5ºC) had a shelf-life of 6 days (Duyar et al., 2012). Cooking techniques give rise to increase the shelf-life of fish. Heat treatments can be used to inhibit microorganisms that are not heat resistant, as well as to extend the shelf-life of fish. Proper boiling of fish results in sensory characteristics as well as microbiological safety. These parameters are regulated by the appropriate temperature and heating time. Boiling, scalding, and steaming are all culinary processes that use wet heat. It is formed when the fish easily flakes when tested with a fork, or when the core temperature is between 63 and 65°C (about the thickness of 2.5 cm) (Sampels, 2015). In the present study fish fillets were boiled in plastic bags for 35 minutes at 65°C ± 1 in a water bath using the immersion method. The boiling technique effectively eradicated bacterial groups (<1 log cfu/g) in fresh fish fillets.

The added additives also have an impact on the quality of fish and fish products at each stage of the process (Sampels, 2015). In the present study, microorganisms were also inhibited by the heat treatment and marination of fish. However, the microbial ecology of marinades were determined coming from spices and also detected during storage.

Marination is used not only to soften or change the structure, flavor, and textural features of the raw material, but also to prevent microbe development due to the preservation effects of the mixture of acetic acid and salt (Gökoğlu al., 2004). The inhibitory effects of these substances on bacteria and enzymes increase with concentration (Sallam et al., 2007). Because of the lack of refrigeration facilities, marinating is initially employed to preserve food products by immersing them in alkaline or acidic solutions, which generates both technical and anti-microbial effects (Aktaş et al., 2003). The lower the pH value, the longer the shelf-life of marinades is. The increase in the vinegar content of the marinade solution can extend its shelf-life, but it may cause negative effects on the taste and smell of the final product (Sallam et al., 2007). Marinating fish with vinegar and spices is a very old way of preserving food. Vinegar lowers the pH and thus delays bacterial growth. 15% acid is needed to completely stop bacterial growth. Most kinds of vinegar contain 6% acetic acid, and for pickled fish, a concentration equivalent to at least 2.5% acetic acid. For this reason, most fish products are preserved only temporarily. However, pickled products containing 3% acetic acid can be stored chilled for several months (McLay, 2003). Additionally, the marinade itself is often considered an inhibitor effect on bacterial growth due to high concentration of salts, its acidic pH, preservatives and spices (Björkroth, 2005). Hawthorn (*Crataegus orientalis*), a plant species with dark yellow-orange fruits from the Rosaceae family. It is stated that its antioxidant effect is important due to the phenolic compounds it contains (Çoklar and Akbulut, 2016). Rose species have been used in the preparation of various herbal medicines since ancient times. Such as rose vinegar and rose wine. These fruits are still used today against colds due to their high vitamin C content (Magiatis, 2008). Pomegranate (*Punica granatum*), a fruit from the Punicaceae family that grows in temperate climates. Pomegranate is a food with high antioxidant content. Pomegranate vinegar and pomegranate wine are among the antioxidant foods as they contain organic acids, polyphenols and minerals (Ergin, 2019). There are marinade studies conducted by adding spices to various aquatic products. Çelik (2004) examined the sensory values and chemical composition of marinated akivades (*Tapes decussatus* L., 1758). In this study, the cleaned akivades meats were placed in sterilized jars. Daphne leaves, garlic and lemon slices were placed on the akivades meat. 15% grape vinegar and salt were added along with spices. After the prepared mixture was boiled for 45 minutes, it was placed in the jars with the meat and stored at +4 degrees. Crude oil, pH, humidity, raw ash, crude protein, sensory analyses, salt and vinegar determinations were made on the stored marinated akivades. The average pH value of the marinade was found to be 4.43. In the sensory analysis, all values were reported to be of good quality, while the general appearance was reported to be of medium quality. As a result of the study, it was reported that marinated akivades had a different taste and were appreciated. In a study, olive (*Olea europaea* L.) leaf and oil rose (*Rosa damascena* Mill.) extracts were applied to hot smoked rainbow trout (*Oncorhynchus mykiss*) fillets. Chemical, microbiological and sensory changes were examined during storage in the refrigerator. It was determined that the shelf-life of the olive leaf + rose extract, and rose extract applied groups exceeded the microbiological limit value on the 28th day, and the olive leaf applied group exceeded the microbiological limit value on the 42nd day. Olive leaf extract was selected as the most appreciated group. It was also reported that the applied plant extracts had a positive effect on the shelf-life (Mutlu and Bilgin, 2016). In another study, with the increase of propolis extract concentration in the samples was reported to be caused by the growth rate of microbial population decreased during storage at 5°C (Mahdavi-Roshan et al., 2022). In addition to this, the authors reported in this study that the total quantity of aerobic mesophilic and psychrophilic microbes decreased after marinating with the extracts. The acquired results were also indicated to be promising concerning the utilization of plant extracts (Simat et al., 2023). In another study, chemical, microbial, physical and sensory analyses were performed during the cold storage of flounder (*Paralichthys olivaceus*) fillets coated with chitosan (CS) and

hawthorn flavonoids (HF). Samples were treated with 0.5% acetic acid, 1% CS, 1% CS + 0.3% hawthorn flavonoids (CS+HF) and stored at 4–8°C for 14 days. It was determined in this study that the shelf-life of CS and CS+HF groups was extended by 4–6 days. It was also determined that the applied chitosan and hawthorn flavonoid coating affected the quality of the fish and extended the shelf-life (Li et al., 2017). Moreover, in another study, the aerobic plate count (APC) and psychrotrophic bacteria count (PBC) of frozen marinated Asian hard clam (*Meletrix lusoria*) increased rapidly during storage at 4°C, while reaching 8.3 log CFU/g and 8.4 log CFU/g on day 15. However, the pH of frozen this marinated clam species dropped rapidly during storage, reaching 3.4 on this day (Lee et al., 2022). However, the development of lactic acid bacteria in acidic conditions can limit the shelf-life of marinade products. In other words, the marinating process did not extend the shelf- life of marinated food products excessivelly because of the growth of psychrotrophic, anaerobic bacteria such as lactic acid bacteria (LAB) in acidic conditions (Björkroth, 2005). In the present study, the pH values of the fish decreased due to the vinegars used during the marinating process. Later, during the storage of marinades, it increased depending on storage. The results of our study were very similar to the findings (Gün et al., 1994; Erdem et al., 2005; Olgunoglu, 2007; Çakır, 2010; Dericioğlu, 2019, Duyar and Eke Gülüm, 2020) about decreasing of pH values after marination as well as increasing of pH values of marinades during storage. It is thought that the observed decrease in pH may be due to the acidity of the vinegar used. In addition to this, it is also thought that the increase in pH value from day to day may be due to the appearance of volatile nitrogenous compounds during storage.

According to the Turkish Fisheries Quality Control Manual of the General Directorate of Protection and Control of the Ministry of Agriculture and Rural Affairs; the limit values of microorganisms that should be in processed fish; the total number of aerobic mesophilic bacteria is $1.0x10^6$ cfu/g, coliform is 95 cfu/g, *Staphylococcus aureus* 5×10^3 cfu/g (Bilir, 2011). Indeed, the group of coliform bacteria are typical indicators of food hygiene and indicators of food safety (Lues and Van Tonder, 2007). Additionally, Staphylococcal food poisoning is typically caused when cooked meals are contaminated by infected food handlers (Hudson et al., 2024). The maximum recommended number of bacteria to be acceptable for consumption in processed fish and aquaculture products is given as 6.0 log cfu/g (ICMSF, 1986). Yeast and molds do not pose a problem for human health up to a level of $1.0x10^3$ cfu/g, but the ICMSF (1978) reports that there is no legal limit for yeast and molds counts. In the present study, after the marination process, the counts of TMAB, TPAB, LAB, MY counts in all marinated groups with different vinegars decreased below the detectable level (<1 log cfu/g). All marinated groups exceeded the limit of TMAB ($1.0x10^6$ cfu/g) on day 60 according to the sources mentioned above. Furthermore, *Staphylococcus aureus*, fecal coliform bacteria, coliform bacteria and *E. coli* were not detected in any of the marinated groups. A decline was observed in all sensory values of cooked shad marinated with different vinegars as storage time increased. On the 60th day, according to the taste analysis, all groups remained below the acceptability limit value (4.00) and therefore, taste analysis was not performed on the 90th day (end of storage). Similarly, other analysis results on the 90th day also remained below the acceptability limit (4.00). According to the general evaluation of sensory analyses, the group prepared with hawthorn vinegar decreased from the value of 7.62±0.51 to the value of 4.03±0.54 by the 60th day. The group prepared with pomegranate vinegar decreased from the value of 7.37±0.91 to the value of 4.21±0.53, the group prepared with rose vinegar decreased from the value of 7.25±1.48 to the value of 4.68±0.96 on the 60th day. According to the general evaluation results, the most acceptable marinade group on the 60th day was the one prepared with pomegranate vinegar, while the least acceptable marinade group was the one prepared with hawthorn vinegar.

In one study, the effects of aqueous pomegranate peel extract (APPE) and ethanolic pomegranate peel extract (EPPE) on bighead carp (*Aristichthys nobilis*) fillets stored at 4 °C were investigated. It was stated that pomegranate peel extract delayed the deterioration of sensory quality and color change and prevented the formation of bacteria. It was also reported that it could be used as a potential preservative (Zhuang et al., 2019). A sensory analysis of anchovy marinades preserved in oil in glass jar containers revealed a shelf-life of 105 days (Özden and

Baygar, 2003). As a result of the sensorial analysis of the present study, all the groups were indicated as rejected on day 60. Additionally, *L. plantarum*, *L. lactis*, *P. pentosaceus*, *R. mucilaginosa*, *C. albicans* were identified in all the cooked marinated twaite shad.

Lactobacillus spp. is a microaerophilic, obligately heterofermentative lactic acid bacterium isolated from many different media. *Lactobacillus* spp. is involved in the production of a wide variety of fermented products worldwide. However, in some cases, it can cause various foods to spoil (Teixeira, 2014). In one study, *Lactobacillus alimentarius* was found to be the organism of specific degradation in all marinated herring. All isolates obtained from different product types were of the same clonal type. The slight increase in pH value, combined with the pronounced gas production, suggested a rare type of lactic acid bacterial degradation called 'protein swelling' with herring spoilage (Lyhs et al., 2001). In another study, *Lactobacillus curvatus, Lactobacillus sakei*, and strains of the *L. curvatus spp./Lactobacillus fuchuensis* group were the main species detected. Of all the isolates, six were identified as *Lactococcus* spp. in spoiled maatjes herring (Lyhs and Björkroth, 2008). The main microorganisms in the other fermented jeotgal (Korean fermented fish products) were reported to be the species of *Pseudomonas, Lactobacillus, Bacillus, Brevibacterium, Micrococcus, Pediococcus, Halobacterium,* and *Leuconostoc (*Koo et al., 2006). A mixed LAB population dominated by a *Leuconostoc* species resembling *Leuconostoc gelidum* was found to cause the spoilage of the product. *Lactobacillus sakei, Lactobacillus curvatus* and a Gram-positive rod phenotypically similar to heterofermentative *Lactobacillus* species were the other main organisms detected in this spoilage population. Increase in pH together with the extreme bulging of packages was considered to be a rare LAB spoilage type called "protein swell". This spoilage was characterized by excessive production of gas due to amino acid decarboxylation and the rise of pH was attributed to the subsequent deamination of amino acids. However, The rise in pH values was likely to result from the buffering capacity of the meat (Björkroth, 2005). In general, *Lactobacillus* species, especially *Lactobacillus sakei*/ *curvatus,* were identified during storage at 6^0C in marinated product (Björkroth et al., 2005). In another study, *Lactobacillus curvatus, Lactobacillus sanfranciscensis* were reported to be present in all salad groups considered in Italian marinated seafood salad (pH 5.0). (Andrighetto et al., 2009). In addition, it was stated that *Lactobacillus plantarum* was the most isolated lactic acid bacterium in marinades (Lundström and Björkroth, 2007). Nieminen et al., (2002) also indicated that the lactic acid bacteria species in marinated fillet pieces during cold storage were to be *Lactobacillus spp.* and *Leuconostoc spp.* In another study, yeasts were reported as a spoilage agent associated with fish and fishing products stored at low temperatures, especially dominated by two genera, which were the species of *Candida* and *Rhodotorula* (Tahiluddin et al., 2022). In the present study, lactic acid bacterial species were identified in marinade products during cold storage of cooked twaite shad marinades packaged in olive oil by adding spices after marinating with different vinegars. *Lactobacillus plantarum, Lactococcus lactis, Pediococcus pentosaceus* lactic acid bacteria species were identified in cooked twaite shad fish marinades, *Rhodotorula mucilaginosa, Candida albicans* yeast species were also identified. It was believed that the lactic acid bacteria and yeast species determined in cooked twaite shad marinades came from bay leaf, black pepper and red pepper flakes added to marinade products during marinade production and showed improvement in pH values during the storage of marinade products.

5.CONCLUSION

According to the findings of the present study, fish kinds that are not commonly consumed, such as twaite shad fish, can readily be made edible. The effects of the vinegar kinds utilized in the study on quality can be examined using various marinade ratios and techniques. Furthermore, product quality, shelf-life, and organoleptic studies can be conducted using marinated items such as various spices and sauces. Moreover, various packaging methods can be used to conduct studies on product quality factors. It is believed that the present study will lead to further studies. Marine goods are foods that degrade quickly if not treated. For this reason, treating these items allows for extending their shelf-life and introducing non-common species into consumption. This study is expected to generate ideas for developing alternative goods in the realm of seafood processing. Previously, there

have been cold marinade research using aquaculture items such as anchovies, sardines, mussels, and twaite shad, but cooked marinade studies are fairly restricted. The investigations done have raised concerns about packaging methods and storage conditions. The investigations are largely with acid addition, and the number of trials using natural additives is minimal. As demonstrated in the present study, alternative goods can be created using natural vinegars. Especially today, the usage of both natural and chemical additions may increase product preference. Issues such as raising the degree of scalding performed in the research, scalding with vinegar, modifying the formulation ratios, and storing methods will be the focus of subsequent investigations.

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AUTHOR CONTRIBUTIONS

Ecem Özer: formal analysis, writing the original draft, content and design of the analysis. Berna Kılınç: formal analysis, writing the original draft, content and design of the analysis.

CONFLICTS OF INTEREST

The authors have no conflict of interests to declare.

ETHICS APPROVAL

There are no ethical issues with the publication of this manuscript.

DATA AVAILABILITY

The authors confirm that the data that supports the findings of this study are available within the article.

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Research Article | **Araştırma Makalesi**

The Effect of Twine Thickness of Monofilament Gillnets on the Catchability and Fishing Gear Losses For Pikeperch (*Sander Lucioperca (L.*, 1758)) Fishing

Sudak (*Sander Lucioperca* (L., 1758)) Avcılığında Kullanılan Monofilament Sade Uzatma Ağlarında İp Kalınlığının Av Verimine ve Kayıp Av Araçları Üzerindeki Etkisi

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Abstract: In this study, the effect of twine thicknesses on the catch efficiency in monofilament gillnets were investigated. Field trials were carried out in Seyhan Dam Lake between September 2020 and September 2021 on a monthly basis. Net codes with different mesh sizes (bar length) and twine thicknesses were as follows, respectively: 1=26 mm 0.16 mm, 2=26 mm 0.18 mm, 3=28 mm 0.16 mm, 4=28 mm 0.20 mm, 5=30 mm 0.16 mm, 6=30 mm 0.20 mm, 7=30 mm 0.33 mm, 8=32 mm 0.16 mm, 9=32 mm 0.20 mm. A total of 34 experimental fishing operations were carried out. The results showed no statistically significant difference between the catch efficiency of thin twine and thick twine in nets with 26 mm and 32 mm mesh sizes ($p>0.05$). However, significant differences were found in the 28 mm mesh size ($p<0.05$). In gillnet with 30 mm mesh size, no statistical differences were observed in catch efficiency between net codes 5 and 6 ($p> 0.05$). However, statistical differences were found in catch efficiency between net codes 5 and 7, as well as between codes 6 and 7 ($p <0.05$). These results indicate that a 12.5% (twine thickness: 0.16-0.18 mm) and 25% (twine thickness:0.16-0.20 mm) increase in rope thickness did not affect catch yield, whereas a 65% (twine thickness: 0.20-0.33 mm) increase did.

Keywords
- Sustainability
- Inland fisheries
- Small-scale fisheries
- Fisheries management

Özet: Bu çalışmada, monofilament sade uzatma ağlarında ip kalınlığının av verimine etkisi araştırılmıştır. Saha çalışmaları, Seyhan Baraj Gölü'nde Eylül-2020 ve Eylül-2021 tarihleri arasında, 26, 28, 30 ve 32mm ağ göz genişliğinde (tek kol uzunluğu) farklı ip kalınlığında (1=26mm 0.16mm, 2=26mm 0.18mm, 3=28mm 0.16mm, 4=28mm 0.20mm, 5=30mm 0.16mm, 6=30mm 0.20mm, 7=30mm 0.33mm, 8=32mm 0.16mm, 9=32mm 0.20mm) 9 posta monofilament sade uzatma ağı kullanılarak yapılmıştır. Toplam 34 deneysel avcılık operasyonu gerçekleştirilmiştir. Elde edilen sonuçlar; 26 ve 32mm ağ göz genişliğindeki ağlarda ince ip ile kalın ip av verimi arasında istatistiksel olarak önemli bir fark bulunmadığını göstermiştir ($p>0.05$). 28mm göz genişliğindeki ağda ise fark istatistiksel olarak önemli bulunmuştur ($p<0.05$). 30mm ağ göz genişliğindeki ağlarda ise 5 ve 6 no'lu ağların av verimleri arasında istatistiksel olarak fark yok iken ($p>0.05$), 5 ve 7, 6 ve 7 no'lu ağların av verimleri arasındaki fark istatistiksel olarak önemlidir ($p<0.05$). Tüm bu sonuçlar; ip kalınlığındaki %12.5 (ip kalınlığı: 0.16-0.18 mm) ve %25'lik (ip kalınlığı: 0.16-0.20 mm) artışın av verimini etkilemediğini ancak %65'lik (ip kalınlığı: 0.20-0.33 mm) artışın etkilediğini göstermiştir.

Anahtar kelimeler
- Sürdürülebilirlik
- İç su balıkçılığı
- Küçük ölçekli balıkçılık
- Balıkçılık yönetimi

1.INTRODUCTION

Pikeperch is a species naturally distributed in the northern latitudes of Europe and Asia. This species is of high economic value and is targeted for the rehabilitation of inland waters, sport fishing, and commercial fishing purposes. It has been inoculated from the United Kingdom and Portugal in the west to China in the east; Africa

(Morocco, Tunisia, and Algeria) in the south, to North America in the west, and the Azores Islands in the Atlantic Ocean, and spreading its distribution worldwide (fishbase.se, 2024). It is an opportunistic predator species that continues to spread its distribution area by being reported in a total of 46 countries and/or islands. In Türkiye, it is naturally found in the Bafra lagoons in the Black Sea and in the Terkos, Büyükçekmece, and Küçükçekmece lakes in the Marmara Region (Aral, 1986). As part of a joint project between the Directorate General for State Hydraulic Works and Çukurova University from 1971 to 1973 for rehabilitation purposes, it was inoculated to the Seyhan Dam Lake. The species, which had an economic population in the following years, became one of the most important target species of commercial fishing. It is mostly caught with monofilament gillnets (Avşar and Ozyurt, 1999).

Set nets are fishing gear used by humans for thousands of years. Due to its low initial investment cost and personnel requirement, it still constitutes an important part of small-scale fishing activities carried out in inland waters and coastal areas of the marine area (Cunningham et al., 2004; Gray et al., 2005). In the historical process, set nets, which were initially made from net twine obtained from natural filament, began to be made with net twine obtained from synthetic filament in parallel with the development of technology (Gabriel et al., 2008). The set nets used today can be designed with different technical features depending on parameters such as target species and fishing area (He et al., 2021; Özdemir and Erdem, 2006). The most important goal in these designs is to keep the catch efficiency at the highest possible level. Accordingly, many studies have been conducted on the effect of the physical (monofilament-multifilament, twine colour, twine thickness, etc.) and technical properties (gill-trammel, mesh shape, floating-sinking force, etc.) of set nets on catch efficiency (Balık, 1999; Balık and Çubuk, 2001; Holst et al., 2002; Sürer and Kusat, 2013). In set nets, one of the most important parameters affecting catch efficiency is the visibility of the net (Hamley, 1975). Target species are less likely to notice low visibility nets, increasing the likelihood of being caught. (Hamley, 1975). It has been shown in many studies that the visibility of thin twine thickness is lower than that of thicker twine thickness, thus the catching efficiency is higher (Ayaz et al., 2011; Grati et al., 2015; Yokota et al., 2002). However, due to the low breaking strength, thin net twines physically damage out quickly and their economic life may be shorter (Hamley, 1975). In fact, since it is made of thin twines, broken parts or the entire net can remain under water (Laist, 1996). Therefore, using the thickest net twine that does not negatively affect catching efficiency will be important to provide a more economical fishing activity

In this study, the effect of twine thickness on catch efficiency for pikeperch was examined. Then, for different mesh size, the effect of using increasing ratios (12.5% (0.18 mm), 25% (0.20 mm) and 65% (0.33 mm)) of twine thickness based on the thinnest twine thickness (0.16 mm) on the catch efficiency was examined and the optimum twine thickness was tried to be determined.

2.MATERIAL and METHODS

Seyhan Dam Lake, built for flood prevention, irrigation and energy production, was put into operation in 1956 (Figure 1). The reservoir of the dam lake has a width of 4 km and a length of 23 km, its altitude is 67 m and the deepest point is 45 m (Kiyağa, 2008). The maximum surface area of the lake is 6782 hectares and the water level can vary greatly depending on the seasons. In the lake, which has a mesotrophic character (Cevik et al., 2007), the target species of commercial fisherman are *Cyprinus carpio, Carassius gibelio* and *Sander lucioperca.*

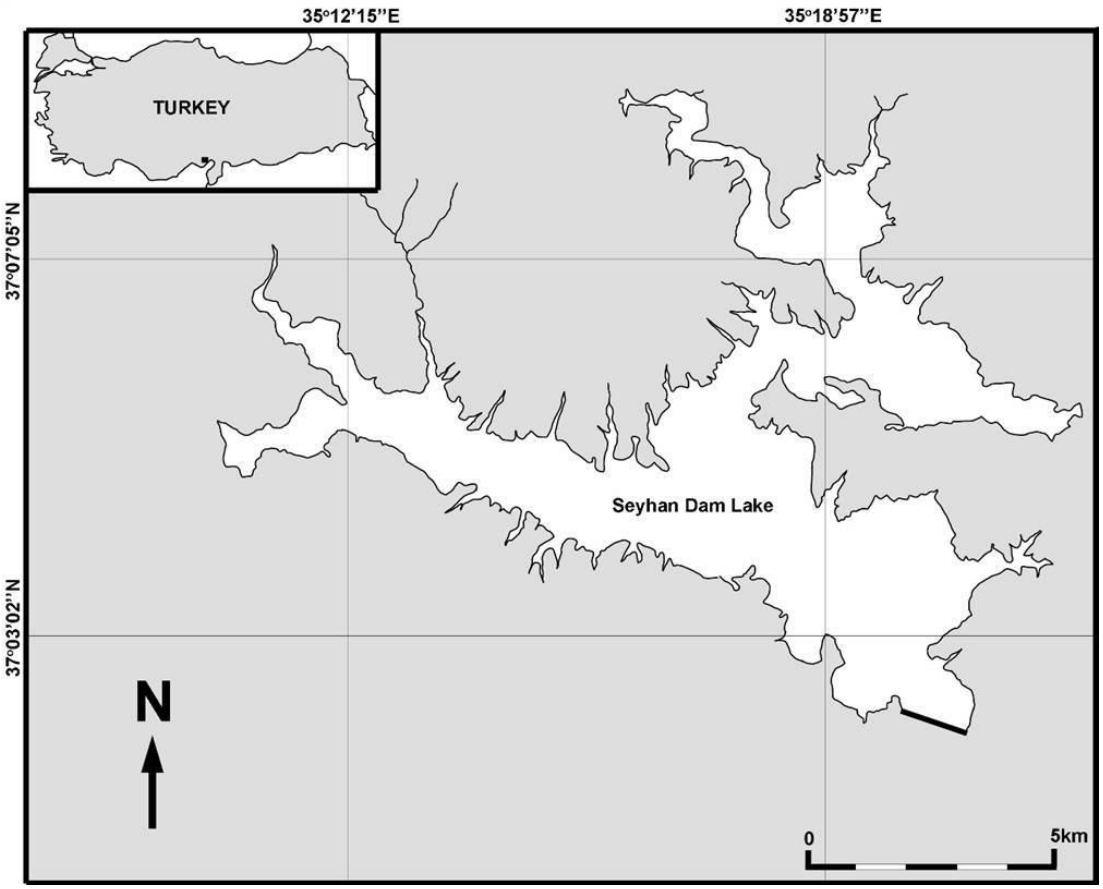

Figure 1. Seyhan Dam Lake.

Nine different gillnet panels, each were rigged 100 meters long and varying in mesh size and twine thickness, were randomly connected to one another by float and lead lines. Nets are coded with numbers according to mesh size and twine thickness (Table 1).

Table 1. Mesh size, net codes and twine thicknesses.

Mesh size (mm)	Net code	Twine thickness (mm)
26	1	0.16
	2	0.18
28	3	0.16
	4	0.20
30	5	0.16
	6	0.20
	7	0.33
32	8	0.16
	9	0.20

The trials were conducted in Seyhan Dam Lake between September 2020 and September 2021, covering a 12-month period. 34 experimental fishing operations were carried out where commercial fishermen catch intensively. The gillnets were utilised for fishing from dusk to dawn, for a set time of 10-12h. The samples obtained were transported freshly to the faculty laboratories via a cold chain. Total length (cm) and total weight (g) of pikeperch individuals caught in gillnets with different twine thickness and mesh size were measured. CPUE was calculated using the equation provided below (Sparre and Venema,1998)

$$CPUE = \frac{Yield}{Efort}$$

In this equation, yield is considered the number of individuals caught per operation. Effort can be calculated using various parameters, such as duration, number of vessels, engine power, or the number of fisherman, depending on the fishing gear. Sparre and Venema stated that the most suitable measure of effort for gillnets is the number of gillnets deployed. Since the number and lengths of the nets used in this study were equal, effort was considered as the number of operations.

The effect of different twine thicknesses on catching efficiency was determined by; 1) if there are two different twine thicknesses, the non-parametric test (the assumptions of normality and homogeneity of variances were not met) called Wilcoxon Signed Ranks test was used, and 2) if more than two twine thicknesses are involved, Kruskal Wallis tests was used. The statistical significance of the differences between the length distributions of individuals caught in nets with the same mesh size was determined using the Kolmogorov Smirnov test. For statistical analysis, the One-Way Test package (Dag et al., 2018) and for data visualization, the ggplot2 package (Wickham, 2016) within the R programming language was used.

3.RESULTS

A total of 1260 pikeperch were caught, with lengths ranging from 9.4 to 57.3 cm and weights ranging from 6.38 to 1633.87 g. The number, percentage distribution, minimum, maximum and average length-weight values of pikeperch caught according to mesh size and twine thicknesses are given in Table 2. According to the results obtained; net code 1 caught the most with 333 (26.43%) individuals, followed by net code 2 with 232 individuals (18.41%), and net code 7 caught the least with 55 (4.37%) individuals. In general, it is observed that nets with thin twine catch more than nets with thick twine, except for nets with 28 mm mesh size. It has been observed that as the mesh size increases, the average length increases, and the number of individuals caught decreases (it should be taken into consideration that there are 3 panel nets with a 30 mm mesh size).

Table 2. Number, percentage distribution, minimum, maximum, mean length and weight of pikeperch individuals caught in different nets (SE: Standard Error).

Mesh size (mm)	Net code	Twine thickness (mm)	N	N (%)	Total length (cm)			Weight (g)		
					Min.	Max.	Mean ±SE	Min.	Max.	Mean ± SE
26	1	0.16	333	26.43	15.60	46.40	27.56±0.17	28.08	874.55	161.94±3.66
	2	0.18	232	18.41	13.70	38.40	27.25±0.19	21.48	433.89	154.57±3.45
28	3	0.16	81	6.43	14.00	50.20	28,02±0.59	21.95	1126.37	188.66±14.46
	4	0.20	147	11.67	220.00	40.90	29.44±0.32	77.12	548.22	208.11±6.93
30	5	0.16	139	11.03	14.90	45.90	31.05±0.42	20.89	732.5	240.42±9.06
	6	0.20	110	8.73	13.60	44.60	31.87±0.34	17.68	564.55	254.72±8.39
	7	0.33	55	4.37	20.50	53.40	32.34±0.63	61.58	1282.9	279.47±21.81
32	8	0.16	84	6.67	15.80	39.90	31.71±0.57	31.6	464.51	254.68±11.34
	9	0.20	79	6.27	9.40	57.30	31.03±0.74	6.38	1633.87	261.01±21.08

The CPUE (individual/operation) values of the nets was calculated as 9.79 and 6.82 for nets 1 and 2, 2.38 and 4.32 for nets 3 and 4, 4.09, 3.24 and 1.62 for nets 5, 6 and 7, for nets 8 and 9 2.47 and 2.32 (Figure 2.).

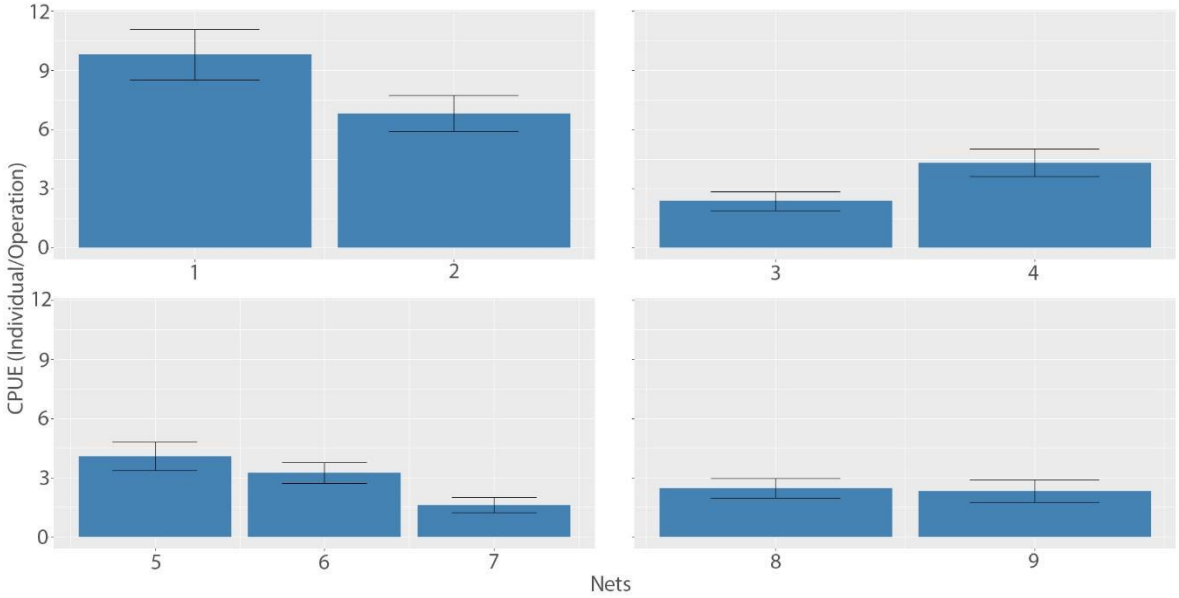

Figure 2. CPUE values of nets.

The statistical analyses of the differences in CPUEs according to net twine thickness are given in Table 3. The results obtained in the study have shown that the difference between the catch efficiency of different twine thicknesses of nets with 26 mm and 32 mm mesh sizes were not statistically significant ($p>0.05$), whereas the difference between the catch efficiency of different twine thicknesses of nets with 28 mm and 30 mm mesh sizes were statistically significant ($p<0.05$). The Wilcoxon Rank Test results for the 26 mm mesh size indicate that although the CPUE value of the thinner net twine (0.16 mm) (9.79 individuals/operation) was slightly higher than that of the thicker net twine (0.18 mm) (6.82 individuals/operation), the difference was not statistically significant ($p > 0.05$). Therefore, it was understood that in this mesh size, the catch efficiency was not affected by a 12.5% increase in net twine thickness. For 32mm mesh size, the CPUE value of the thin net twine (0.16 mm) (2.59 individual/operation) and the CPUE value of the thick net twine (0.20 mm) (2.32 individual/operation) were not statistically different ($p>0.05$). Therefore, it can be said that at this mesh size, catching efficiency was not affected by increasing the twine thickness by 25%. Kruskal Wallis test was used to compare the catch efficiency of nets with three different twine thicknesses with a mesh size of 30 mm and it was determined that the difference was statistically significant ($p <0.05$). As a result of the posthoc test given in Table 3; The difference between twine thickness catch efficiency net code 5 (0.16 mm) and 6 (0.20 mm) were not statistically significant ($p>0.05$). However, it was determined that the differences between net codes 5 (0.16mm) and 7 (0.33 mm) and net codes 6 (0.20 mm) and 7 (0.33 mm) were statistically significant ($p<0.05$). In other words, it was determined that at 30 mm mesh size, catch efficiency was not affected by a 25% increase in net twine thickness, but the efficiency was affected by a 65% increase.

Table 3. CPUE and p values of nets with different twine thicknesses all mesh sizes.

Mesh size (mm)	Net code	Twine thickness (mm)	N	CPUE	W-H*	p
26	1	0.16	333	9.79	W=714	p= 0.095
	2	0.18	232	6.82		
28	3	0.16	81	2.38	W=377	p= 0.012
	4	0.20	147	4.32		
30	5	0.16	139	4.09	H=12.32	0.16 and 0.20mm p=0.9750
	6	0.20	110	3.24		0.16 and 0.33mm p=0.0022
	7	0.33	55	1.62		0.20 and 0.33mm p=0.0381
32	8	0.16	84	2.59	W=617	p = 0.63
	9	0.20	79	2.32		

*W: Wilcoxon Rank Value, H: Kruskal-Wallis Value.

In nets with a mesh size of 28 mm, the CPUE value of the thick twine net (0.20 mm) (4.32 individual/operation) was determined to be higher than the CPUE value of the thin twine net (0.16 mm) (2.38 individual/operation). Contrary to 32 mm mesh size, it was determined for 28 mesh size that the difference between the number of individuals caught according to net twine thickness was statistically significant ($p < 0.05$). In field studies, it was observed that this net suffered significant physical damage after a while and there was a decrease in catching efficiency (Figure 3, Table 4). In order to determine whether the decrease in catch efficiency was due to physical damage, the first 18 operations, in which the physical damage of the net was relatively low, and the last 16 operations, in which physical damage became evident, were compared separately. It was observed that in the first 18 operations, the difference between the CPUE value of the thin net twine (0.16 mm) (2.83 individual/operation) and the CPUE value of the thick net twine (0.20 mm) (3.33 individual/operation) were not statistically significant ($p > 0.05$). On the other hand, in the last 16 operations, it was determined that there was a significant difference between the CPUE value of the thin net twine (1.9 individual/operation) and the CPUE value of the thick net twine (5.4 individual/operation) ($p<0.05$). These results strongly strengthen the idea that the lower catching efficiency of thin net twine compared to thick net twine was due to physical damage. The results of the statistical comparisons made for mesh with 28 mm mesh size are given in Table 4. At the end of the field studies, the net with a thin twine was spread and the physical damage of the net was examined and photographed (Figure 3). It was observed that there were pieces of the net completely broken off from the floating and sinking rope in many parts of the net (Figure 3). It was observed that the large broken net pieces reduced the total area of the net. In addition, the area around the damaged parts was probably deformed, causing more areas to lose their catching ability than the damaged areas. As a natural result of this, the catching efficiency of the net with thin twine thickness was lower than that of the net with thick twine thickness.

Figure 3. Physical damage of the nets with a mesh size of 28 mm and a twine thickness of 0.16 mm (Left: net broken from the float rope, On the right: net broken from the sinking rope).

Table 4. CPUE and p values of nets with different twine thicknesses and 28mm mesh size.

Operations	Mesh size (mm)	Net code	Twine thickness (mm)	N	CPUE	W	p
All Operations	28	3	0.16	81	2.38	W=377	p= 0.012
		4	0.20	147	4.32		
First 18 Operations	28	3	0.16	51	2.33	W=134	p= 0.3714
		4	0.20	60	3.33		
Last 16 Operations	28	3	0.16	30	1.90	W=52.5	p= 0.0040
		4	0.20	87	5.40		

If the physically damaged net with the mesh size 28 mm is ignored, the catch efficiency was not affected by the 12.5% and 25% increases in net twine thickness. Therefore, it can be said that using 0.20 mm twine thickness in all mesh sizes for pikeperch fishing does not have a negative effect in terms of catching efficiency. It can even be said that the physical damage of the net might be reduced. However, it is understood that catch efficiency would be reduced by increasing the twine thickness from 0.20 mm to 0.33 mm (65% increase).

4.DISCUSSION

The important factor that limits the use of thick net twine is catching efficiency. In many previous studies, it has been determined that the twine thickness affected the catching efficiency. The catching efficiency of nets with thin twine was found to be higher, and this was generally attributed to the fact that the target species were less likely to see nets with thin twine thickness (Ahmadi and Kristina, 2017; Grati et al., 2015; Holst et al., 2002; Turunen, 1996). The results obtained in this study showed that the differences between the catching efficiencies and the twine thicknesses of 0.16 mm and 0.33 mm, and 0.20 mm and 0.33 mm were statistically significant ($p<0.05$). In addition, the catching efficiency of thinner twines were found to be higher. For commercial fishermen, using thick net twine with lower CPUE value to reduce net losses does not seem to be a practically applicable method. Therefore, the most appropriate solution would be to use the thickest possible net twine that does not affect the catching efficiency. The results obtained in this study showed that it is possible to implement this recommendation. When the thinnest twine thickness of 0.16 mm was increased by 12.5% (0.18 mm) and 25% (0.20 mm), it was observed that the difference between the catching efficiency was not statistically significant ($p>0.05$). Therefore, it may be recommended that using 20 mm twine thickness for pikeperch fishing would be better. However, when the twine thickness is increased from 0.16 mm to by 30%, 40% or 50%, the decrease in catch efficiency can be evaluated based on the visual sensitivity of the pikeperch. The twine thicknesses chosen in the study did not allow for this type of evaluation. It is known that visual sensitivity in fish varies depending on the species, different life stages of the species, atmospheric conditions, and the characteristics of the water source (Utne-Palm, 2002). Since the trials in this study were carried out in areas where commercial fishing was conducted, the results obtained were applicable to the target species and study region. In addition, for the results obtained in such studies conducted in different regions, it would also be useful to measure some environmental parameters such as light intensity, blurriness, and depth.

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CONFLICT OF INTEREST DISCLOSURE

Dear Editor, the present study: “The Effect of Twine Thickness of Monofilament Gillnets on

The Catchability and Fishing Gear Losses for Pikeperch (*Sander Lucioperca (L.*, 1758)) Fishing", has no conflict with any institution, organization, person or financial institution. There is no foreseeable conflict of interest from any perspective and there is no conflict of interest among the authors.

AUTHOR CONTRIBUTIONS

Literature: CEO, TN; Methodology: CEO, FU; Performing the experiment: AA, KA, CEA; Data analysis: AA, TA; Manuscript writing: AA, Supervision: CEO. All authors approved the final draft.

ETHICS and PERMIT APPROVAL STATEMENT

We declare that the study is among the studies that do not require ethics committee permission.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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Research Article **Araştırma Makalesi**

Determination of Oxidative Stress Responses in *Dreissena polymorpha* Exposed to Rare Earth Elements (Terbium, Lanthanum, Gadolinium and Praseodymium) with Temperature

Sıcaklıkla Birlikte Nadir Toprak Elementlerine (Terbium, Lantan, Gadolinyum ve Praseodimyum) Maruz Bırakılan *Dreissena polymorpha*'da Oksidatif Stres Tepkilerinin Belirlenmesi

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Abstract: The continuous development of industry pushes people to search for new resources, and for this reason, the usage areas of Rare Earth Elements (REEs) are increasing day by day. Increasing concentrations of REEs, as a result of increased use, create pollution in the environment and harm living organisms. This pollution interacts with increasing temperature and causes more negative synergistic effects of the pollutant in the environment and in the living body. In this study, sublethal concentration values were determined by literature review and the concentration value was determined as 125 mg/L. In the present study tt was aimed to investigate some oxidative stress and antioxidant responses of Terbium, Lanthanum, Gadolinium and Praseodymium REEs in *Dreissena polymorpha* at 125 mg/L concentration at 3 different temperatures (16, 18, 20 ^{0}C) with biomarkers. For this purpose, 24 and 96 hour experimental trial design was created and 7 *D. polymorpha* were used in each trial group, and the application experiments were carried out with 3 replications. The samples at the end of the experimental phase were stored at -80 degrees Celsius until they were analyzed. In this study, Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzyme activities and glutathione (GSH) and Thiobarbituric acid (TBARS) level biomarker responses were determined by ELISA test microplate reader. CAYMAN brand SOD (Catalog No 706002), CAT Catalog No 707002) and GPx (Catalog No 703102), GSH (Catalog No 703002) and TBARS (Catalog No 10009055) were used in the study. SPSS 24.0 package program one-way ANOVA (Duncan 0.05) was used for the evaluation of biochemical analyzes. According to the study data, statistically significant decreases were observed in SOD and CAT activities in the oxidative stress responses of REEs on *D. Polymorpha* with increasing temperature, while there was no significant change in GPx activities. It was determined that there were increases in TBARS levels and decreases in GSH levels. It is thought that the temperature factor, application concentration and application time are effective in the formation of these changes. It can be said that temperature change and pollutants cause oxidative stress in organisms and cause cell damage.

Keywords
- *Dreissena polymorpha*
- Temperature
- Oxidative stress
- Rare Earth Elements

Özet: Sanayi ve endüstrinin sürekli gelişmesi insanları yeni kaynak arayışlarına itmekte ve bu amaçla kullanım alanları her geçen gün artan Nadir Toprak Elementlerinin (NTE) kullanım alanları hızla artmaktadır. Artan kullanım sonucu NTE'lerin artan konsantrasyonları çevrede kirlilik yaratmakta ve canlı organizmalara zarar vermektedir. Bu kirlilik artan sıcaklıkla etkileşime girerek kirleticinin çevrede ve

Anahtar kelimeler
- *Dreissena polymorpha*
- Sıcaklık

canlı vücudunda daha olumsuz sinerjik etkilere neden olmaktadır. Bu çalışmada, subletal konsantrasyon değerleri literatür taraması ile belirlenmiş ve konsantrasyon değeri 125 mg/L olarak tespit edilmiştir. *Dreissena polymorpha*'da Terbium, Lanthanum, Gadolinium ve Praseodymium NTE'lerin 125 mg/L konsantrasyonda 3 farklı sıcaklıkta (16, 18, 20 0C) bazı oksidatif stres ve antioksidan tepkilerinin biyobelirteçler ile araştırılması amaçlanmıştır. Bu amaçla 24 ve 96 saatlik deneme deseni oluşturularak her deneme grubunda 7 adet *D. polymorpha* kullanılmış ve uygulama deneyleri 3 tekrarlı olarak gerçekleştirilmiştir. Deneme aşaması biten örnekler analiz edilene kadar -80 derecede muhafaza edilmiştir. Bu çalışmada Süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPx) enzim aktiviteleri ile glutatyon (GSH) ve Tiyobarbitürik asit (TBARS) düzeyi biyobelirteç yanıtları ELISA testi mikroplaka okuyucu ile belirlenmiştir. Çalışmada CAYMAN marka SOD (Katalog No 706002), CAT Katalog No 707002) ve GPx (Katalog No 703102), GSH (Katalog No 703002) ve TBARS (Katalog No 10009055) kullanılmıştır. Biyokimyasal analizlerin değerlendirilmesi için SPSS 24.0 paket programı tek yönlü ANOVA (Duncan 0.05) kullanılmıştır. Çalışma verilerine göre, *D. Polymorpha* üzerinde NTE'lerin oksidatif stres tepkilerinde artan sıcaklıkla birlikte SOD ve CAT aktivitelerinde istatistiksel olarak anlamlı düşüşler gözlenirken, GPx aktivitelerinde anlamlı bir değişiklik olmamıştır. TBARS seviyelerinde artışlar, GSH seviyelerinde ise düşüşler olduğu tespit edilmiştir. Bu değişikliklerin oluşumunda sıcaklık faktörü, uygulama konsantrasyonu ve uygulama süresinin etkili olduğu düşünülmektedir. Sıcaklık değişiminin ve kirleticinin organizmalarda oksidatif strese neden olduğu ve hücre hasarına yol açtığı söylenebilir.

● Oksidatif stres
● Nadir Toprak Elementleri

1.INTRODUCTION

Rare Earth Elements (REE) are considered as strategic elements because they are used in many different sectors in the production of advanced technological materials resistant to high temperature, abrasion and corrosion (Celep et al., 2021). It consists of 15 elements of the lanthanide group with similar chemical properties, a total of 17 elements including scandium and yttrium by Krishnamurthy and Gupta (2016). REEs are widely used in the production of many advanced technological devices (such as mobile phones, computers, TVs), rechargeable batteries (NiMH batteries), modern medical devices (such as MRI equipment), catalytic converters, engines (aircraft, hybrid vehicles, wind turbines), glass and ceramics, oil refinery, solar panels (Binnemas et al., 2013; Krishnamurthy & Gupta 2016; USGS, 2020). Terbium (Tb) is one of the rare earth elements, although it is still twice as much as silver in the earth's crust. It cannot be found in nature as a free element, but is found in many minerals. Gadolinium (Gd) is used in control rods for nuclear reactors and nuclear power plants. It is used to make garnet for microwave applications, and its compounds are used to make phosphorus for color TV tubes. Praseodymium (Pr) metal darkens slowly in air, forming a green oxide layer that flakes off like iron rust. It reacts slowly with most acids and cold water, more quickly with hot water (URL 1, 2023).

There are several release pathways through which anthropogenically derived REE can enter the aquatic environment or be transferred across environmental segments where they can have potentially adverse effects on organisms and ecosystems. Researchers investigating such potential adverse effects have reported a range of effects including changes in survival, reproduction and growth rates in freshwater zooplankton, echinoderms and fish, as well as changes in neural and cardiac activities in embryonic development (Blaise et al., 2018; Cui et al,. 2012; Dubé et al., 2019; Lürling & Tolman 2010; Zhao et al., 2021). These effects can be attributed to cellular inhibition, homeostasis, Ca^{2+} signaling and alteration of gene transcription involved in DNA repair processes. Chronic exposure to REE may adversely affect hepatic, respiratory and neural functions. They can affect a range of organisms starting from the most primitive living things in the environment to more evolved organisms such as humans. REE may be released into the environment as particulate matter or dust during processing and use. They enter the aquatic environment as a result of atmospheric transport and precipitation through urban and industrial wastewater flow, rivers, groundwater seepage (Olmaz et al., 1991;; Klaver et al., 2014; Morgan et al., 2016; Brito et al., 2018; Trifuoggi et al., 2018).

For many aquatic organisms, temperature is an important environmental variable that can affect physiological mechanisms at enzymatic and cellular levels and cause changes in metabolic rates (Cairns et al., 1975; Ward & Stanford 1982). Such temperature effects can alter an organism's ability to detoxify xenobiotics by altering pollutant uptake, elimination or biotransformation rates, ultimately affecting toxicokinetic and toxicodynamic processes and toxicity (Hooper et al., 2013). Water bodies can harbor an increasing number of agricultural and industrial chemicals that can disrupt free radical processes taken up by organisms. Uptake of these pollutants by hydrobionts can occur from water, sediments, suspended particulate matter, and food sources. Aquatic organisms also have special systems for the production and breakdown of free radicals. Current knowledge and recent developments in general toxicology and especially in the toxicology of hydrobionts provide a fertile field for aquatic toxicology studies (Lushchak 2011).

Reactive oxygen species (ROS) are an indispensable part of aerobic life. Steady-state concentrations are a balance between production and elimination providing a certain steady-state level of ROS. The dynamic balance can be disrupted, leading to increased levels of ROS and damage to cellular components called "oxidative stress". Changes in temperature, oxygen levels, and salinity can cause stress in natural and artificial conditions by inducing an imbalance between ROS production and elimination (Serdar et al., 2024a). Catalase (CAT) is one of the antioxidant enzymes and has been implicated as an essential defense against the potential toxicity of superoxide anions such as the hydroxyl free radical. Therefore, it is thought to act as a cellular defense against the potentially harmful effects of the superoxide anion produced by a wide variety of biological reactions (David et al., 2008). Superoxide dismutase (SOD) is an important antioxidant enzyme that catalyzes the conversion of superoxide to oxygen and hydrogen peroxide in aerobic organisms (Kim et al., 2011). Among the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GSH) have been widely used as effective biomarkers of environmental contamination in aquatic organisms and have been identified as effective protective barriers against ROS formation (Figueiredo et al., 2018). Depletion has an imbalance in the redox state and ability to cope with organic xenobiotics metabolized by glutathione S-transferase (GST) and glutathione peroxidases (GPx) (Aydın & Serdar, 2024). Lipid peroxidation, measured as thiobarbituric acid reactive substances (TBARS), has been frequently used as a marker of oxidative stress in response to different environmental pollutants in various studies (Roméo et al., 2019; Serdar, 2019; Choi & Oris 2000; Oakes & Van Der Kraak 2003; Almroth et al. 2005).

In the examination of pollution in aquatic ecosystems, the longevity, limited mobility and non-selective filter feeding of freshwater mussels ensure their widespread and reliable use in toxicological studies with biomarkers (Serdar et al., 2021). *D. polymorpha* may cause the imbalance of the very sensitive food chain to deteriorate and the aquatic ecosystem may be adversely affected by them (Serdar 2021). In addition, the sticking behavior of mussels to hard surfaces also causes problems. In addition, bivalve mollusks are susceptible to heat stress and water quality due to their sedentary lifestyle, inability to regulate body temperature and bioaccumulation of pollutants (Serdar et al., 2024b). Since *D. polymorpha* has a strong oxidative defense and a relatively high resistance to xenobiotics, it is widely used to conduct ecotoxicological experiments (Faria et al., 2009). The invasive behavior of the zebra mussel is seen as a disadvantage in its widespread use. This apparent disadvantage may represent one of the important reasons to ensure the conservation of native species by sampling *D. polymorpha*, which is both invasive and widely used in biomonitoring and toxic impact assessment studies (Binelli et al., 2015).This makes them excellent watchdogs for ecosystem health in freshwater ecosystems and also good model organisms for studying the interactive effects of temperature and pollution stress in the field (Negri et al., 2013).

In this study, it is aimed to examine the oxidative stress responses of SOD, CAT, GPx activities and TBARS and GSH levels in living organisms as a result of the application of REEs in mixed form to *D. Polymorpha* individuals with increasing temperature.

2.MATERIAL METHOD

2.1.Model Live Supply and Adaptation

D. polymorpha individuals were collected from the Euphrates River (38º 48 '25 ”N, 38º 43' 51” E). The organisms were quickly brought to the laboratory in plastic bottles. Before being used in the experiments, stock ponds were created by feeding microalgae in 500 L aerated reinforced tanks for at least 30 days in an environment similar to natural living conditions in a 12:12 hours light:dark cycle for adaptation to laboratory conditions. Healthy-appearing organisms at similar developmental stages were selected for the experimental study and were not fed during the experimental study.

2.2.Experiment Design

Model organisms were exposed to a 1:1:1:1 mixture of REE (Tb, Gd, La, Pr) at 3 different temperatures (16, 18, 20℃) and 125 mg/L concentrations for 24 and 96 hours. In particular, the global average surface temperature is expected to increase from 1.0 °C to 5.7 °C by the end of this century, depending on different CO_2 emission scenarios (IPCC 2022). In line with this information given in the literature, the study was designed by determining temperature values (16, 18, 20℃) close to the specified temperature averages.

125 mg/L Mix REE at 16 °C

125 mg/L Mix REE at 18 °C

125 mg/L Mix REE at 20 °C

Experiments were carried out with 3 replications.

2.3.Biochemical response

All application experiments were carried out with 3 repetitions and 7 *D. polymorpha* were used for each experimental group. Samples were taken from each group at 24 and 96 hours, and the soft tissues of the *D. polymorpha* individuals were collected by dissection, following the collection process they were stored at -80 degrees until they were analyzed. In this study, SOD, CAT and GPx enzyme activities and TBARS and GSH levels were determined with CAYMAN kits and ELISA test microplate reader to determine biochemical responses (Aydın & Serdar, 2023). CAYMAN brand SOD (Catalog No 706002), CAT Catalog No 707002) and GPx (Catalog No 703102), GSH (Catalog No 703002) and TBARS (Catalog No 10009055) were used in the study. The kits used in the study were purchased from CAYMAN.

2.4.Dissection Procedures and Preparation of Supernatants

Test organism individuals were separated from their shells with the help of scalpel and forceps. An average of 0.5 g from each organism was carefully weighed, then placed into 1:2 PBS (phosphate-buffered saline) buffer and homogenized with DAIHAN brand ultra turrax homogenizer while keeping everything on ice. The homogenized samples were then centrifuged at 4°C, at 17000 rpm for 15 minutes. The resulting supernatants were kept at -80 °C until the measurement was performed.

2.5.Statistical Analysis

SPSS 24.0 package program one-way ANOVA (Duncan 0.05) was used for the evaluation of biochemical analyzes.

3.RESULTS

3.1.Determination of Biochemical Response TBARS Level

Time-dependent TBARS levels at increasing temperature values at 125 mg/L concentrations of Mix REE are given in Figure 1. It was determined that the increases in TBARS levels in all exposure groups due to increasing temperatures compared to the control group were statistically significant ($p<0.05$).

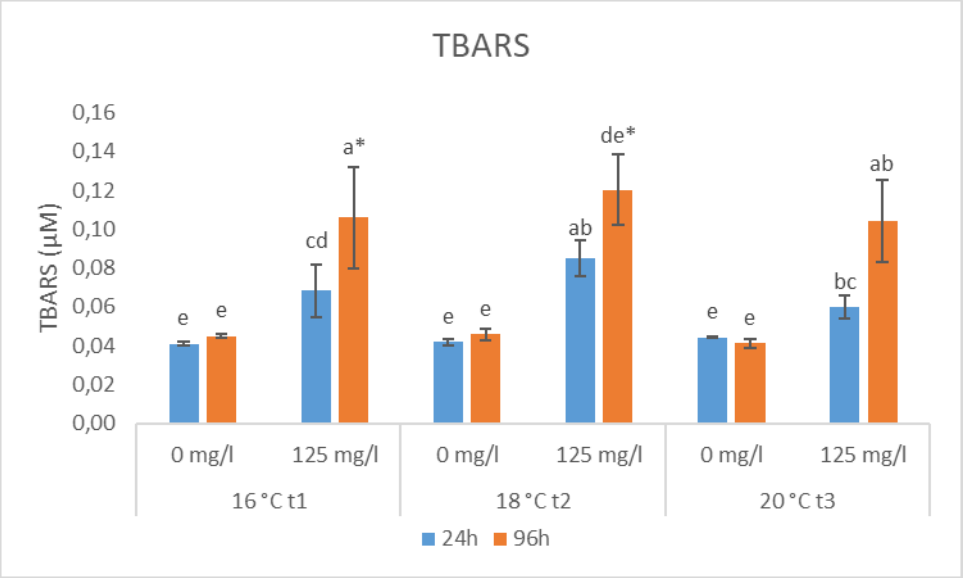

Figure 1: TBARS (µM tissue) levels of *D. polymorpha* exposed to Mix REE, different letters of the bar are statistically significant ($p<0.05$).

3.2.GSH Level

The time-dependent GSH levels of REE mixture at increasing temperatures at a concentration of 125 mg/L are given in Figure 2. While a statistically significant decrease was observed in GSH levels with 125 mg/L REE mixture at 16 and 20 ^{0}C compared to the control groups ($p<0.05$), no significant difference was observed at 18^{0}C ($p>0.05$).

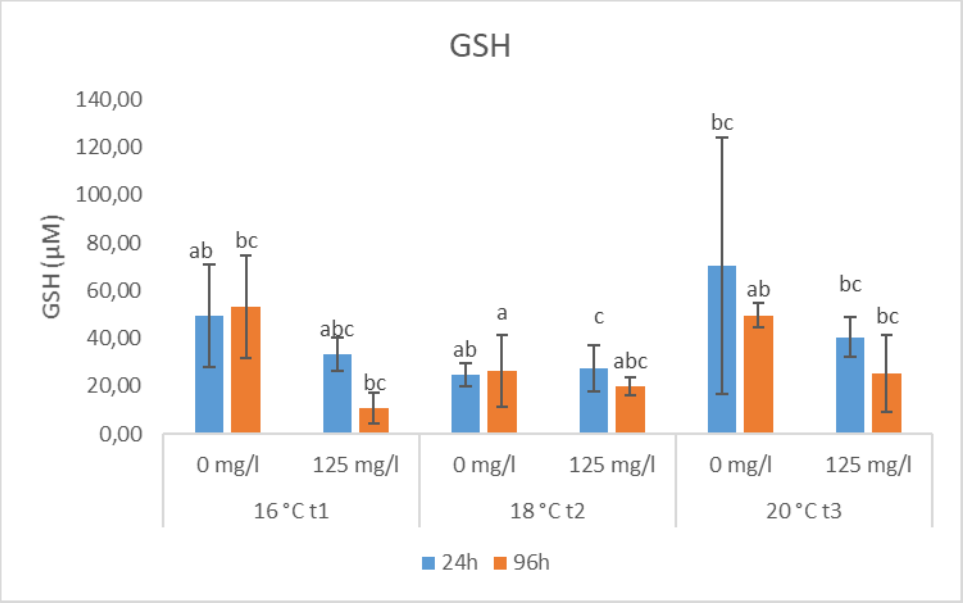

Figure 2: GSH (µM tissue) levels of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant ($p<0.05$).

3.3.CAT Activity

CAT activities in *D. polymorpha* exposed to 125 mg/L concentration of REE mixture and time dependent increase for tested temperatures are given in Figure 3. While there was a decrease in CAT activities at 16 ^{0}C in 24 hours, the decrease at the 96th hour was not statistically significant ($p>0.05$). While there was a decrease in CAT activities at 16 ^{0}C in 24 hours, the decrease at the 96th hour was not statistically significant ($p>0.05$).

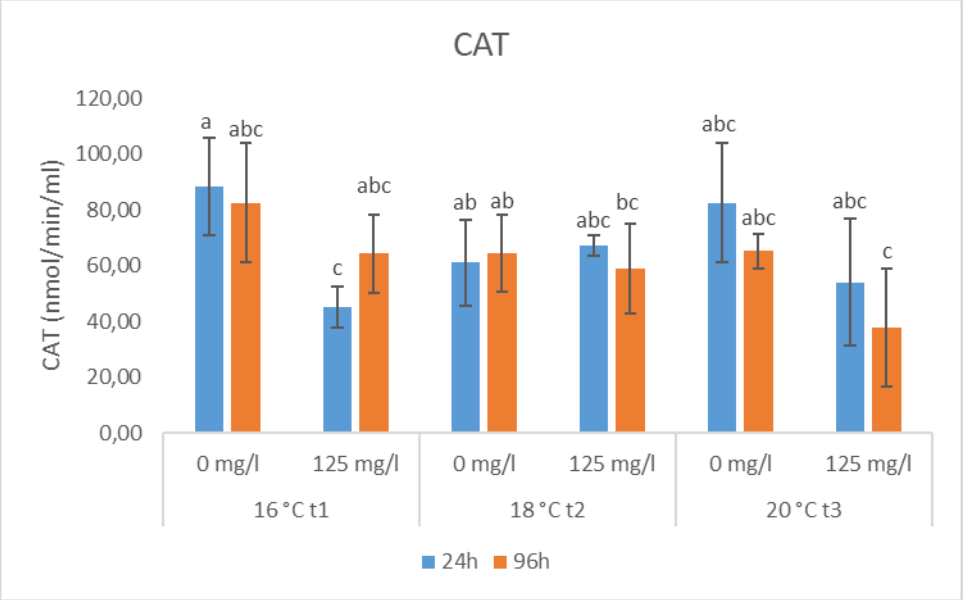

Figure 3: CAT (nmol/min/ml) activities of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant ($p<0.05$).

3.4.GPx Activity

GPx activities in *D. polymorpha* exposed to 125 mg/L of REE mixture at different temperatures over time are given in Figure 4. It was stated that the changes in all temperature groups after 24 and 96 hours compared to the control group were not statistically significant ($p>0.05$).

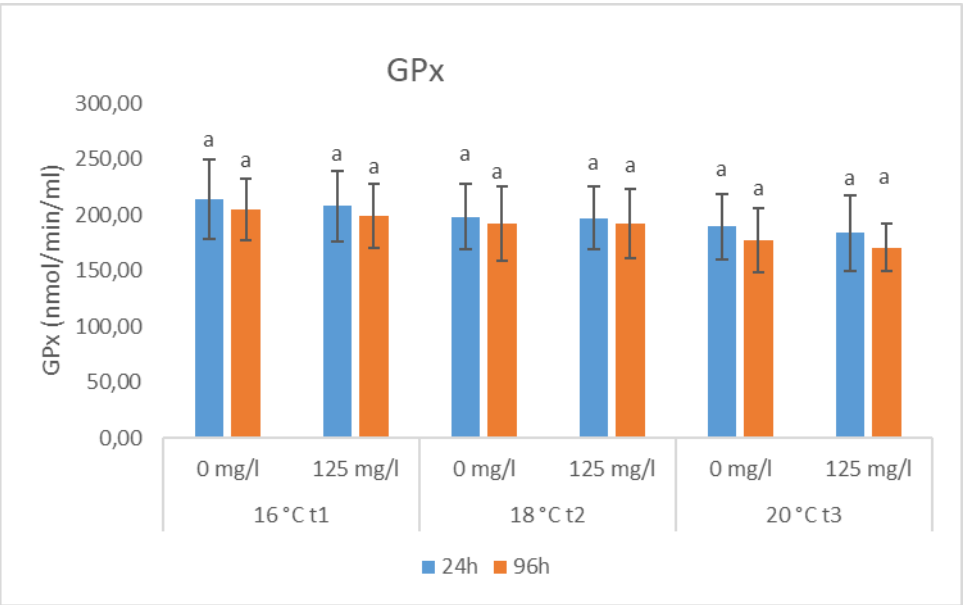

Figure 4: GPx (nmol/min/ml) activities of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant ($p<0.05$).

3.5.SOD Activity

Time dependent SOD activities at increasing temperature ratios at 125 mg/L of REE mixture are given in Figure 5. The reductions in SOD activities at 16, 18 and 20 °C temperatures compared to the control group were statistically significant ($p<0.05$).

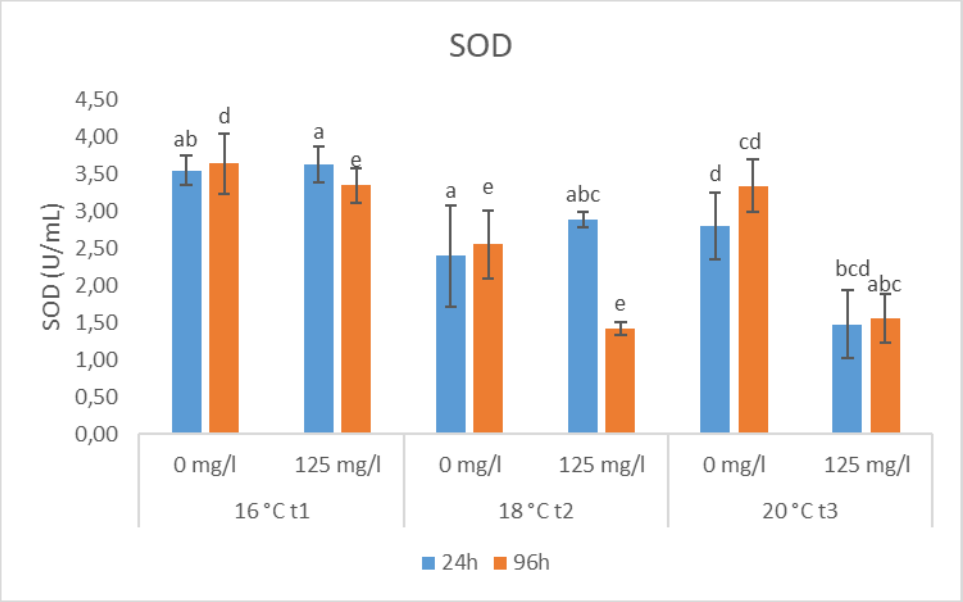

Figure 5: SOD (U/mL) activities of *D. polymorpha* exposed to REE mixture, different letters on the column are statistically significant ($p<0.05$).

4.DISCUSSION AND CONCLUSION

Many researchers have contributed to the literature with their studies investigating the effects of various pollutants on the environment as a result of the pollutants' interaction with increasing temperature. Vergauwen et al. 2013, they exposed zebrafish acclimated to 12, 18, 26 (standard temperature) and 34 °C to 5 µM cadmium for 4 or 28 days at the respective adaptation temperature and reported that oxidative stress parameters increased and mortality rates increased depending on the temperature. Abdel-Tawwab et al., 2017 examined the oxidative stress responses of Nile tilapia by co-exposing them to 0.0 or 0.5 mg Cd/L for 8 weeks at 20, 24, 28 and 32°C and they noted that SOD, CAT, GPx and GST activities were significantly induced due to Cd exposure and water temperatures reflecting the direct effect of Cd as a cell signaling molecule. Gholamhosseini et al., 2023 investigated the physiological response of the freshwater crayfish *Astacus leptodactylus* exposed to polyethylene microplastics at different temperatures (17 and 22 °C) and observed increases in SOD and CAT activities as a result. Zhang et al., 2023 investigated the antioxidant system against the combined effects of ammonia and temperature in *Procambarus clarkii* in their study and stated that the interaction between ammonia and temperature was significant in SOD, GPX, but not significant in CAT. Figueiredo et al., 2022 examined the single and combined ecotoxicological effects of ocean warming (15 and 19℃) on lanthanum exposure in *Spisula solida*, and stated that there were decreases in SOD, GPx and CAT activities as a result. It is thought that the changes in the enzyme activities that occur with REE mixture and increasing temperature in *D. polymorpha* are caused by the temperature values and concentration, and the results are thought to be in parallel with the studies in the literature.

Oxidative stress develops due to excessive accumulation of reactive oxygen species (ROS). It controls the physiological and chemical events that perform roughly all biotic and abiotic stresses (Demidchik, 2015). The role of various REEs in the redox imbalance leading to oxidative stress has been demonstrated in a number of independent studies in both plant and animal models, and many REEs have been reported to cause oxidative stress (Tseng et al., 2012; Wang et al., 2012; Zhao et al., 2013). Verlecar et al., 2007 they investigated the biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and heat, and as a result, they stated that there were increases in TBARS levels and increases in SOD and CAT activities. Banni et al., 2004 examined the biomarker responses in *Mytilus galloprovincialis* exposed to nickel and heat stress in their study and stated that as a result, CAT, SOD and GST levels increased significantly compared to the control. Park et al.,

2020 examined the antioxidant defense system responses of cadmium and high temperature combined stressors in zebrafish (*Danio rerio*) embryos and stated that there was an increase in SOD and CAT activities. Ihunwo et al., 2022 investigated the oxidative stress responses of young *Oreochromis niloticus* to some heavy metals under the simulation of increasing temperature and stated that there were decreases in GSH level, SOD and GPx activity. Mlouka et al., 2019 examined the biological responses of *M. galloprovincialis* to copper with increasing temperature and stated that there were increases in CAT and SOD activity. Lannig et al., 2006 investigated the co-effects of temperature and cadmium in *Crassostrea virginica* and determined decreases in GSH levels as a result. Additional stressors such as pollution may further sensitize mollusks to temperature-induced oxidative stress Falfushynska et al., 2014 Therefore, increased temperature and combined exposure to REE mixture are thought to affect the antioxidant capacity of *D. polymorpha* and caused differences in oxidative stress responses.

The increase in intracellular ROS due to HO overproduction was associated with a decrease in CAT expression (Venkatesan et al., 2006). Dubé et al., 2019 investigated the effect of 7 different REEs on the *Rainbow trout* and found that Yttrium (Y), Samarium (Sm), Erbium (Er) and Gadolinium (Gd) were the most toxic elements in fish, CAT and GST in Ce. They stated that its activity was down-regulated, and the most sensitive for the 7 elements examined were HSP72, GST, CYAP1A1, GADD45 and SOD for Y, Nd, Ce, Gd, Sm, La and Er, respectively. Hanana et al., 2021a five (cerium (Ce, 280 µg/L), lanthanum (La, 140 µg/L), neodymium (Nd, 120 µg/L), praseodymium (Pr, 28 µg/L) in *Oncorhynchus mykiss* and samarium (Sm, 23 µg/L) rare earth elements, investigated the toxicity of the mixture state and stated that there were increases in CAT activities at all concentrations of the mixture, SOD activity was not affected and GSH levels increased. Liu et al., 2023 examined the effects of neodymium in zebrafish and stated that CAT activity decreased. Hanana et al., 2021b examined the biomarkers of *rainbow trout* exposed to dysprosium (Ds) and lutetium (Lu), and there were significant changes in Dy exposure, CAT and SOD activity compared to controls. They stated that exposure to Lu was lower than control, and best differentiated it from SOD, CAT and MT. Andrade et al., 2023 examined the effect of yttrium (Y) in *Mytilus galloprovincialis* and observed decreases in CAT activity. Figueiredo et al., 2018 study, examined the effects in *Anguilla anguilla* under lanthanum exposure and stated that there were decreases in CAT activities in the internal organs. Huang et al., 2010 study, examined the biomarker responses induced by cerium in *Drosophila melanogaster* and observed reductions in CAT activities. Similar to Huang et al.'s data, the reduction in CAT activities that occurred in this study, as a result of exposing *D. polymorpha* to the REE mixture (Tb, La, Gd, Pr), proves the effectiveness of determined REE mixture concentration and the applied temperature increments.

It is well known that organisms can increase ROS production in the presence of a stressful situation, including the presence of pollutants. To avoid damage (including lipid peroxidation, protein carbonylation, and DNA damage) caused by ROS, organisms can increase the activity of antioxidant enzymes. Among these enzymes is SOD, which has the capacity to remove ROS (i.e. superoxide anion, hydroxyl radical and hydrogen peroxide) and protects organisms from cellular damage. However, this response normally occurs when oxidative stress is not too high or too long-lasting (Freitas et al., 2020). In the study, it is thought that oxidative stress causes a decrease in the biomarker of SOD in the organism. Similarly, Pastorino et al., 2021 examined the effects of cerium (Ce), scandium (Sc), neodymium (Nd), lanthanum (La), yttrium (Y) and praseodymium (Pr) on *Barbus balcanicus*; They stated that SOD and GST were higher in gills. Liu et al., 2023 examined the effects of neodymium in zebrafish and reported that there were decreases in SOD activity. Huang et al., 2010 study, examined the biomarker responses induced by cerium in *D. melanogaster* and stated that decreases in SOD activities occurred.

GSTs are a superfamily of Phase II detoxification enzymes involved in the detoxification of ROS and toxic xenobiotics. These enzymes can catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for detoxification purposes, and therefore, in the presence of contaminants, GST activity is induced to achieve efficient cell protection. *D. polymorpha* was exposed to REE mixture in this study and it is thought that the

change in the GSH level occurred due to the presence of the pollutant, its effective concentration and the effective temperature values tested. Similar to the results of the study, Liu et al., 2023 examined the effects of neodymium in zebrafish and reported that GSH-Px values increased. Freitas et al., 2020 examined the effects of Neodymium (Nd) in *M. galloprovincialis* and reported that GSH levels decreased. Henriques et al., 2019 examined the effects of gadolinium on *M. galloprovincialis* and reported that there were decreases in GSH levels as a result.

Malondialdehyde levels are a reliable indicator of lipid peroxidation (LPO). Lipid peroxidation initiates the damaging process by increasing the stiffness of cellular membranes (Nagarani et al., 2011). Liu et al., 2023 examined the effects of neodymium in zebrafish and reported that MDA content increased. Figueiredo et al., 2018 study, examined the effects in *A. anguilla* under lanthanum exposure and stated that there were significant differences in MDA levels in the internal organs. Huang et al., 2010 study, examined the biomarker responses induced by cerium in *D. melanogaster* and stated that cerium increased the MDA content. Yang et al., 2016 study, investigated the effect of yttrium in *M. aeruginosa* and observed increases in MDA levels. Serdar et al., 2019 study, examined the biochemical effects of Gadolinium exposure on *D. polymorpha* and observed increases in TBARS levels. In this study, it is thought that the tested pollutant concentration and temperature increments are effective in these increases in TBARS levels.

Glutathione peroxidase plays an important role in antioxidant defense and the reduction of lipids, hydrogen peroxides and organic hydroperoxides to H_2O and related alcohols (Arthur, 2000). Freitas et al., 2020 study, examined the toxicological effects of neodymium in *M. galloprovincialis* and stated that GPx did not change compared to the control. In this study, as in previous studies in the literature, it is observed that REEs alone or in a mixture cause oxidative stress in the organism by affecting the biological activities of the living organism.

CONCLUSION

It has been observed that there is a parallelism between the present study's data and previous studies in the literature. It is an undeniable fact that temperature affects all living organisms at every stage of their lives. Considering that temperature is effective in all biological and physical events of the living things, it can be thought that the effects of the pollutant are also affected by the temperature increase. According to the results of the study, it is considered that the combined use of REEs may cause environmental and water pollution if they mix with the environment even in trace amounts. In this case, it can be thought that this pollution effect may culminate with the temperature increase. Therefore, it is suggested that all kinds of pollutants released directly or indirectly to the environment should be minimized.

ETHİCAL APPROVAL

All authors declare that there is no ethical violation in this manuscript. Also, this manuscript does not contain data belonging to others. The authors declare that they have no conflict of interest. The authors alone are responsible for the content and authoring of the present paper.

CONSENT TO PARTİCİPATE

All authors have confirmed that this has not been published elsewhere and is currently not considered to be published elsewhere.

PERMİSSİON TO PUBLİSH

All authors agree that the article can be published.

CONFLICT OF INTEREST

All authors involved in the manuscript contributed to the article. There is no conflict of interest between the authors.

DATA AVAILABILITY STATEMENT

Since no new data was created or analyzed in this study, data sharing is not applicable to this article**.**

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Research Article
Araştırma Makalesi

Evaluation of Biochemical Content and Antioxidant Activity of *Pterocladiella capillacea* Algae

Pterocladiella Capillacea Alginin Biyokimyasal İçeriğinin ve Antioksidan Aktivitesinin Değerlendirilmesi

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Abstract: Red macroalgae are the basis of many commercially important food, pharmaceutical and other important industries. Research on these species has generally focused on improving seaweed cultivation, developing new methods to extract useful compounds or identifying new applications. In our study, the biochemical contents (total protein, carbohydrate and total phenolic substance) of *Pterocladiella capillacea* algae collected from the Black Sea were determined. In addition, antioxidant activity of *P. capillacea* species extracted by Soxhlet and ultrasonic-assisted maceration methods was investigated using 2 different methods (DPPH scavenging and iron reduction). The carbohydrate value of *P. capillacea* dry biomass was determined as 42% and protein value as 17%. In our study, where the effect of the extraction method on antioxidant activity and total phenolic substance was evaluated, it was determined that the Soxhlet method was more effective in total phenolic substance and iron reduction tests, while the ultrasonic-assisted maceration method was more effective in DPPH scavenging activity. In conclusion, the high carbohydrate content of *P. capillacea* species collected from the Black Sea (Türkiye) coasts and its potential use as a source of bioactive compounds causing antioxidant activity were highlighted.

Keywords
- Rhodophyta
- Extraction
- Antioxidant activity
- Total carbohydrate

Özet: Kırmızı makroalgler, ticari açıdan önemli birçok gıda, ilaç ve diğer önemli endüstrinin temelini oluşturur. Bu türler üzerine yapılan araştırmalar genellikle deniz yosunu yetiştiriciliğini iyileştirmeye, yararlı bileşikleri çıkarmak için yeni yöntemler geliştirmeye veya yeni uygulamalar belirlemeye odaklanmıştır. Çalışmamızda Karadeniz'den toplanan *Pterocladiella capillacea* alginin biyokimyasal içerikleri (toplam protein, karbonhidrat ve toplam fenolik madde) belirlendi. Ayrıca soxhlet ve ultrasonik destekli maserasyon yöntemleriyle ekstrakte edilen *P. capillacea türünün* 2 farklı metodla (DPPH süpürme ve demir indirgeme) kullanılarak antioksidan aktivitesi araştırılmıştır. *P. capillacea* kuru biyokütlesinin karbonhidrat değeri %42, protein değeri %17 olarak tespit edilmiştir. Ekstraksiyon metodunun antioksidan aktivite ve toplam fenolik madde üzerindeki etkisinin değerlendirildiği çalışmamızda toplam fenolik madde ve demir indirgeme testinde soxhlet metodunun, DPPH süpürme aktivitesinde ultrasonik destekli maserasyon metodunun daha etkili olduğu belirlenmiştir. Sonuç olarak, Karadeniz (Türkiye) kıyılarından toplanan *P. capillacea* türünün yüksek karbonhidrat içeriğine sahip olduğu ve antioksidan aktiviteye neden olan biyoaktif bileşik kaynağı olarak potansiyel kullanımını vurgulanmıştır.

Anahtar kelimeler
- Rhodophyta
- Ekstraksiyon
- Antioksidan aktivite
- Toplam karbonhidrat

1.INTRODUCTION

Marine macroalgae resources are gaining attention in the health and food cost industries due to their low cost and easy production. Among them, red algae (Rhodophyta): the largest group containing valuable bioactive compounds are used in cosmetics, food industry, pharmaceuticals, fertilizers and various supplements in food formula. Red algae are multicellular organisms that mostly live in the sea. It contains about 6000 species. (Aziz et al., 2021). Its cellular components, especially the cell wall polysaccharide composition, are quite different from other algal groups (Yoon et al., 2010). Red algae have been shown to contain a number of significant bioactive substances, including polysaccharides (alginate, agar, and carrageenan), lipids and polyphenols, steroids, glycosides, flavonoids, tannins, saponins, alkaloids, triterpenoids, anthraquinones, and cardiac glycosides. All of these bioactive compounds are now widely used as dietary and food supplements, emulsifiers, stabilizers and thickeners in the textile, food, cosmetics and pharmaceutical industries. Bioactive compounds obtained from marine macroalgae have attracted the attention of many researchers due to their various emerging biological activities and new beneficial properties. As a result, these substances can be used as a foundation for the development of new pharmaceutical substances intended for use as therapeutic and preventive agents, as well as dietary supplements, nutraceuticals, and functional food products (Torres et al., 2019; Khotimchenko et al., 2020). Macroalgae are especially preferred as a source of antioxidants.

Nowadays, the efforts of researchers to find natural alternatives to chemical compounds have increased considerably. It has been seen in studies in the literature that various techniques should be tried to reveal the best bioactive compounds. Extraction is of great importance in the recovery of phytochemicals from plant matrix and biomass. Many methods such as maceration, supercritical fluid extraction, filtration, microwave-assisted extraction, soxhlet method and ultrasonically assisted extraction are used for the recovery of bioactive molecules. Compared with traditional extraction techniques, ultrasonic assisted extraction is an efficient method as it can reduce the working time and solvent usage (Pollini et al., 2020; Tavakoli et al., 2021). Furthermore, this technique allows for low-temperature extraction, which minimizes heat loss from high temperatures and guarantees the preservation of bioactive compounds. Studies in the literature have shown that ultrasonically assisted extraction to extract bioactive compounds from different plant materials significantly reduces the extraction time and improves the overall targeted compound extraction yield compared to conventional methods (Lee and Lin 2007; Gam et al., 2020; Rashad et al., 2023).

In this study, it was aimed to determine the biochemical contents (chlorophyll a, chlorophyll b, carotenoids, total proteins, total carbohydrates) of dry biomass of red algae ***Pterocladiella capillacea*** collected from the Black Sea. In addition, the effects of ethanolic (70%) extracts prepared with different extraction methods (soxhlet and ultrasonic assisted extraction) on total phenolic substance and antioxidant activity were compared.

2.MATERIAL AND METHODS

2.1.Sample collection

Algae samples were collected (approximate wet weight 1 kg) from the coast of Kandıra district of Kocaeli province on September 29, 2024. Algae were brought to the laboratory, sorted and cleaned, and identified using identification books (Braune and Guiry, 2011; Bunker et al., 2017; Bothwell, 2023). The identified *Pterocladiella capillacea* (S.G.Gmelin) Santelices & Hommersand 1997 were dried in a drying oven (7 days at 50^{o}C).

2.2.Biochemical contents

2.2.1.Chlorophyll-a, chlorophyll-b, and total carotenoids contents measurement of dried biomass

The determination of chlorophyll-a, chlorophyll-b and total carotenoids of dried samples was performed by spectrophotometric methods according to Tavakoli et al. (2021). For pigment analysis, 20 mg of dried biomass was extracted with 3 mL of 95% glacial acetone. The absorbances of the samples were measured at 470, 648 and 664 nm spectrophotometrically. The amounts of chlorophyll-a, chlorophyll-b and total carotenoids (mg/g dried extract) were calculated according to the following equations.

$$Chla = 13.36 \times A664 - 5.19 \times A648 \quad (1)$$

$$Chlb = 27.43 \times A648 - 8.12 \times A664 \quad (2)$$

Carotenoids total = [(1000 × A470–1.63 × Chla - 104.96 × Chlb)/221] (3)

Chla: Chlorophyll-a; Chlb: Chlorophyll-b

2.2.2.Total protein

Total protein (TP) was included in the Bradford (1976) administration. Using 2 milliliters of 0.031 M citrate-phosphate buffer (pH: 5.5) solution, 0.01 grams of dried algal biomass were homogenized. The homogenized samples were centrifuged for 20 minutes at 14,000 rpm and +4°C, and the supernatants were then separated. 0.031 M Citrate-Phosphate buffer (pH: 5.5) and 0.01% Coomassie Brilliant Blue G-250 were added to the supernatants to create the combination. Using the standard chart (bovine serum albumin), the levels of protein were determined in milligrams per gram.

2.2.3.Total Carbohydrate

The total carbohydrate (TC) content was determined using the phenol-sulfuric acid method (Kochert, 1978). 1 mL of 5% phenol, 1 mL of an algal sample at a concentration of 0.5 mg/mL, and 3 mL of concentrated sulfuric acid were mixed together. After five minutes of incubation at 90 °C in a water bath, the mixture's absorbance at 490 nm was measured. The produced d-glucose standard graph was used to determine the samples' carbohydrate content.

2.2.4.Extract production

Soxhlet methods: 10 g of algal biomass was placed in a Soxhlet cartridge and extracted with 100 mL of ethanol (70%) for 8 hours in a Soxhlet device. Ultrasound assisted maceration (UAM): It was carried out using an ultrasonic bath (Wisd WiseClean) providing 100 W maximum power (W) at a fixed frequency of 30 kHz (f). 100mL of ethanol was added to 10g of algae biomass and mixed for 2 hours. Then, the mixtures placed in the ultrasound bath were extracted for 60 minutes by applying a power of 100 W (Tavakoli et al 2021). The extract obtained at the end of the extraction process was filtered through filter paper. Then, the solvents of the samples were removed under vacuum in a rotary evaporator at 55°C. A stock (at a concentration of 10 mg/mL) was prepared from the extracts and stored at +4°C until its use for the experiment (Semerci et al., 2020).

2.2.5.The total phenolic contents (TPC)

The TPC of the extracts was determined, as mentioned in the previous work (Semerci et al., 2020). First, 200 µL of 50% Folin-Ciocalteu reagent was combined with 100 µL of extract (0.5 mg/mL) and left for three minutes. The absorbance at 760 nm was then measured after 1 mL of a 3% sodium carbonate solution was added and left in the dark for 60 minutes. A calibration curve was employed for the gallic acid standard, and TPC extract was expressed as milligram per gram (mg GA g^{-1}).

2.3.Antioxidant activity

2.3.1. 2,2-difenil-1-pikrilhidrazil (DPPH) radical scavenging

The modified Blois method was used to determine the DPPH radical scavenging activity (Blois, 1958). 1 mL of the algae extract prepared at different concentrations (0.1-1 mg/mL) was taken and 1 mL of 0.04% DPPH was added. The quickly mixed mixtures were incubated in the dark at 25°C for 30 minutes. At the end of the period, the samples were read on a spectrophotometer at 517 nm. The DPPH% radical scavenging activity was determined using the Equation (4):

$$DPPH\%\ radical\ scavenging = \left(\frac{control\ absorbance - extract\ absorbance}{control\ absorbance}\right) x\ 100 \quad (4)$$

2.3.2. Reducing power

According to Oyaizu (1986), the extract's reducing power was measured. 1 mL of extract was mixed with 2.5 mL of phosphate buffer and 1% potassium ferricyanide at a certain concentration (0.1-1 mg/mL). For 25 minutes, this mixture was incubated at 50°C. 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 2500 g for 15 minutes. 0.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 500 µL of 1% (w/v) $FeCl_3$. Distilled water served as a blank when the absorbance value was measured at 700 nm.

2.4. Statistical analysis

Results of experiments performed in 3 replicates are presented as mean values ±95% confidence limits. Analysis of variance was performed using ANOVA procedures. Significant differences between means in antioxidant activity were determined at $P<0.05$ level by Tukey's pairwise comparison test.

3.RESULTS AND DISCUSSION

3.1. Biochemical contents

The use of different marine macroalgae (seaweed) as sources of bioactive compounds has the potential to industrialize a renewable natural

resource that has so far been underutilized. Macroalgal biomasses have been shown to produce a wide range of nutrients and bioactive secondary metabolites (Patarra et al., 2011; Biris-Dorhoi et al., 2020). In our study, the chlorophyll content and carotenoid values of the red alga *P. capillacea* are given in Figure 1. The chlorophyll a value in dry biomass was determined as 2.8 mg/g, chlorophyll b as 0.6 mg/g and carotenoid value as 0.86 mg/g ($p<0.05$). In a study conducted in Egypt, *P. capillacea* species was reported to have approximately chlorophyll a in 0.5 mg/g, chlorophyll b 0.17, and carotenoids 0.1 mg/g (El-Din and El-Ahwany 2016). Differences between studies are possible because the physical conditions of the algae collection site affect the pigment content of the algae. In particular, conductivity, turbidity, dissolved oxygen, sulfate and geographical location were found to be important in explaining the differences in pigment content(Hodgson et al., 2004; . Voerman et al.,2022).

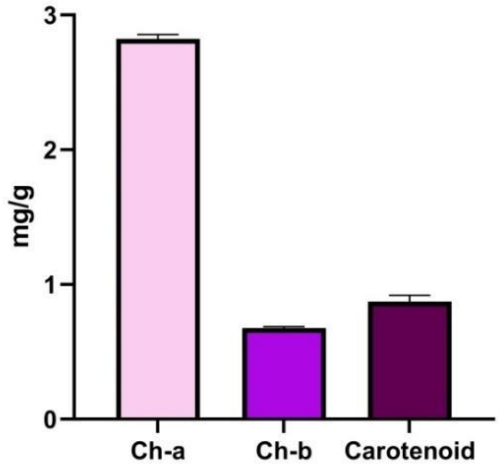

Figure 1. Chlorophyll-*a*, chlorophyll-*b*, and total carotenoid content [mg g^{-1}] (±standard deviation) in *P. capillacea*.

Combined with their textural properties, the use of algae as functional foods seems worth investigating. Regardless of the nutritional value of food products, their acceptability depends on the consumer's cognitive experiences and organoleptic properties. Some algae can bring bitterness from protein-derived peptides, saltiness from high mineral content (e.g. Na and K), and sweetness from soluble sugars (e.g. mannitol). Algae are known as representatives of the fifth taste, umami. In addition, algae have very characteristic aromas and smells transmitted by volatile compounds. Determination of the biochemical contents of new algae groups is important in revealing organoleptic properties (Mouritsen et al., 2012; Francezon et al., 2021). Macroalgae have a protein content that can range from 7% to 31% of dry weight and a lipid content that can range from 2% to 13% of dry weight (Fleurence et al., 2018; Kazir et al., 2019). Significant amounts of carbohydrates can also be found in macroalgae (32–60% dry weight). In our study, the carbohydrate value of *P. capillacea* dry biomass was determined as 42% and protein value as 17% ($P<0.05$) (Table 1). The protein and carbohydrate content were found to be within the range of the values given in the literature. In a study investigating the biochemical content of *P. capillacea* species collected from the Gulf of Alexandria (Egypt), the total protein value of the dry weight was reported as 18.47% and the total carbohydrate value as 51.36% (Ashour et al., 2020). The protein value of *P. capillacea* species collected from the coast of Portugal was found as 20.19% and 19.76% carbohydrate (Paiva et al., 2017). Since it is known that light, temperature and inorganic contents of the collection region affect the biochemical content of algae, it is possible that there are differences in the biochemical contents of algae collected from different regions (Voerman et al.,2022).

Table 1. Biochemical content of *P. capillacea* algae (DW: dry weight).

Biochemical contens	***P. capillacea***
Total protein content (% DW)	17±0.8
Total carbohydrate content (% DW)	42±0.4
Soxhlet TPC (mg GA/g)	22±1.2
UAM TPC (mg GA/g)	19±0.7

The high carbohydrate content of the strain we used in our study (such as polysaccharides beta-glucans, sulfated polysaccharides, cellulose, and others) suggested that these strains could aid bowel movements as dietary fiber for the digestive system. Additionally, these species might be helpful in controlling blood serum cholesterol and glucose levels by the use of nutraceuticals or dietary additives.

Ethanol and water mixtures are widely used in the isolation of antioxidant compounds due to their many advantageous properties such as low toxicity and suitability for food use (Dai et al., 2010; Do et al., 2014; Semerci et al., 2020). In

the studies conducted in the literature, it has been found that aqueous alcohol mixtures are effective in the recovery of other compounds, including phenolic compounds, from various macroalgae and microalgae (Monteiro et al., 2020; Andriopoulos et al., 2022). Polar solvents are generally the preferred solvent when the target compounds are polar antioxidants such as polyphenols and tannins (Semerci et al., 2024). In our study, based on this information, *P. capillacea* species was extracted using the Soxhlet and UAM methods by diluting the aqueous ethanolic solvent. It was determined that the total phenolic compound was higher in the extract prepared by the Soxhlet method (22 mgGA/g). In a previous study on *P. capillacea*, the TPC value of the ethanolic extract obtained by the maceration method was reported as 15.23 mgGA/g (De Alencar et al., 2016). In another study, the total phenolic content of *P. capillacea* was evaluated with a different method and the total phenolic compound content was found to be approximately 1100 µg g^{-1} FW. It was also found that the phenolic content decreased due to increased Cd toxicity (Schmidt et al., 2016). In a study investigating the phenolic content of *P. capillacea* species in Egypt in two different seasons, it was reported that the total phenolic content varied between 17.79 - 16.85 mg/g (Ashour et al.,2020). The method used, the place where the algae are collected and the nutrient medium produced appear to be effective on the phenol content.

3.2. Antioxidant activity

Antioxidants prevent or slow down the oxidation of these molecules by providing an electron-rich environment to the compounds that are likely to undergo oxidation. The complex properties of herbal antioxidants, which have few negative health impacts, include solubility, structure, production, mechanism of action, and kinetics (Neupane and Lamichhane 2020). Therefore, at least two test systems have been proposed to determine the in-vitro antioxidant activities of crude plant extracts. In the current investigation, two different methods have been used to assess the antioxidant activity of extracts. While the DPPH approach focuses on the radical scavenging characterisation of pure compounds, the reducing power method focuses on reducing antioxidant characterization (Gupta, 2015). In our study, the %DPPH scavenging activity of ethanolic extracts obtained using soxhlet and ultrasonically assisted maceration methods is given in Figure 2.

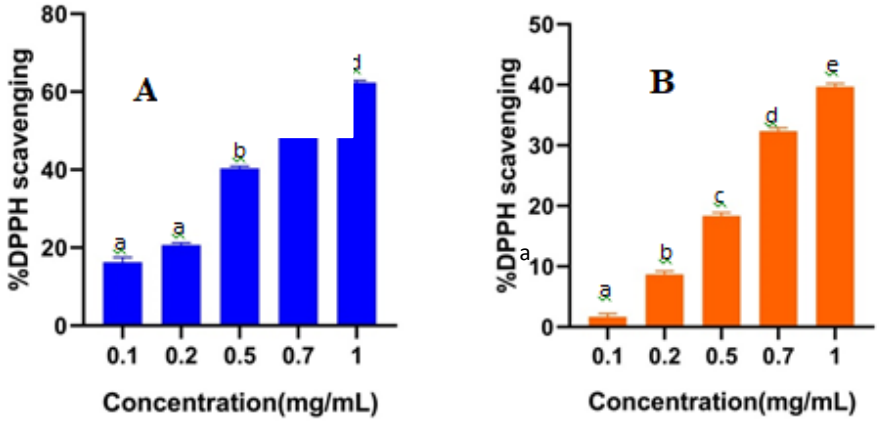

Figure 2. %DPPH scavenging activity of extracts prepared by ultrasonic-assisted maceration (A) and soxhlet methods (B). Bars with the same lower case letter (a–e) are not significantly (P>0.05) different.

At a concentration of 1 mg/mL, the extract produced by the ultrasonically assisted maceration approach was shown to scavenge the DPPH radical by 60%. At the same concentration, it was found that the extract made using the Soxhlet technique scavenged DPPH by 40%. In our study, it was observed that the extracts obtained with the ultrasonic-assisted maceration method had higher DPPH scavenging activity than the extracts obtained with the Soxhlet method. Another study reported that the DPPH scavenging rate of *P. capillacea* ethanolic

extract at a concentration of 1 mg/mL was 30% (De Alencar et al., 2016). It has been seen in the literature that different extraction methods are effective in revealing antioxidant activity (Ashour et al., 2020).

The reducing capacity of *P. capillacea* macroalgae was determined by measuring the amount of reducing agent in the sample. Reducing agents are substances that show antioxidant activity by donating a hydrogen atom and breaking free radical chains. In our study, the iron reducing capacities of extracts adjusted in the concentration range of 0.1-1 mg/mL were determined. The reducing agents present in the solution promote the reduction of the Fe 3+ /ferrocyanide complex to the ferrous form (Fe 2+), which can be measured in the absorbance at 700 nm. The greater the absorbance of the mixture at 700 nm, the greater the antioxidant activity of iron reduction.(Saadatmand et al., 2011). The results showed a dose-dependent increase in reducing power in both extraction methods ($P<0.05$) (Figure 3). The iron reducing power of the extract prepared by ultrasonic assisted maceration method increased in the concentration range of 0.1-1 mg/mL depending on the dose. There was a significant increase in the iron reducing power of the extract obtained by Soxhlet extraction between 0.1-0.7 mg/mL depending on the dose. But the iron reducing power reached saturation in the concentration range of 0.7-1 mg/mL. Unlike the DPPH scavenging test, it was observed that the extracts made with the Soxhlet method showed higher reducing power than the extracts made with UAM.

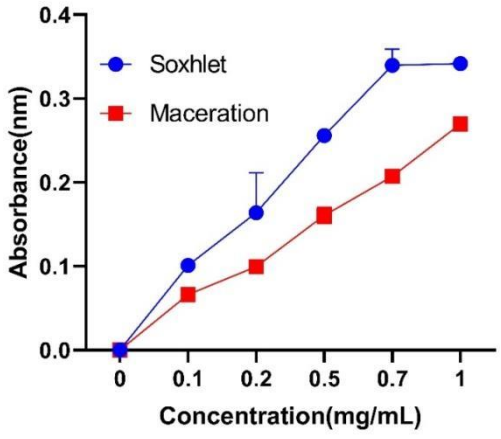

Figure 3. Iron reduction analysis results of the extract.

It was revealed that Soxhlet technique was more effective in revealing iron reducing power and phenolic content. The use of total phenolic compounds as reducing agents has been documented in the literature. It is also known that chemicals that easily enter into redox reactions can produce high levels of activity in the Folin-Ciocalteu method (Singleton et al., 1965; Dorman and Hiltunen 2004). The connection between phenolic compounds and iron reduction is supported by these data. Despite the strong iron binding properties of polyphenols, it is a matter of debate whether the iron chelation ability of polyphenol groups containing catechol or gallol plays an important role in their antioxidant activity (Perron and Brumaghim, 2009). However, researchers have found that catechins, which are important representatives of polyphenols, have antioxidant properties at all concentrations and have attributed the antioxidant behavior to iron chelation (Sugihara et al., 2001). According to Wang et al. (2009), phenolic compounds have low metal chelation abilities. Other compounds such as carbohydrate/protein (agar, carrageenan and alginate), carotenoids and lipids found in algae are also known to have metal chelation abilities and there is evidence that they have inhibitory effects on the absorption of iron ions (De Alencar et al., 2016; Hentati et al., 2022; Premarathna et al., 2024). In our study, it is thought that the iron reducing power is significantly high due to the carotenoid group and other secondary and primary compounds in red algae in addition to phenolic compounds.

4. CONCLUSION

Our study determined that *P. capillacea*, a red algae (Rhodophyta) with rich biochemical content, has a carbohydrate value of 42% and a protein value of 17% in dry weight. Pigment values in dry biomass were determined as 2.8 mg/g for chlorophyll a, 0.6 mg/g for chlorophyll b and 0.86 mg/g for carotenoids. Due to the high carbohydrate content of *P. capillacea* species, this macroalgae can be evaluated as a potential candidate in food insecurity, micronutrient deficiencies and sustainable food scarcity problems. Ultrasonic maceration method was first tried in the extraction of *Pterocladiella capillacea* species and the effect of this method on antioxidant activity was evaluated comparatively with the Soxhlet method. In the

results, it was determined that the Soxhlet method was more effective in total phenolic substance and iron reduction tests. It was determined that the ultrasonically assisted maceration method was more effective in revealing the DPPH scavenging activity of *P. capillacea* species.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Fiction: YS, ABS; Literature: AGT, YS, SE, TOS; Methodology: AGT, YS, SE, SA, TOS; Performing the experiment: ABS, TOS; Data analysis: YS, SA, AGT; Manuscript writing: YS, ABS, TOS Supervision: ABS, TOS. All authors approved the final draft.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Research data is not shared.

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Review
Derleme

Diseases, Economic Losses and Treatment Methods in Trout (*Oncorhynchus mykiss*) Farming

Alabalık (*Oncorhynchus mykiss*) Yetiştiriciliğinde Görülen Hastalıklar, Ekonomik Kayıplar ve Tedavi Yöntemleri

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Abstract: Trout farming is important in aquaculture production worldwide and appeals to a wide range of consumers due to its high protein content and nutritional value. However, various diseases seen in trout negatively affect production processes and food safety, and can cause environmental problems and behavioral disorders by affecting the welfare of the fish due to the use of chemicals (formaldehyde, copper sulfate, etc.) used for treatment. In this article, some of the diseases that are frequently encountered in trout and cause economic losses are examined and the effects of these diseases on trout farming are discussed. Diseases seen in trout are generally caused by bacterial, viral, parasitic and fungal pathogens. While these factors seriously threaten the health of trout, they also cause economic losses to producers such as high treatment costs and production losses. In addition, measures taken to prevent the spread of diseases generally require improving water quality, increasing hygienic measures and applying chemical treatment methods. Moreover, problems such as excessive use of drugs and the development of antibiotic resistance complicate this process. As a result, disease prevention, early intervention and the development of effective treatment methods are critical for sustainable production in the sector for trout producers. This article aims to provide important information on disease management and control strategies in the trout farming industry.

Keywords
- Trout
- Diseases
- Production
- Economic losses
- Treatment

Özet: Alabalık yetiştiriciliği, dünya genelinde su ürünleri üretimi içerisinde önemli bir yere sahip olup, yüksek protein içeriği ve besleyici değeri nedeniyle geniş bir tüketici kitlesine hitap etmektedir. Ancak, alabalıklarda görülen çeşitli hastalıklar, üretim süreçlerini, gıda güvenliğini olumsuz etkilemekle birlikte, tedavi için kullanılan kimyasallar (formaldehit, bakır sülfat vb.) kullanımından dolayı çevresel sorunlara, balıkların refahını etkileyerek davranış bozukluklarına neden olabilmektedir. Bu makalede, alabalıklarda sıkça karşılaşılan ve ekonomik kayıplara neden olan hastalıklardan bazıları incelenmiş, bu hastalıkların alabalık yetiştiriciliği üzerindeki etkileri ele alınmıştır. Alabalıklarda görülen hastalıklar, genellikle bakteriyel, viral, parazitik ve mantar kökenli patojenlerden kaynaklanmaktadır. Bu etmenler, alabalıkların sağlık durumunu ciddi şekilde tehdit ederken, üreticilere yüksek tedavi maliyetleri ve üretim kayıpları gibi ekonomik zarara uğratmaktadır. Ayrıca, hastalıkların yayılmasını önlemek amacıyla alınan tedbirler, genellikle su kalitesinin iyileştirilmesi, hijyenik önlemlerin artırılması ve kimyasal tedavi yöntemlerinin uygulanması gerekliliğini ortaya koymaktadır. Diğer yandan, aşırı ilaç kullanımı ve antibiyotik direncinin gelişmesi gibi sorunlar da bu süreci karmaşıklaştırmaktadır. Sonuç olarak, alabalık üreticileri için hastalıkların önlenmesi, erken müdahale ve etkili tedavi yöntemlerinin geliştirilmesi, sektörde sürdürülebilir bir üretim için kritik öneme sahiptir. Bu makale, alabalık yetiştiriciliği sektöründeki hastalık yönetimi ve kontrol stratejileri hakkında önemli bilgiler sunmayı amaçlamaktadır.

Anahtar kelimeler
- Alabalık
- Hastalıklar
- Üretim
- Ekonomik kayıp
- Tedavi

1. INTRODUCTION

Trout (*Oncorhynchus mykiss*) is one of the most cultivated freshwater fish species worldwide. These fish, cultivated specifically for freshwater resources, reach a large consumer base, especially due to the growing trend of healthy eating. Trout is a food source with high nutritional value, low-fat content, and rich in protein. These characteristics make trout popular among health-conscious individuals. This increasing demand has elevated the economic importance of trout production, which constitutes a significant part of the aquaculture sector in many countries (FAO, 2020). Trout farming serves as a major source of income for many countries, both for local consumption and export (Arslan & Yıldız, 2021). Trout production is one of the fastest growing parts of the aquaculture industry in Europe, North America and some parts of Asia (Başçınar, 2004). For example, countries such as Norway and Chile have a significant share of global trout production, while Türkiye, China and Japan are also important trout producers (FAO, 2020). However, trout production faces many environmental, biological and economic challenges.

Trout farming is directly affected not only by environmental factors and water conditions, but also by various diseases seen in trout. As an organism living in aquatic environments, trout can encounter many pathogens. Trout are susceptible to bacterial, viral, parasitic and fungal diseases, and the rapid spread of these diseases can threaten fish health and lead to high economic losses. For example, in the case of viral diseases such as Infectious Pancreatic Necrosis (IPN) and Viral Hemorrhagic Septicemia (VHS), losses are seen at a rate of 70-80%, while this rate can be seen as 5-20% in bacterial and fungal diseases (Georgiadis et al., 2001; Lafferty et al., 2015).

Diseases are one of the biggest threats to trout production. Many of these diseases weaken the immune system of the fish and can cause high mortality rates (Georgiades et al., 2016; Behringer et al., 2020). Particularly, diseases with high mortality rates pose a great economic threat to producers (Perera et al., 2005). Bacterial infections can spread rapidly, especially in cases of poor water quality, while viral diseases are another significant threat to trout producers. Additionally, parasitic and fungal diseases can spread rapidly in polluted waters or areas where fish are intensively farmed (Nguyen, 2024).

Trout are especially vulnerable to diseases when they are young and sensitive individuals. It has been observed that diseases spread more rapidly and mortality rates are higher in juvenile trout (Yilmaz et al., 2011; Griffin et al., 2013). In addition, when trout are raised in intensive farming conditions, stress levels increase, facilitating the rapid spread of diseases. These diseases encountered in trout farming not only threaten the health of the fish, but also threaten the jobs of fish producers. Epidemic diseases caused by pathogens such as IPN, VHS, Bacterial Cold Water Disease (BCWD) can lead to high mortality rates such as 70-80%, especially in young fish (0.3-15 g), resulting in production losses, increased treatment costs and potential market losses (Meyer, 1991; Lieke et al., 2020). Combating these various diseases in trout farming can lead to significant economic challenges. Diseases can seriously reduce efficiency of trout production and disrupt the financial balance in the sector. The removal of infected fish, their treatment, and rehabilitation lead to increased costs. In addition, fish mortalities due to diseases cause losses to producers (Bauer et al., 2023).

The economic impact of diseases is not limited to direct production losses. They can also cause problems in the marketing of fish. The introduction of infected fish to the market can cause reputation damage to trout producers and reduce consumer confidence. Especially in this period of increasing global competition, the spread of diseases can directly affect a producer's commercial success (Bondad-Reantaso et al., 2005). Additionally, the use of chemicals such as antibiotics, medications, and disinfectants to treat certain diseases adds to production costs and damages profitability (Waagbø & Remø, 2020; Bauer et al., 2021).

The economic impact of diseases is especially pronounced for small-scale enterprises. Since small businesses operate with less capital compared to large-scale producers, losses caused by diseases can quickly weaken them financially (Blanco et al., 2000). Furthermore, the additional infrastructure and technological solutions required to prevent the spread of diseases come at a high cost, making it difficult for producers to maintain sustainable operations, which negatively affects the competitive structure of the sector (Opiyo et al., 2018).

Prevention and treatment of diseases in trout farming is of critical importance for producers. In

order to prevent the spread of diseases, it is necessary to ensure the hygiene of production areas, regulate water quality and implement strategies that strengthen the immune system of fish. In addition, it is important to balance biological, environmental and management factors for healthy trout farming (Ferreira, 2007; Maldonado-Miranda, 2022). Therefore, protection should be provided with immersion vaccination when the fish reach 2-3 grams of weight and injection vaccination after 20 grams of weight, and attention should be paid to stock density, feeding and size.

Antibiotics and vaccines are frequently used in the treatment of bacterial diseases, while vaccines have been developed abroad for the treatment of viral diseases, but they are not yet used in our country. In addition, immunostimulants are used to help strengthen the immune system of fish. However, excessive use of antibiotics for treatment can lead to new health problems such as antibiotic resistance in fish, which can complicate future treatment processes (Kan & Kubilay, 2024). Instead, studies on alternative solutions such as biological control methods, vaccines and genetic selection have an important place in the fight against diseases. In addition, it is possible to prevent the spread of diseases with early detection of diseases, rapid intervention and effective management strategies (Glover et al., 2017; Robinson et al., 2023).

2. DISEASES IN TROUT

2.1. Bacterial Diseases

Bacterial diseases are important common infections in trout and pose a significant threat to fish health. Bacterial pathogens weaken the immune system of fish and cause diseases to spread rapidly (Radosavljević et al., 2022; Duman et al., 2023). Bacterial diseases are more common in environments with poor water quality, high stress, and inadequate hygiene (Brunt et al., 2007; Chapela et al., 2018).

2.1.a *Aeromonas salmonicida*

Aeromonas species are frequently encountered pathogens in trout, especially in fish production facilities, and can cause serious diseases. In addition to the most common pathogen, *Aeromonas hydrophila*, species such as *Aeromonas sobria* and *Aeromonas caviae* can also cause infections in trout. These bacteria lead to serious clinical symptoms such as septicemia (blood infections), skin lesions, ulcers and bleeding in fish. In addition, they can cause digestive system infections with symptoms such as diarrhea, bloating and weight loss (Cao et al., 2020). Aeromonas infections are usually more common due to factors such as stress, poor water quality, overcrowding and lack of hygiene. Although antibiotics can be used to treat infections, the treatment process can be difficult, especially due to the resistance developed by *A. hydrophila* to antibiotics. Therefore, monitoring water quality, minimizing stress factors and providing proper care are critical for disease in prevention (Yang et al., 2021).

2.1.b Columnaris

Columnaris is a bacterial infection that affects fish, including trout, and is caused by *Flavobacterium columnare*. This bacterium becomes more active at high temperatures and in conditions such as poor water quality and stress. Columnaris disease spreads rapidly in fish and can cause significant economic losses. The disease typically presents as skin lesions and wounds (Austin & Austin, 2016). In more advanced stages, fish may show symptoms such as respiratory distress, swelling, digestive problems and bleeding. Columnaris weaken the immune system and multiplies rapidly in the body, especially under stressful and intensive farming conditions. Antibiotics and appropriate water conditions play a crucial role in treating the disease, but resistance developed by *Flavobacterium columnare* to some antibiotics can complicate treatment. Regular monitoring of water temperature, pH and oxygen levels is necessary for early intervention and to prevent the spread of the disease (Evenhuis et al., 2015).

2.1.c *Vibrio anguillarum*

Vibrio species are significant pathogens in trout and other aquatic organisms. Species such as *Vibrio anguillarum*, *Vibrio ordalii* and *Vibrio harveyi*, cause vibriosis in fish. Vibrio infections are generally more common in warmer waters (above 14 degrees) and are characterized by symptoms such as skin lesions, ulcers and bleeding in fish. (Lieke et al., 2020; Mondal & Thomas, 2022). *V. anguillarum* is the most prevalent agent and can cause septicemic infections and inflammation in trout, seriously affecting their overall health (Yang et al., 2021; LaFrentz et al., 2022). *Vibrio* species are easily transmitted to fish due to factors such as weak immune systems, stress, poor water quality, and high-density. In advanced stages of infections, fish may show clinical signs such as decreased appetite, severe respiratory distress, and death.

Although antibiotics can be used to treat these infections, *Vibrio* species often develop antibiotic resistance, making treatment more difficult. Therefore, improving water quality, managing stress factors, and ensuring hygienic conditions are essential for preventing infections (Evenhuis et al., 2015; Urku et al., 2024).

2.1.d *Yersinia ruckeri*

Yersinia species, particularly *Yersinia ruckeri* and *Yersinia pseudotuberculosis*, are known pathogens in fish and can cause a disease called yersiniosis (Pajdak-Czaus et al., 2019). These bacteria can cause serious health problems, especially in freshwater fish such as trout. *Y. ruckeri* causes enteric redmouth disease in trout, resulting in symptoms such as hemorrhagic septicemia (blood poisoning) and bleeding. *Y. pseudotuberculosis*, though less common, can cause slow-developing infections and intestinal problems in trout (Hickey & Lee, 2018; LaFrentz et al., 2022). These bacteria become more active and spread rapidly under factors such as weakened immune systems, stress, poor water quality, and high-density rearing. Treatment is typically done with antibiotics, but since Yersinia species can develop antibiotics resistance, proper water management, hygiene and stress reduction are vital to preventing diseases (Chettri et al., 2012).

2.1.e *Lactococcus garvieae*

Lactococcus species, especially *Lactococcus garvieae*, are important pathogens in trout and can cause lactococcosis. *L. garvieae* presents with symptoms such as septicemia, inflammation, and skin lesions in trout (Semwal et al., 2023). This bacterium is usually more active in fish with weakened immune system overcrowded conditions or poor water quality. Infected fish may exhibit symptoms such as general weakness, decreased appetite, difficulty breathing, and swelling of the eyes (Abdelsalam et al., 2023. As the disease progresses, bleeding, skin ulcers, and white pus lesions can be observed in infected fish. In addition, since some strains of *L. garvieae* can develop resistance to antibiotics, the treatment process can be challenging. Therefore, the best strategy for preventing the disease is to ensure healthy rearing conditions by regularly monitoring water quality, providing appropriate nutrition, and minimizing stress (Juárez-Cortés et al., 2024).

2.1.f *Flavobacterium psychrophilum*

Fry Mortality Syndrome is a bacterial infection that causes significant economic losses in trout farming. The causative agent of the disease is a gram-negative bacterium called *Flavobacterium psychrophilum*. This bacterium is generally effective in cold waters below 18°C and causes high mortality, especially in young individuals (fry trout). The disease can be seen in acute and chronic forms and usually manifests itself with symptoms such as fin rot, skin lesions, anemia and loss of appetite (Henryon et al., 2005). The disease is transmitted through direct contact, contaminated water or equipment. In addition, vertical transmission, i.e. the transfer of bacteria from infected broodstock to eggs, is also an important means of spread (Bebak et al., 2007). Treatment and control methods include the use of antibiotics, vaccine development studies and biosecurity measures. However, the development of resistance to antibiotics has limited effective treatment methods. Improving environmental conditions, reducing intensive stocking rates and protecting water quality are of critical importance in controlling the disease. Additionally, alternative approaches such as probiotic and herbal supplements that enhance the immune response to the disease are also being investigated. Future genetic resistance development studies hold promise in reducing the effects of cold water disease (Boyacioglu & Akar 2012).

2.2. Treatment of Bacterial Infections

Bacterial infections are diseases that are frequently seen in trout and need to be treated. Bacteria such as *Aeromonas salmonicida* can lead to the death of trout. Antibiotic treatment is widely used for such infections, but excessive use of antibiotics can lead to the development of antibiotic resistance (Radosavljevi et al., 2022; Semwal et al., 2023). Therefore, it is important to use antibiotics in a controlled manner and only when necessary. Antibiotic treatment is a common method to control bacterial diseases. Antibiotics used for diseases such as aeromonosis can stop the spread of infection and help fish recover. However, the use of antibiotics can have harmful effects on the environment and accelerate the development of resistance (Brunt et al., 2007; Duman et al., 2023). Therefore, antibiotics should only be used with the advice of a veterinarian and at the correct dose.

2.3. Viral Diseases

Viral diseases can be extremely devastating for trout and often spread rapidly, leading to major production losses. Since there is currently no medication available to treat viral fish

diseases, preventive measures are of great importance. Viral fish infections usually require quarantine, vaccinations, and early intervention (Mancheva et al., 2021).

2.3.a Infectious Hematopoietic Necrosis (IHN)

Infectious Hematopoietic Necrosis is a fatal viral disease seen in trout, typically causing high mortality rates in young fish. The IHN virus affects the hematopoietic (blood-forming) tissues of fish, leading to bleeding in internal organs, necrosis (tissue death), and weakening of the immune system (Wang et al., 2024). This disease can spread rapidly in trout production facilities causing significant losses. IHN usually manifests through changes in the swimming behavior of fish, making it difficult for them to move. Deterioration of water quality paves the way for the spread of the disease (Dupuy et al., 2019). There is no effective treatment method for IHN, but strategies such as improving water quality, implementing quarantine measures, and isolating infected fish can be applied to control the disease. Vaccination is also used as an effective method for preventing IHN in some countries (Lin et al., 2022).

2.3.b Viral Hemorrhagic Septicemia (VHS)

Viral Hemorrhagic Septicemia is another viral disease commonly seen in trout. VHS causes bleeding, swelling and necrosis in the organs of fish. This disease can rapidly increase mortality rates in trout and, in some cases the entire fish population (Kasai & Nishikawa, 2018; Baillon et al., 2020). VHS virus poses a significant threat especially to cold-water fish, and becomes more active during periods of increased water temperature. The effect of VHS not only increases fish mortality rates, but also leads to economic losses due to the spread of the disease (Danion et al., 2012; Oslon, 2013). Treatment of Viral Hemorrhagic Septicemia is usually done using antiviral drugs, but treatment methods are limited. Therefore, biosecurity measures and quarantine practices are crucial for preventing the spread of the disease (Mohammadisefat et al., 2023).

2.3.c Infectious Pancreatic Necrosis (IPN)

Infectious Pancreatic Necrosis is a viral infection that predominantly affects juvenile trout. IPN is a disease that affects the pancreas of trout and can be fatal. It is more frequently observed in young fish and in densely populated fish farms. The disease is caused by the IPNV (*Infectious Pancreatic Necrosis Virus*) and the virus spreads through water. In trout, this disease is characterized by necrosis (death) of the pancreatic tissue, which leads to digestive and metabolic disorders in the fish (Pajdak-Czaus et al., 2021; Chandra, 2024).

Symptoms of IPN include weakness, swimming near the surface, abdominal swelling, bloody stools, and death. Once the virus enters the body of trout, it can spread rapidly and mortality can be high, especially in fish with weak immune systems. To prevent the spread of IPN, isolation from infected fish, attention to hygiene conditions and regular monitoring of water quality are required. In addition, there are some vaccines and antiviral treatment methods available to prevent and control the disease (Terech-Majewska, 2016; Chandra, 2024).

2.4. Treatment of Viral Infections

Viral diseases cannot be treated with antibiotics, so the fight against viral diseases is usually limited to preventive measures. There is no effective treatment for diseases such as Infectious Pancreatic Necrosis (IPN), Viral Hemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN), so prevention of diseases is much more important. However, in some cases, antivirals such as interferon can be used (Wang et al., 2024). Vaccination plays an important role in trout farming in order to prevent viral diseases. For diseases such as Viral Hemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN), water vaccination methods are widely used (Lin et al., 2022; Mondal & Thomas, 2022). Vaccination helps prevent the spread of diseases by providing immunity to fish. However, vaccination programs may vary depending on the type of disease and regional conditions (Pajdak-Czaus et al., 2021; Mohammadisefat et al., 2023). Vaccines are generally inactive and are applied as immersion during the juvenile period (2-3 g), while when they are 20-30 g, vaccination is done by injection to prevent such diseases. In addition, vaccination strengthens the immune system due to factors such as environmental stress and water quality.

2.5. Parasitic Diseases

Parasitic diseases are infections that affect the health of trout and spread rapidly when environmental conditions are poor. Parasites are usually found on the skin, gills and internal organs of fish. Parasitic diseases cause loss of appetite, slow growth and often even death in trout.

2.5.a Whirling Disease (*Myxobolus cerebralis*)

Myxobolus cerebralis causes a parasitic disease known as "whirling disease" in trout. This disease increases mortality rates, especially in young trout, and causes damage to the skeletal system of fish (Kaeser, 2006; Fetherman et al., 2011). Whirling disease causes trout to swim in circles by constantly turning their heads, which prevents them from performing normal swimming movements. The disease causes serious deformations in organs such as the gills and spine. The disease can spread rapidly in environments with poor water quality and where fish are raised intensively. If left untreated, whirling disease can lead to death (Sarker et al., 2015; Akram et al., 2023). Practices to combat whirling disease include improving water quality, quarantining infected fish and using biological control methods.

2.5.b White Spot Disease (*Ichthyophthirius multifiliis*)

White spot disease is a common and contagious disease in trout, caused by the parasite *Ichthyophthirius multifiliis*. This parasite settles on the skin, gills and body surface of fish, creating white, cottony lesions (spots) on fish. White spot disease is more prevalent in stressed fish, especially when triggered by factors such as sudden changes in water temperature, poor water quality or overcrowded environments. The parasite can be transmitted from infected fish to healthy fish through direct contact or contaminated water (Lahnsteiner, & Weismann, 2007).

White spot disease can cause significant losses if left untreated. Special drugs and salt water baths can be used among the treatment methods. In addition, it is important to isolate infected fish and control water parameters such as temperature and pH to prevent the disease from spreading. Increasing the water temperature during the treatment can accelerate the cycle of the parasite and make the treatment more effective (Lieke et al., 2020; Abu-Elala et al., 2021; Mondal & Thomas, 2022).

2.5.c *Ichthyobodo necator* (Costia)

Costia (*Ichthyobodo necator*) is a protozoan parasite commonly found in trout and other freshwater fish, causing a disease called costiosis. Costia causes infection by settling on the surface of the skin, especially the gills, of fish. Symptoms of costiosis are respiratory distress, skin irritation, decreased appetite, weight loss and heavy breathing. In more severe cases, opacity, swelling and bleeding in the eyes of infected fish may also occur (Mallik et al., 2015). Costia infections are often triggered by stressful conditions, poor water quality and overcrowding. The infection can spread rapidly, so early diagnosis and treatment are essential. Chemicals such as formalin, potassium permanganate or chloramine T are usually used to treat costiosis, though the treatment process may vary depending on factors such as water temperature and pH levels (Balta et al., 2019).

2.6.Treatment of Parasitic Infections

Parasitic diseases are another important factor that threatens the health of trout. Chemical treatment methods are frequently used in the treatment of Myxobolus cerebralis and other parasitic pathogens (Akram et al., 2023). These treatment methods include chemical substances such as organophosphates, formalin, chloramine T and potassium permanganate.

2.7.Fungal Infections

Fungal infections are common, especially in injured fish. *Saprolegnia* spp. is the most common pathogen causing fungal infections in trout. This fungus forms white, cottony lesions on the body surface of trout and affects the fish's organs, often leading to death (Pavić et al., 2022). Fungal infections are more likely to occur when the fish's immune system is weakened. *Saprolegnia* infections are often associated with poor water quality and stressful conditions (Tedesco et al., 2021).

2.7.a *Saprolegnia poses*

Saprolegnia species are pathogenic fungi that can cause serious infections in trout and other aquatic organisms. *Saprolegnia* poses a significant risk of infection, especially when the fish's immune system is weak, under stressful conditions, in poor water quality, and or due to physical injuries. The most common species is *Saprolegnia parasitica* which can spread rapidly by infecting the skin, gills, and wound areas of the fish (Tedesco et al., 2021). *Saprolegnia* infections typically appear as white or gray cotton-like growths. These lesions develop on the skin or gills and cause tissue damage. In the early stages, infected fish may show symptoms such as decreased appetite, general weakness, difficulty breathing, and swelling. If left untreated, the infection can progress, leading to hemorrhages, skin necrosis (tissue death), and death. In addition, the fungus can settle in the gills, making it difficult for the fish to absorb oxygen, which can be fatal (Pavić et al., 2022).

2.7.b *Achlya flagellata*

Achlya species are pathogenic fungi found in aquatic environments that can cause fungal infections in fish. While similar to *Saprolegnia* species, Achlya infections are less common in fish, but can be more problematic, especially at low temperatures and in environments with poor water quality (Choudhury et al., 2014; Pavić et al., 2022). Achlya infections manifest as white, cottony or gray lesions, typically located on open wounds, non-healing tissues, and damaged skin. These fungi weaken the immune system of the fish, causing inflammation, tissue necrosis and sometimes bleeding in the infected areas. Infected fish may experience symptoms such as decreased appetite, weakness, difficulty breathing, swelling and severe skin damage (Tedesco et al., 2021).

2.8.Treatment of Fungal Infections

Fungal infections such as *Saprolegnia spp*. usually occur as a result of poor water quality and injuries. Methods used for fungal treatment include antifungal drugs and iodine solutions. If left untreated, fungal infections can cause serious health problems in fish (Lindholm-Lehto & Pylkkö, 2024). Fungicides such as potassium permanganate are added to the water to prevent the spread of infections.

3.INNOVATIVE TREATMENT METHODS AND FUTURE DİRECTIONS IN TROUT FARMING

Disease management and treatment methods in trout farming should be continuously monitored, and new methods should be developed. Recent scientific and technological advancements have made it possible to manage trout diseases more efficiently and in a more environmentally friendly manner (Shah et al., 2014).

In trout farming, the development of disease-resistant fish species stands out as a promising approach that could revolutionize disease management in the future. Genetic modifications can reduce the risk of diseases being transmitted to trout or strengthen their immune systems. This can be particularly effective in preventing the spread of viral diseases (Sarmasik et al., 2002; Ødegård et al., 2011).

In recent years, studies on more advanced vaccination techniques such as DNA vaccines and peptide vaccines have gained momentum in trout. These vaccines provide more effective protection by specifically targeting the immune system of fish. In addition, the fact that these vaccines can be applied without harming the environment is a significant advantage in trout farming. Future research will focus on developing multiple vaccine combinations and genetically engineered vaccines to prevent a broader spectrum of diseases. Moreover, combined vaccination strategies may be developed to target both bacterial and viral diseases (Imtiaz et al., 2024). In addition to synthetic and chemical treatments, probiotics and prebiotics represent important biotechnological applications that support the immune system of fish. By improving the natural bacterial flora, probiotics can prevent the proliferation of pathogens and increase the resistance of fish to diseases. Probiotic bacteria, especially *Lactobacillus* spp. and *Bacillus* spp., colonize the intestinal system of trout and strengthen immune responses (Mugwanya et al., 2021). Probiotic applications also help reduce antibiotic usage and minimize environmental impacts. In addition, fermentation techniques supported by prebiotics improve feed conversion rates in trout, helping to produce healthier and more resilient fish. These biotechnological approaches enable trout producers to make disease management more sustainable (Rohani et al., 2022). Biotechnology and nanotechnology offer revolutionary innovations in the treatment of trout diseases. Nanotechnological treatment methods provide more efficient and targeted application of antibiotics and antifungal drugs to fish. Nanoparticles accelerate the treatment process by delivering drugs directly to infected areas, increasing the effectiveness of therapeutic compounds (Seethalakshmi et al., 2021).

Excessive use of chemical and synthetic drugs commonly used in the treatment of fish diseases can negatively affect fish health. Therefore, in recent years, treatment methods with medicinal aromatic plants have attracted attention and are being researched as natural and environmentally friendly alternatives (Ghiasi et al., 2022). Medicinal aromatic plants contain oils known for their antiviral, antimicrobial and antifungal properties. Plants such as thyme, garlic extract, tea tree, mint and lavender contain substances such as carvacrol, thymol and eugenol, which are particularly effective plants against pathogens. These components have a broad spectrum even at low temperatures and have been reported to be effective against pathogens that negatively affect fish health (Reda et al., 2024).

4.CONCLUSION AND RECOMMENDATIONS

Diseases encountered in trout farming can lead to serious economic losses for both producers and the industry. However, effective disease management is achievable through appropriate environmental management, treatment methods and technological advancements. Measures such as improving water quality, limiting antibiotic use and implementing biotechnological solutions are fundamental strategies to control the spread of diseases and protect the health of trout.

In the future, the use of genetic engineering, vaccination strategies and smart technologies will provide more effective results in disease management. In addition, the adoption of biotechnological applications and sustainable production methods will be critical for both protecting the environment and minimizing economic losses.

The management of trout diseases is an evolving field that requires continuous research and development. Future studies will focus on new treatment methods, the development of disease-resistant fish species and sustainable farming techniques, enabling trout production to become more efficient and environmentally friendly.

Preventing and treating diseases in trout farming is essential for maintaining healthy production. Early detection of diseases, selecting the correct treatment methods and preventing the spread of infections can reduce mortality rates and prevent economic losses.

In conclusion, an integrated approach is required for effective management of diseases in trout farming. Key factors for sustainable production include improving water quality, reducing stress, early diagnosis of infections, and using appropriate doses of drugs and chemicals.

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Bu bölümde çalışmanın yürütülmesinde alınan etik kurul onayının alındığı kurum, tarih ve numarası belirtilir. Omurgalı hayvanlarla yürütülen çalışmalarda Yerel Etik Kurul Onayı, anket/mülakat çalışmalarında ise Girişimsel Olmayan Araştırmalar Etik Kurulu Onayı gerektirdiği halde beyan edilmeyen makaleler bilimsel değerlendirmeye alınmamaktadır.

Örnek-1: Bu çalışmada deney hayvanları kullanılmaması nedeniyle Yerel Etik Kurul Onayı alınmamıştır.

Örnek-2: Bu çalışma Isparta Uygulamalı Bilimler Üniversitesi Hayvan Deneyleri Yerel Etik Kurul onayı ile yürütülmüştür (Tarih: 01.07.2010, No: 21438139-147).

VERİ KULLANILABİLİRLİK BEYANI
Bu bölümde makalede kullanılan verilerin anonim kullanılabilirliğine ilişkin beyanda bulunulmalıdır. Acta Aquatica Turcica dergisi, yazarları araştırma verilerini paylaşmaya teşvik etmektedir.

Örnek-1: Bu çalışmada kullanılan veriler Figshare platformunda ttps://doi.org/10.6084/m9.figshare.11815566.v1 DOI adresi ile erişime açıktır.

Örnek-2: Bu çalışmada kullanılan verilere ilgili yazardan talep üzerine erişilebilir. Veriler, gizlilik veya etik kısıtlamalar nedeniyle kamuya açık değildir.

Örnek-3: Bu çalışmada kullanılan veriler makul talep üzerine ilgili yazardan temin edilebilir.

Örnek-4: Bu çalışmada yeni veri oluşturulmadığı veya analiz edilmediği için veri paylaşımı bu makale için geçerli değildir.

Örnek-5: Araştırma verileri paylaşılmaz.

Örnek-6: Bu çalışmada kullanılan veriler bu makalenin ekinde mevcuttur.

ATIFLAR

Atıflar yıl sırasına göre ve aralarında noktalı virgül (;) olacak şekilde aşağıdaki formatlarda yazılır:

- Tek yazar:
(Yazar, yıl)
-- … olduğu düşünülmektedir (Küçük, 2008; Güçlü, 2018a; Güçlü, 2018b).
-- Küçük (2008)'e göre …

- İki yazar:
(Yazar-1 ve Yazar-2, yıl)
-- … önemli parametreler arasında yer almaktadır (Küçük ve Güçlü; 2001; Ekici ve Koca, 2021a; Ekici ve Koca, 2021b).
-- Ekici ve Koca (2021b)'a göre …

- Üç ve daha çok yazar:
(Yazar vd., yıl)
-- … dönemsel olarak tekrarlayabilmektedir (Yiğit vd., 2006a; Yiğit vd., 2006b; Boyacı vd., 2020)
-- Boyacı vd. (2020)'e göre …

KAYNAKLAR

Kaynaklar APA 7. versiyona göre yazılmalıdır. Tüm yazarların isimleri verilmelidir, ancak 10. yazardan sonra "vd." kısaltması da kabul edilmektedir. Özel kullanımlar hariç olmak üzere tüm eser türlerinde eser isminin sadece ilk harfi büyük, eserin yayınlandığı veya sunulduğu dergi, yayınevi, kongre isimlerinde geçen tüm kelimeler büyük harfle başlanarak yazılmalıdır.

1-Makale

Dergi ismi kısaltılmadan (italik), cilt (italik), sayı, sayfa numaraları ve aktif link içerecek şekilde DOI numarasına yer verilmelidir:

Petrauskienė, L., Utevska, O., & Utevsky, S. (2009). Can different species of medicinal leeches (Hirudo spp.) interbreed? Invertebrate Biology, 128(4), 324-331. https://doi.org/10.1111/j.1744-7410.2009.00180.x

Wagenaar, D. A., Hamilton, M. S., Huang, T., Kristan, W. B., & French, K. A. (2010). A hormone-activated central pattern generator for courtship. Current Biology, 20(6), 487-495. https://doi.org/10.1016/j.cub.2010.02.027

2-Kitap

Kitap başlığı italik olacak şekilde ve yayın kuruluş ismi olacak şekilde verilmelidir.

Nesemann, H., & Neubert, E. (1999). Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. Spektrum Akademischer Verlag.

Sawyer, R. T. (1986). Leech biology and behavior. Oxford University Press.

3-Kitap bölümü

Bölüm başlığı normal, kitap başlığı italik olacak şekilde, editör(ler), bölümün sayfa numaraları, yayıncı kuruluş ve varsa aktif link içerek şekilde DOI numarasına yer verilmelidir:

Le Couteur, D., Kendig, H., Naganathan, V., & McLachlan, A. (2010). The ethics of prescribing medications to older people. In S. Koch, F. M. Gloth, & R. Nay (Eds.), Medication management in older adults (pp. 29-42). Springer. https://doi.org/10.1007/978-1-60327-457-9_3

McCormack, B., McCance, T., & Maben, J. (2013). Outcome evaluation in the development of person-centred practice. In B. McCormack, K. Manley, & A. Titchen (Eds.), Practice development in nursing and healthcare (pp. 190-211). John Wiley & Sons.

4-Web sitesi

Sayfa başlığı italik, websitesinin ismi ve sayfanın aktif linki olacak şekilde verilmelidir.

International Union for Conservation of Nature. (2010). Chondrostoma nasus. https://www.iucnredlist.org/species/4789/97800985

Wikipedia. (2021). Toxicology. https://en.wikipedia.org/wiki/Toxicology

5- Tezler

Tez başlığı italik olacak şekilde, tez türü (Doktora, Yüksek lisans, Tıpta Uzmanlık) ve üniversite ismi belirtilmelidir.

Filik, N. (2020). Kültür balıklarından izole edilen Aeromonas hydrophila suşlarında fenolik bileşenlerin çevreyi algılama sistemi üzerine inhibisyon etkisi ve suşlar arasındaki klonal ilişkinin pulsed fıeld jel elektroforez yöntemiyle belirlenmesi [Doktora tezi, Isparta Uygulamalı Bilimler Üniversitesi].

Özdal, A. M. (2019). Effects on growth and coloration of red pepper suplementation as pigment sources to diets of jewel cichlid (Hemichromis guttatus) [Yüksek lisans tezi, Isparta Uygulamalı Bilimler Üniversitesi].

6- Konferans, sempozyum sunumları

Etkinlik tarihi, sunu başlığı (italik), sunum türü (Sözlü sunum, Poster sunum), etkinlik adı, şehir ve ülke verilmelidir.

Ceylan, M., Çetinkaya, O. (2017, Ekim 4 - 6). Assessment of population structure and size of medicinal leech Hirudo verbana, inhabiting some model wetlands of Turkey [Sözlü sunum]. International Symposium on Limnology and Freshwater Fisheries, Isparta, Türkiye.

Snoswell, C. (2016, Ekim 31 - Kasım 3). Models of care for store-and-forward teledermatology in Australia [Poster sunum]. 7th International Conference on Successes and Failures in Telehealth, Auckland, Yeni Zelanda.

NOT: Dergi yazım kurallarına uygun olarak hazırlanmayan makaleler değerlendirmeye alınmayacaktır.