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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

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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.

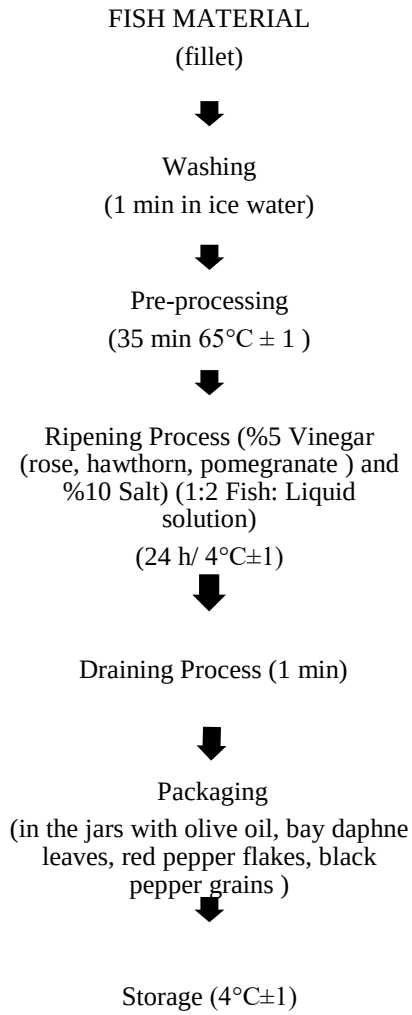

Figure 4. The Processing Stages of Cooked Twaite Shad Marinades.

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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