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Research Article Araştırma Makalesi

# Effect of Ultrasound Applications on Physico-Chemical, Sensory, and Microbiological Quality of Rainbow Trout (*Oncorhynchus mykiss* W.)

## Ultrason Uygulamalarının Gökkuşağı Alabalığının (*Oncorhynchus mykiss* W.) Fiziko-Kimyasal, Duyusal ve Mikrobiyolojik Kalitesi Üzerine Etkisi

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**Abstract:** Ultrasound, whose use has increased in recent years, is one of the most important food processing methods that provides to maintain food quality and increase food safety. The effect of ultrasound treatment on some quality characteristics of vacuum-packed rainbow trout (Oncorhynchus mykiss W.) fillets during cold storage was determined. Experimental groups were formed using different power and duration variables (30%W/5 min.; 30%W/15 min.; 80%W/5 min, and 80%W/15 min.). The samples were stored at 4±1ºC until physico-chemical (moisture, pH, color, and texture), sensory, and microbiological analyses were performed. The effect rates of ultrasound application on the evaluated quality characteristics differed from each other. While there was a significant difference between the groups in the decrease in moisture, increase in pH value and changes in sensory parameters, the change depending on storage was not observed within the group. The decrease in colour and texture values was prevented to a certain extent. It was observed that the effect of ultrasound applications on microbiological inactivation varied according to the type of bacteria and application conditions. The lowest increase in the number of the total mesophilic aerobic bacteria, the total psychrophilic aerobic bacteria, and the lactic acid bacteria was detected in the 80W/5 min. group, while Enterobactericeae and Pseudomonas spp. were detected in the 80 W/15 min. group. The highest inactivation was observed in Pseudomonas spp. bacteria. Our results showed that ultrasound can be an important alternative food processing method to maintain the quality characteristics and food safety of fish meat.

**Keywords**
- Cold storage
- Food quality
- Food safety
- *Oncorhyncus mykiss*
- Ultrasound

**Özet:** Son yıllarda kullanımı artan ultrason, gıda kalitesinin korunmasını ve gıda güvenliğinin artırılmasını sağlayan en önemli gıda işleme yöntemlerinden biridir. Vakum paketlenmiş gökkuşağı alabalığı (Oncorhynchus mykiss W.) filetolarının soğukta muhafazası sırasında ultrason uygulamasının bazı kalite özellikleri üzerine etkisi belirlenmiştir. Farklı güç ve süre değişkenleri (%30W/5 dk.; %30W/15 dk.; %80W/5 dk. ve %80W/15 dk.) kullanılarak deney grupları oluşturulmuş ve örnekler fiziko-kimyasal (nem, pH, renk ve tekstür), duyusal ve mikrobiyolojik analizler yapılana kadar 4±1ºC'de depolanmıştır. Ultrason uygulamasının değerlendirilen kalite özellikleri üzerindeki etki oranları birbirinden farklılık göstermiştir. Nem düzeyindeki azalma, pH değerindeki artış ve duyusal parametrelerde meydana gelen değişimler gruplar arasında önemli bulunurken ($p<0.05$), depolamaya bağlı değişim grup içinde gözlenmemiştir. Renk ve tekstür değerlerindeki azalma ise belli oranda engellenmiştir. Ultrason uygulamalarının mikrobiyolojik inaktivasyon üzerindeki etkisinin bakteri türüne ve uygulama koşullarına göre değiştiği gözlenmiştir. Toplam mezofilik aerobik bakteri, toplam psikrofilik aerobik bakteri ve laktik asit bakteri sayısında en düşük artış 80W/5 dk. grubunda tespit edilirken, Enterobacteriaceae ve Pseudomonas spp. sayısındaki en düşük artış 80W/15 dk. grubunda tespit edilmiştir. En yüksek inaktivasyon Pseudomonas spp. bakterisinde gözlenmiştir. Sonuçlarımız, ultrasonun balık etinin kalite özelliklerini ve gıda güvenliğini korumak için alternatif bir gıda işleme yöntemi olarak kullanılabileceğini göstermiştir.

**Anahtar kelimeler**
- Soğuk depolama
- Gıda kalitesi
- Gıda güvenliği
- *Oncorhyncus mykiss*
- Ultrason

# 1. INTRODUCTION

The sustainability of public health depends on reliable, healthy, balanced nutrition and quality food. Physical, chemical, sensory, and biological parameters are among the most sought-after characteristics in food safety. The most widely used food preservation m,ethod to ensure food safety is heat preservation (Raso and Barbosa-Canovas, 2003). Today, the application of "non-thermal methods" to foods has gained importance in order to eliminate the negative effects of high temperatures on the structure of food (Elez-Martinez et al., 2005). Consumers increasing demand for safe and nutritious food is expanding the different technologies used in the food industry (Türksönmez and Diler, 2021). Non-thermal methods include high hydrostatic pressure, ultrasound, pulsed electric field, pulsed light, ohmic heating, irradiation, and microwave (Karabacak, 2015). The advantages of ultrasound compared to heat treatment are that the loss of sensory and chemical properties of the food is much less, the time used is saved, and a higher percentage of enzymes and microorganisms are inactivated (Ashokkumar et al., 2008). Furthermore, in the framework of the "Green Food Processing" concept, ultrasound is used to ensure high quality and safe food (Chemat and Ashokkumar, 2017). In the literature, ultrasound-assisted salting of beef (Sanches et al., 2021), curing of meat varieties (Inguglia et al., 2018), marinating of chicken meat (Xiong et al., 2020), drying of beef (Ojha, Kerry, and Tiwari, 2017), control of microorganisms in beef and poultry meat were performed (Piñon et al., 2020). Besides, in meat products, a number of studies have focused on evaluating quality characteristics in meat emulsions (Leães et al., 2020), Italian salami (Alves et al., 2018), bacon (Pan et al., 2020), frankfurter-type sausages (Zhang et al., 2021), cooked ham (Barretto et al., 2018), and dry fermented sausage processing (Alves et al., 2020). Although there are many studies on red and white meat in the literature, studies on fish meat are more limited.

Recently, fish is considered an important protein source due to its nutritional components and the knowledge of the effects of nutrients on our health. However, lipids, proteins, and non-protein nitrogenous compounds in fish meat are exposed to post-mortem biochemical reactions. In addition, fish is one of the most perishable foods because it provides a very good environment for spoilage-causing microorganisms (Esteves et al., 2016). For this reason, the transportation of fish meat in the cold chain until it reaches the consumer or the shelf life before consumption can be ensured by cold storage. The quality changes of fish meat under cold storage conditions are directly related to food safety and should be monitored in a controlled manner. Rainbow trout (*Oncorhynchus mykiss* W.) provide a sustainable source of protein through aquaculture. The prevalence of fresh consumption necessitates the preservation of quality characteristics during cold storage and its sustainability in terms of public health. Therefore, our study consists of monitoring the changes in some quality characteristics of cold stored rainbow trout (*O. mykiss* W.) fillets exposed to ultrasound waves at different powers.

# 2. MATERIALS AND METHODS

## 2.1. Sample preparations

The material was obtained from rainbow trout reared in Sapanca Inland Fisheries Production Research and Application Unit of Istanbul University Faculty of Aquatic Sciences. 12 kg rainbow trout with an average weight (225-250 g) were filleted together with their skins under hygienic conditions. For rainbow trout fillets, the lower vacuum packing material was selected with a thickness of 150 µ and the upper vacuum packing material with a thickness of 80 µ. Fillet samples were vacuum packed using a conveyor vacuum machine (Multivac R 240, Germany). Operating conditions: 20 mbar air vacuum stop point from 1000 mbar and 0.6 seconds vacuum time. The vacuumed fillets (*n*=90) were kept at (4±1°C) for the duration of ultrasound application and analysis.

## 2.2. Ultrasound treatment

Fillet samples were placed in a 1.5 liter plastic container in the ultrasound device with probe in the presence of 1 liter of deionized water (temperature not exceeding +5 °C). The application was performed with an ultrasonic homogenizer (JY92- IIDN, China; frequency 20–25 KHz, power range 60–650 W, Ø 6 (1/4") probe), a controlled adjustable power percentage (30% W, 80% W), and a duration of 5–15 min. After the application, five different groups were formed: control, 30W/5 min, 30W/15 min, 80W/5 min, and 80W/15 min.

## 2.3. Moisture and pH measurement

Moisture content (%) was determined in an etuve (Nuve N-120) according to AOAC 950.46 (AOAC, 2005). The pH of fillet samples was measured in fish/water (1:10, w/v) homogenate using a pH-meter (Hanna HI-221) (Varlık et al., 2007).

**2.4. Color measurement**

Color measurements were performed directly on samples with a Minolta Color Reader (Minolta CR-400, Osaka, Japan). According to the Hunter $L^*$, $a^*$, and $b^*$ color scale (Hunter Associates Laboratory Inc., USA), where "$L^*$" refers to lightness (0 is black and 100 is white), "$a^*$" indicates greenness ($a^*$<0) or redness ($a^*$>0), and "$b^{**}$" measures blueness ($b^*$<0) or yellowness ($b^*$>0) of samples. As a summary measure, total color change (denoted ΔE) was used (Anonymous, 2008).

**2.5. Texture analysis**

A texture profile analysis test was performed using a texturometer (LFRA Texture Analyzer, Brookfield Engineering Labs Inc., USA) equipped with a 12.7 mm diameter stainless steel spherical probe approaching the sample at a velocity of 1 mm and clamping 5 mm into the fillet samples. Measurements (kgf, here 1 kgf = 9.806 N) were made using Texture Pro Lite v1.1 software (Brookfield Engineering Labs Inc., USA) to read values (Szczesniak, 2002).

**2.6. Sensory analysis**

The sensory evaluation panel of the samples consisted of 5 assessors who were appropriately trained on the basis of the Torry scale (color, odor, texture, and overall evaluation) (Regenstein and Regenstein, 1991). A ten-point range was used to detect sensory results (10-8, excellent; 7-5, good; and ≤4, not acceptable).

**2.7. Microbiological analyses**

On each sampling day, samples (10 g) of fillets were aseptically placed into sterile Stomacher® bags containing 90 ml of peptone water with NaCl (0.85% w/v) (Merck, Darmstadt, Germany) and homogenised for 2 min (Stomacher® 400, Seward Ltd., London, UK). Serial dilutions were prepared and total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB) counts were determined on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) by the pour plate method, incubating TMAB, 30°C and 48 hours; TPAB, 6.5°C and 10 days (ISO 4833, 2003; ISO 17410, 2001). Lactic acid bacteria (LAB) were incubated on De Man Rogosa and Sharp (MRS) agar (Merck, Darmstadt, Germany) by double layer pour plate method at 25 °C for 5 days and LAB counts were determined (American Public Health Association, 1974). Enterobacteriaceae counts were determined on Violet Red Bile Glucose (VRBG) agar (Merck, Darmstadt, Germany) by double layer pour plate method; incubated at 37°C for 48 hours (ISO 5552, 1997). *Pseudomonas* spp. were incubated on Cephaloridine-Fusidin-Cetrimid (CFC) agar (Merck, Darmstadt, Germany) by smear plate method at 25°C for 48 hours and bacteria were counted (ISO 13720, 2000). Analyses were performed in 3 replicates and results were represented in log cfu/g.

**2.8. Statistical analysis**

The SPSS statistical software program (IBM, SPSS Statistics Version 22.00) was used for the data. Results are recorded as the mean ± standard deviation. Ultrasound-related changes in means were tested for normality of data distribution by Levene's test. Before the analyses, the percentages of the values were normalized by the arcsine transformation. Since all data met the assumptions of parametric tests, means were compared by one-way ANOVA. Homogeneity of variances it was tested by Tukey's Honest Significant Difference (Tukey HSD) ($p$<0.05) (Esteves, 2011).

## 3. RESULTS

**3.1. Chemical changes**

As the storage period progressed, the moisture values showed minimum decreases in each group. However, the decrease at the end of storage was significantly different between the experimental groups ($p$<0.05) (Table 1). The maximum decrease was determined in the 30W/5 min group (77.35±0.32- 74.46±0.45) and the minimum change was determined in the 80W/5 min group (75.45±1.71- 74.96±0.35). On the first day of the application, the highest effect of ultrasound was determined in the 80W/15 min group. At the same time, the lowest moisture value was observed in the 30W/15 min group on the 9th day (73.72±1.87).

The pH values of trout fillets changed in the direction of increasing depending on ultrasound application and storage time (Table 2). However, 30W/5 min group was the only treatment group that showed a minimum decrease (6.72±0.01-6.70±0.05) instead of an increase at the end of storage. The effect of ultrasound application was found to be different between 30W/15 min and 80W/5 min groups on the 6th day of storage and between 30W/5 min and 80W/5 min groups on the 15th day ($p$<0.05) (Table 2). However, the pH limit value for fresh fish (6.8–7.0) was not exceeded during storage

**Table 1.** Variation of moisture content percentage according to groups and storage period.

| Storage period† (days) | Power and duration of ultrasound application (%Mean± SD‡) | | | | |
|---|---|---|---|---|---|
| | Control | 30W/5 min | 30W/15 min | 80W/5 min | 80W/15 min |
| **0** | 77.42±2.43$^{ax}$ | 77.35±0.32$^{ax}$ | 76.74±1.59$^{ax}$ | 75.45±1.71$^{ax}$ | 75.01±1.49$^{ax}$ |
| **3** | 74.12±1.45$^{ax}$ | 75.45±0.59$^{abx}$ | 75.43±0.35$^{abx}$ | 74.91±0.05$^{ax}$ | 74.67±0.61$^{ax}$ |
| **6** | 74.66±0.84$^{ax}$ | 74.01±1.23$^{bx}$ | 75.12±0.49$^{abx}$ | 75.49±0.67$^{ax}$ | 75.44±0.39$^{ax}$ |
| **9** | 75.10±0.30$^{ax}$ | 74.65±0.79$^{bx}$ | 73.72±1.87$^{bx}$ | 74.33±0.34$^{ax}$ | 74.93±0.22$^{ax}$ |
| **12** | 74.03±0.56$^{ax}$ | 74.53±0.34$^{bx}$ | 75.23±0.77$^{abx}$ | 74.98±0.78$^{ax}$ | 74.67±0.18$^{ax}$ |
| **15** | 75.41±0.47$^{ax}$ | 74.46±0.45$^{bx}$ | 75.00±0.44$^{abx}$ | 74.96±0.35$^{ax}$ | 74.28±1.07$^{ax}$ |

†Ultrasound application was applied with different power (30% and 80% W) and duration (5 to 15 minutes) not exceeding 5 °C in deionized water and kept at 4±1°C during storage (0, 3, 6, 9, 12, and 15 days). ‡Means with dissimilar letters in rows (a-b) and columns (x-y) are statistically different (*p*<0.05; Tukey's HSD). The data represent the mean± SD (%) of the total 90 samples.

**Table 2.** Variation of pH value according to groups and storage period.

| Storage period† (days) | Power and duration of ultrasound applications (Mean± SD‡) | | | | |
|---|---|---|---|---|---|
| | Control | 30W/5 min | 30W/15 min | 80W/5 min | 80W/15 min |
| **0** | 6.66±0.01$^{bx}$ | 6.72±0.01$^{ax}$ | 6.78±0.10$^{ax}$ | 6.67±0.01$^{cx}$ | 6.68±0.12$^{abx}$ |
| **3** | 6.68±0.08$^{bx}$ | 6.65±0.02$^{ax}$ | 6.69±0.01$^{abx}$ | 6.73±0.03$^{bcx}$ | 6.72±0.02$^{abx}$ |
| **6** | 6.65±0.02$^{bxy}$ | 6.69±0.05$^{axy}$ | 6.62±0.02$^{by}$ | 6.75±0.04$^{bcx}$ | 6.75±0.01$^{abx}$ |
| **9** | 6.68±0.05$^{bx}$ | 6.69±0.06$^{ax}$ | 6.75±0.05$^{abx}$ | 6.67±0.03$^{cx}$ | 6.65±0.06$^{bx}$ |
| **12** | 6.79±0.10$^{abx}$ | 6.74±0.04$^{ax}$ | 6.72±0.05$^{abx}$ | 6.79±0.01$^{abx}$ | 6.74±0.01$^{abx}$ |
| **15** | 6.89±0.02$^{ax}$ | 6.70±0.05$^{ay}$ | 6.81±0.02$^{axy}$ | 6.88±0.04$^{ax}$ | 6.85±0.05$^{ax}$ |

†Ultrasound application was applied with different power (30% and 80% W) and duration (5 to 15 minutes) not exceeding 5 °C in deionized water and kept at 4±1°C during storage (0, 3, 6, 9, 12, and 15 days). ‡Means with dissimilar letters in rows (a-b) and columns (x-y) are statistically different (*p*<0.05; Tukey's HSD). The data represent the mean± SD (%) of the total 90 samples.

## 3.2. Physical changes

Although L* values of trout fillets increased in all groups, no significant difference was observed between the groups (*p*>0.05) (Table 3). However, when the changes within the groups were observed according to the storage day, a significant difference was found between the first day and the 6th day in the Control and 30W/5 min group, between the 3rd day and the 15th day in the 30W/15 min group, between the 3rd day and the 12th day in the 80W/min group and between the first day and the last storage day in the 80W/15 min group (*p*<0.05). 3rd day and 9th day between groups during storage except for a* values, no significant difference was observed (*p*>0.05) (Table 3). During the storage period, it was determined that there was a significant difference between day 0th and day 6th only in the 80W/5 min. group (*p*<0.05).

Contrary to this, L* and a* color parameters, yellowness (b* >0) values showed non-linear changes both between groups and during storage days (Table 3). The 30W/5 min group was observed as the group that showed differences on the 3rd and 9th days of storage. Total color change (ΔE) values of trout fillets did not differ in treatment groups or storage days (*p*>0.05).

The hardness values (N) in the texture profile decreased during storage in all treatment groups (Table 4). However, no difference was observed between the groups (*p*>0.05).The effect of ultrasound application differed in the 30W/15 min (3rd –15th day), 80W/5 min (0th –15th day) and 80W/15 min (3rd –12th day) groups on storage days (*p*<0.05). While the stickiness values decreased with storage, the stickiness value was measured as (0.02) in all groups at the end of storage. With the effect of ultrasound, the only group in which the stickiness continued to decrease without increasing in the same direction was the 30W/15 min group (Table 4). Elasticity values decreased in all groups, and the difference in storage days was observed on the 6th and 15th days. The change in elasticity values was observed between the 30W/5 min –80W/15 min and 80W/15 min –80W/5 min groups. Chewability values (kgf/mm) of trout fillets showed a 3-fold decrease between the 30W/5 min and 80W/5 min groups only on the 6th day of storage ($p<0.05$). At the end of storage, the chewability value was determined as (0.03 kgf/mm) in all groups.

**Table 3.** Variation of color parameters according to groups and storage period.

| Storage period† (days) | Groups (Mean± SD‡) | Color parameters§ L* | a* | b* | ΔE |
|---|---|---|---|---|---|
| 0 | **Control** | 44.95±3.73$^{bx}$ | 1.52±1.08$^{ax}$ | 8.57±1.85$^{ax}$ | 0.00 |
| | **30W/5 min** | 43.98±3.53$^{cx}$ | 0.61±1.26$^{ax}$ | 7.93±2.38$^{abx}$ | 0.00 |
| | **30W/15 min** | 45.23±3.94$^{bx}$ | 0.89±1.31$^{ax}$ | 7.13±2.18$^{ax}$ | 0.00 |
| | **80W/5 min** | 46.11±3.09$^{abx}$ | 0.43±0.99$^{bx}$ | 8.91±2.63$^{ax}$ | 0.00 |
| | **80W/15 min** | 44.67±2.25$^{bx}$ | 1.13±1.89$^{ax}$ | 7.52±2.54$^{ax}$ | 0.00 |
| 3 | **Control** | 47.95±3.20$^{abx}$ | 0.28±0.78$^{ay}$ | 7.81±2.63$^{ax}$ | 3.55±2.01$^{ax}$ |
| | **30W/5 min** | 47.39±3.72$^{abx}$ | 1.49±0.84$^{axy}$ | 9.33±2.74$^{ax}$ | 3.99±1.25$^{ax}$ |
| | **30W/15 min** | 45.90±2.45$^{bx}$ | 0.96±2.08$^{axy}$ | 6.96±3.75$^{ax}$ | 2.20±0.73$^{abx}$ |
| | **80W/5 min** | 45.04±3.66$^{bx}$ | 1.35±1.16$^{abxy}$ | 9.17±2.64$^{ax}$ | 2.43±0.99$^{abx}$ |
| | **80W/15 min** | 46.45±3.62$^{abx}$ | 1.70±1.34$^{ax}$ | 7.86±2.15$^{ax}$ | 2.41±1.02$^{ax}$ |
| 6 | **Control** | 49.52±6.02$^{ax}$ | 1.27±1.23$^{ax}$ | 8.85±3.10$^{ax}$ | 4.77±2.82$^{ax}$ |
| | **30W/5 min** | 49.49±3.52$^{ax}$ | 1.21±0.96$^{ax}$ | 8.32±2.99$^{abx}$ | 3.50±0.54$^{ax}$ |
| | **30W/15 min** | 47.30±5.81$^{abx}$ | 1.85±1.44$^{ax}$ | 7.85±3.31$^{ax}$ | 4.21±1.22$^{ax}$ |
| | **80W/5 min** | 47.33±4.86$^{abx}$ | 1.97±1.90$^{ax}$ | 9.21±2.92$^{ax}$ | 2.94±1.99$^{ax}$ |
| | **80W/15 min** | 46.68±4.49$^{abx}$ | 2.30±1.29$^{ax}$ | 9.32±3.60$^{ax}$ | 2.89±0.42$^{ax}$ |
| 9 | **Control** | 47.21±1.83$^{abx}$ | 0.46±1.10$^{ay}$ | 6.61±1.37$^{ax}$ | 2.19±0.67$^{ax}$ |
| | **30W/5 min** | 45.96±2.44$^{bcx}$ | 1.57±1.46$^{axy}$ | 6.33±2.80$^{bx}$ | 4.26±0.21$^{ax}$ |
| | **30W/15 min** | 46.95±2.09$^{abx}$ | 2.08±1.77$^{ax}$ | 8.22±2.50$^{ax}$ | 5.86±2.14$^{ax}$ |
| | **80W/5 min** | 47.70±2.31$^{abx}$ | 1.44±1.53$^{abxy}$ | 8.07±1.84$^{ax}$ | 2.72±0.71$^{abx}$ |
| | **80W/15 min** | 47.48±3.35$^{abx}$ | 1.59±1.14$^{axy}$ | 8.01±1.94$^{ax}$ | 3.61±2.65$^{ax}$ |
| 12 | **Control** | 48.84±2.58$^{abx}$ | 0.84±1.32$^{ax}$ | 8.39±2.03$^{ax}$ | 2.84±1.60$^{ax}$ |
| | **30W/5 min** | 46.76±2.07$^{abcx}$ | 1.05±1.69$^{ax}$ | 6.87±2.75$^{abx}$ | 1.83±1.13$^{abx}$ |
| | **30W/15 min** | 47.33±2.71$^{abx}$ | 1.50±1.41$^{ax}$ | 7.65±2.83$^{ax}$ | 2.67±0.09$^{abx}$ |
| | **80W/5 min** | 49.16±2.70$^{ax}$ | 0.86±1.93$^{abx}$ | 8.37±2.72$^{ax}$ | 2.09±0.88$^{abx}$ |
| | **80W/15 min** | 47.06±2.66$^{abx}$ | 1.42±1.69$^{ax}$ | 7.28±2.50$^{ax}$ | 1.58±1.10$^{ax}$ |
| 15 | **Control** | 48.80±2.40$^{abx}$ | 1.39±2.37$^{ax}$ | 8.49±2.32$^{ax}$ | 1.76±0.17$^{ax}$ |
| | **30W/5 min** | 48.26±3.12$^{abx}$ | 1.71±1.71$^{ax}$ | 8.46±2.19$^{abx}$ | 3.18±1.67$^{ax}$ |
| | **30W/15 min** | 49.63±3.00$^{ax}$ | 1.06±1.17$^{ax}$ | 8.46±2.49$^{ax}$ | 3.39±2.07$^{abx}$ |
| | **80W/5 min** | 48.45±2.04$^{ax}$ | 0.99±1.60$^{abx}$ | 8.68±2.71$^{ax}$ | 2.44±0.54$^{abx}$ |
| | **80W/15 min** | 48.22±2.58$^{ax}$ | 1.89±1.58$^{ax}$ | 9.20±2.40$^{ax}$ | 2.66±1.06$^{ax}$ |

†Ultrasound application was applied with different power (30% and 80% W) and duration (5 to 15 minutes) not exceeding 5 °C in deionized water and kept at 4±1 °C during storage (0, 3, 6, 9, 12, and 15 days). ‡Means with different (a-c) letters in the same column within the same storage day are statistically different (p<0.05; Tukey's HSD). Means with different (x-y) letters in the same column for the same group are statistically different (p<0.05; Tukey's HSD). The data represent mean± SD of the total 90 samples. §L: L* indicates lightness (0 is black and 100 is white), a* indicates greenness (a*< 0) or redness (a*> 0), b indicates blueness (b*< 0) or yellowness (b*> 0) and ΔE indicates total colour change.

**Table 4.** Variation of texture parameters according to groups and storage period.

| Storage period[†] (days) | Groups (Mean± SD[‡]) | Texture parameters | | | |
|---|---|---|---|---|---|
| | | Hardness (N) | Stickiness | Elasticity | Chewability (kgf/mm) |
| 0 | **Control** | 5.24±1.37$^{ax}$ | 0.66±0.01$^{bx}$ | 2.04±0.43$^{ax}$ | 0.07±0.04$^{ax}$ |
| | **30W/5 min** | 5.18±2.42$^{ax}$ | 0.07±0.03$^{abx}$ | 2.24±0.71$^{ax}$ | 0.10±0.08$^{ax}$ |
| | **30W/15 min** | 6.23±2.61$^{abx}$ | 0.08±0.03$^{ax}$ | 2.37±0.31$^{ax}$ | 0.12±0.05$^{abx}$ |
| | **80W/5 min** | 6.21±3.07$^{ax}$ | 0.08±0.04$^{ax}$ | 2.35±0.49$^{ax}$ | 0.11±0.08$^{ax}$ |
| | **80W/15 min** | 4.57±1.72$^{abx}$ | 0.06±0.02$^{ax}$ | 2.03±0.63$^{ax}$ | 0.07±0.05$^{abx}$ |
| 3 | **Control** | 5.85±1.43$^{ax}$ | 0.08±0.03$^{bx}$ | 2.26±0.65$^{ax}$ | 0.13±0.11$^{ax}$ |
| | **30W/5 min** | 4.80±2.71$^{ax}$ | 0.09±0.03$^{ax}$ | 2.29±0.42$^{ax}$ | 0.09±0.05$^{ax}$ |
| | **30W/15 min** | 7.57±2.00$^{ax}$ | 0.07±0.03$^{abx}$ | 2.27±0.41$^{ax}$ | 0.13±0.10$^{ax}$ |
| | **80W/5 min** | 6.14±1.76$^{ax}$ | 0.08±0.03$^{ax}$ | 2.28±0.48$^{ax}$ | 0.12±0.08$^{ax}$ |
| | **80W/15 min** | 7.32±3.25$^{ax}$ | 0.07±0.01$^{ax}$ | 2.43±0.72$^{ax}$ | 0.14±0.10$^{ax}$ |
| 6 | **Control** | 4.43±1.00$^{ax}$ | 0.08±0.04$^{bx}$ | 2.05±0.35$^{ax}$ | 0.07±0.03$^{axy}$ |
| | **30W/5 min** | 5.53±1.78$^{ax}$ | 0.07±0.03$^{abcx}$ | 2.19±0.41$^{ax}$ | 0.09±0.05$^{ax}$ |
| | **30W/15 min** | 4.54±0.66$^{abx}$ | 0.06±0.02$^{abcxy}$ | 1.75±0.83$^{ax}$ | 0.06±0.02$^{abxy}$ |
| | **80W/5 min** | 3.81±1.18$^{abx}$ | 0.02±0.00$^{by}$ | 0.08±0.02$^{by}$ | 0.03±0.01$^{ay}$ |
| | **80W/15 min** | 4.92±0.93$^{abx}$ | 0.04±0.02$^{abxy}$ | 0.11±0.04$^{by}$ | 0.05±0.02$^{bxy}$ |
| 9 | **Control** | 4.91±0.82$^{ax}$ | 0.36±0.05$^{ax}$ | 0.08±0.04$^{bx}$ | 0.03±0.02$^{ax}$ |
| | **30W/5 min** | 6.47±3.70$^{ax}$ | 0.02±0.01$^{cy}$ | 0.05±0.02$^{bx}$ | 0.03±0.01$^{ax}$ |
| | **30W/15 min** | 4.99±1.99$^{abx}$ | 0.03±0.02$^{bcy}$ | 0.10±0.04$^{bx}$ | 0.04±0.02$^{abx}$ |
| | **80W/5 min** | 6.08±1.54$^{ax}$ | 0.02±0.00$^{by}$ | 0.05±0.01$^{bx}$ | 0.03±0.01$^{ax}$ |
| | **80W/15 min** | 5.23±1.29$^{abx}$ | 0.03±0.01$^{by}$ | 0.07±0.04$^{bx}$ | 0.04±0.02$^{bx}$ |
| 12 | **Control** | 4.40±1.08$^{ax}$ | 0.03±0.01$^{bx}$ | 0.09±0.03$^{bx}$ | 0.04±0.02$^{ax}$ |
| | **30W/5 min** | 6.69±2.13$^{ax}$ | 0.03±0.02$^{bcx}$ | 0.05±0.02$^{bx}$ | 0.03±0.02$^{ax}$ |
| | **30W/15 min** | 5.67±2.11$^{abx}$ | 0.03±0.01$^{bcx}$ | 0.07±0.02$^{bx}$ | 0.04±0.02$^{abx}$ |
| | **80W/5 min** | 4.54±1.32$^{abx}$ | 0.03±0.01$^{bx}$ | 0.09±0.02$^{bx}$ | 0.04±0.01$^{ax}$ |
| | **80W/15 min** | 3.89±0.86$^{bx}$ | 0.02±0.00$^{bx}$ | 0.08±0.01$^{bx}$ | 0.03±0.00$^{bx}$ |
| 15 | **Control** | 4.98±2.39$^{ax}$ | 0.02±0.01$^{bx}$ | 0.07±0.02$^{by}$ | 0.03±0.01$^{ax}$ |
| | **30W/5 min** | 4.08±1.28$^{ax}$ | 0.02±0.00$^{cx}$ | 0.09±0.02$^{bxy}$ | 0.03±0.00$^{ax}$ |
| | **30W/15 min** | 3.99±1.15$^{bx}$ | 0.02±0.01$^{cx}$ | 0.09±0.02$^{bxy}$ | 0.03±0.01$^{bx}$ |
| | **80W/5 min** | 2.46±0.69$^{bx}$ | 0.02±0.00$^{bx}$ | 0.14±0.04$^{bx}$ | 0.03±0.01$^{ax}$ |
| | **80W/15 min** | 3.87±1.78$^{bx}$ | 0.02±0.01$^{bx}$ | 0.08±0.03$^{by}$ | 0.03±0.02$^{bx}$ |

[†]Ultrasound application was applied with different power (30% and 80% W) and duration (5 to 15 minutes) not exceeding 5 ºC in deionized water and kept at 4±1 ºC during storage (0, 3, 6, 9, 12, and 15 days). [‡] Means with different (a-c) letters in the same column within the same storage day are statistically different ($p$<0.05; Tukey's HSD). Means with different (x-y) letters in the same column for the same group are statistically different ($p$<0.05; Tukey's HSD). The data represent mean± SD of the total 90 samples.

## 3.3. Sensory changes

While sensory parameters decreased in all groups dependent on storage, the highest values (odor, color, texture, and general evaluation) at the end of storage were determined in group 80W/15 min (Figure 1). The fastest change in odour evaluation (9.40-2.66) occurred in the control group. Odour change was observed between the control and 80W/15 min group from the 3$^{rd}$ day of storage ($p$<0.05).The change in the color parameter of the sensory analysis coincides with the total color change (ΔE) results of the instrumental color analysis. Initially, panelists gave close scores in terms of texture between treatment groups, but as storage progressed, the groups with higher power differed. While the parameter values of the sensory analysis decreased depending on storage, it was seen that it did not have a significant effect on the general evaluation.

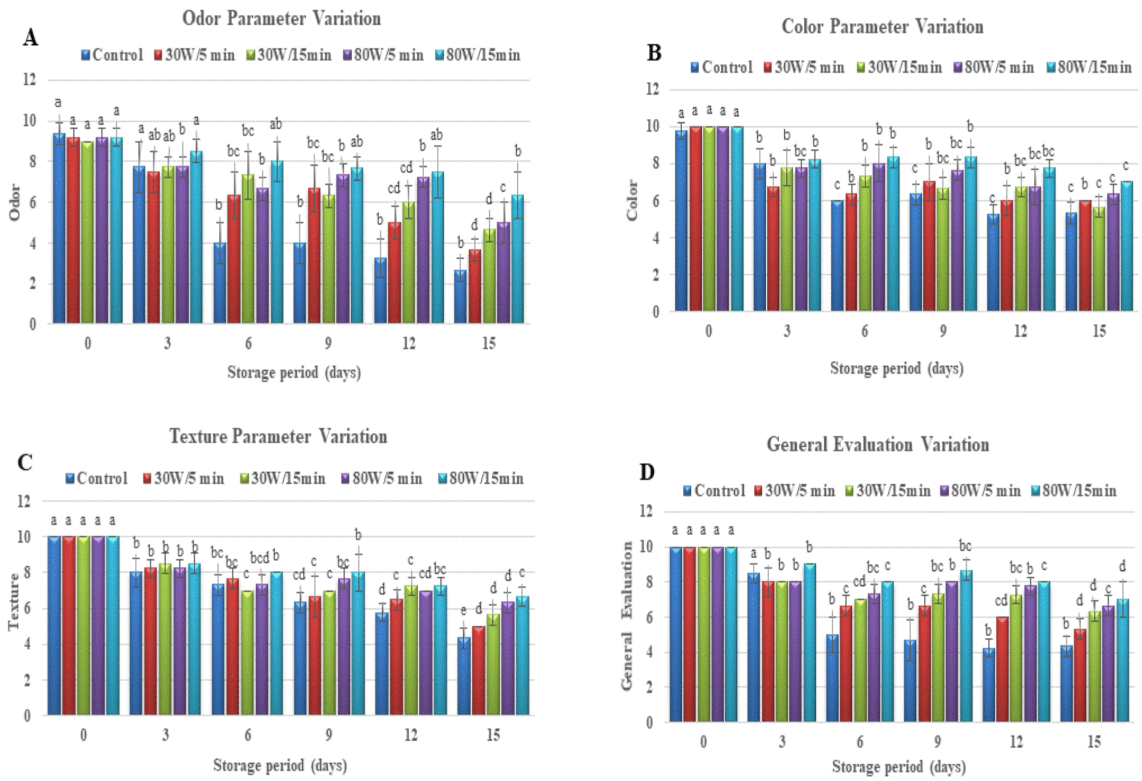


**Figure 1.** Changes in (A) odor, (B) color, (C) texture, and (D) general evaluation scores of sensory parameters according to ultrasound treatment groups during chilled storage of trout fillets. A ten-point range was used to detect sensory results (10-8, excellent; 7-5, good; and ≤4 not acceptable). The data represent the mean ± SD of a total of 90 samples. Different colored columns (groups) indicated by different letters (a-e) in the graph are significant ($p$<0.05; One-way ANOVA; Tukey's HSD).

### 3.4. Microbiological changes

The effect of ultrasound application showed an increase in TMAB counts during storage, but the acceptability limit of 7 log cfu/g was exceeded in all groups on the 9$^{th}$ day (Figure 2). The highest TMAB count was 8.34±0.12 log cfu/g in the 30W/5 min group on the 12$^{th}$ day. At the end of storage, the 80W/5 min (7.69±0.30 log cfu/g) and 80W/15 min (7.84±0.16 log cfu/g) groups showed the least change ($p$<0.05). The least effect of ultrasound application on TPAB counts in trout fillets was observed in the 30W/15 min (7.22±0.03 log cfu/g) group as of the 3$^{rd}$ day. However, it was observed that trout fillets in the 80W/5 min and 80W/15 min groups maintained their acceptability until the 9$^{th}$ day of storage (Figure 2). When the lactic acid bacteria counts were compared between the first day of the application and the 3$^{rd}$ day, a difference was found between the control group (2.03 log cfu/g), 30W/5 min (1.61 log cfu/g), 80W/5 min (1.72 log cfu/g), and 80W/15 min (1.53 log cfu/g), except for the 30W/15 min group. At the end of storage, the least increase was observed in the 80W/5 min (0.92 log cfu/g) group (Figure 2). Although the 80W/15 min group (3.80±0.05 log cfu/g), which had the lowest initial load in terms of Enterobacteriaceae, increased at the end of storage, it was still the group with the lowest load (Figure 2). *Pseudomonas* spp. counts in the samples increased during storage in all groups. The least change occurred in the 80W/15 min group (3.17±0.00 - 7.22±0.31 log cfu/g), followed by the 30W/5 min group (3.44±0.01 - 7.56±0.12 log cfu/g). At the end of storage, no difference was observed between the ultrasound treated groups.

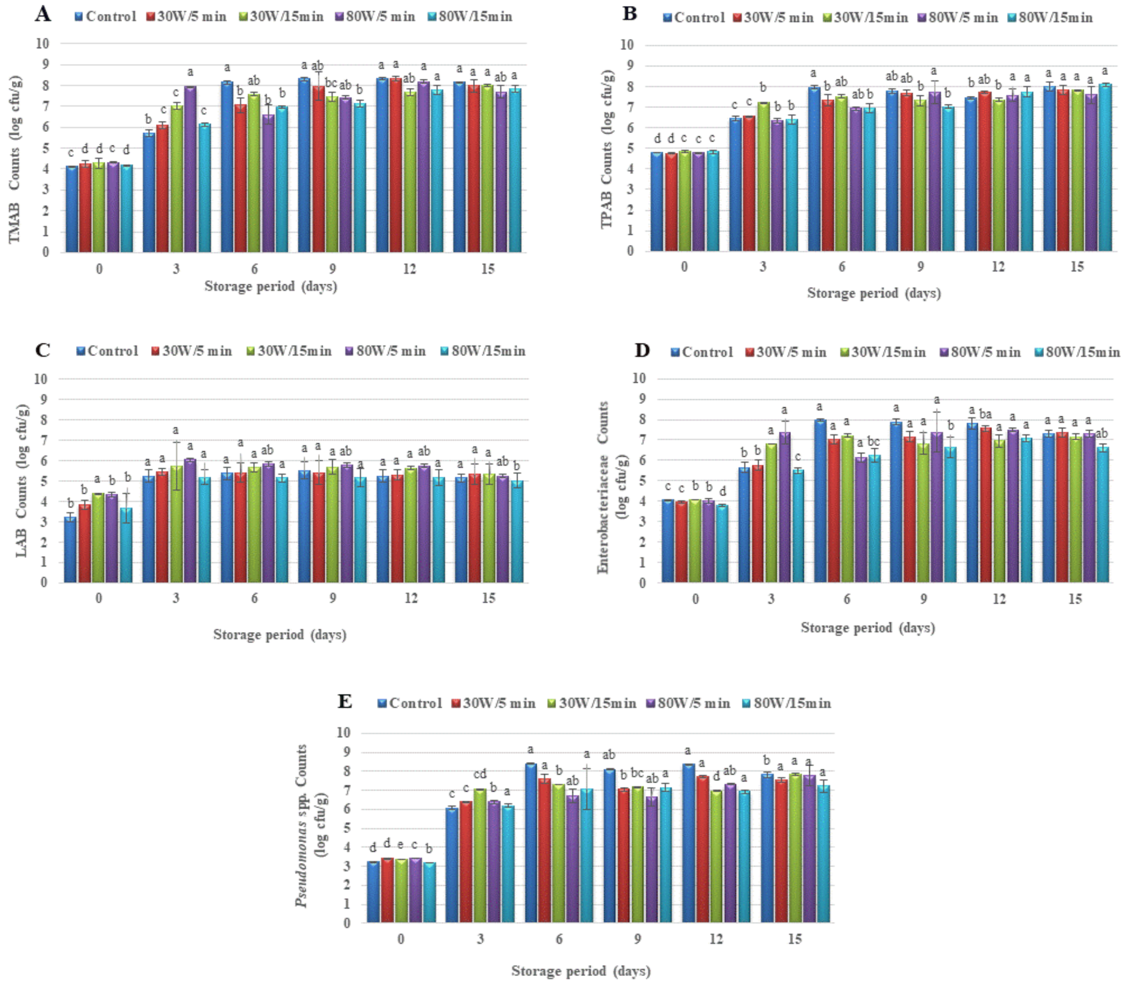


**Figure 2.**Changes in (A) Mesophilic, (B) Psychrophilic, (C) Lactic acid bacteria, (D) Enterobacteriaceae, (E) *Pseudomonas* spp. counts (log cfu/g) according to ultrasound treatment groups during chilled storage of trout fillets. The data represent the mean ± SD of a total of 90 samples. Different colored columns (groups) indicated by different letters (a-e) in the graph are significant ($p$<0.05; One-way ANOVA; Tukey's HSD).

# 4. DISCUSSION

## 4.1. Chemical changes

Although fish meat is a food with high nutritional value weak connective tissue structure in fish muscle, high enzyme activity, pH value, and water content make fish meat susceptible to spoilage (Özden and Gökoğlu, 1996). Moisture content and pH value in fish meat are the most important parameters for maintaining nutritional quality.

Recently, ultrasound has been combined with different food processing technologies and has become more widely used. Başlar, Kılıçlı and Yalınkılıç (2015) found that the drying time of salmon and trout fillets can be shortened with ultrasound assisted vacuum drying techniques. Ojha, Kerry, and Tiwari (2017) observed that the changes in water distribution in the dried beef samples were dependent on the frequency of the ultrasound effect. Grass carp (*Ctenopharyngodon idella*) samples were salted by ultrasound-treatment and then dried. It was showed that ultrasound can significantly increase the rate of water loss (Wang et al., 2018). Turhan, Sarıcaoğlu and Öz (2013) revealed that ultrasound-assisted marination accelerated moisture transport in anchovy (*Engraulis encrasicolus*) marinades. Ayvaz et al. (2019) applied ultrasound at different intensities to marinated vacuum anchovies and found that moisture content decreased depending on the intensity and storage. Moreover, the lowest moisture content in cooked ham was obtained with "0.75 salt + ultrasound" treatment (Barretto et al., 2018). Liu et al. (2023) reported that the moisture values decreased in the ultrasound application applied to *Culter alburnus* fillets for a fixed time (15 min.) with varying power compared to the control group and the lowest moisture value was determined at 150 W. The lowest moisture value in our study was observed in the 80W/15 min. group. It was determined that ultrasound application was effective in reducing the moisture content of vacuumed fresh trout fillets without using a different processing technology according to the application conditions and storage time. Both our and other studies confirm that ultrasound treatments support the reduction of moisture content in fish meat. It also prevented the increase of moisture content during storage and provided preserve food quality.

It was found that the pH change in two different cold-stored bovine muscles increased depending on storage time after ultrasound application (Jayasooriya et al., 2007). Our results coincide with the increase in pH in cold-stored trout fillets after ultrasound application. According to some researchers, applying ultrasound before rigor mortis can increase the initial pH of red meat (Got et al., 1999). It was observed that the short rigor period of fish meat compared to red meat slowed down the increase in pH value with ultrasound application. When the samples to which alkaline phosphate was added to increase the pH value in meat products were compared with the samples to which ultrasound was applied, the pH value was found to be higher in the ultrasound treated samples (Zhang et al., 2021). Alves et al. (2018) found that pH values varied between storage and ultrasound treatment time in Italian salami production. Similarly, the relationship between the power of ultrasound and application time was observed in our results. However, it was revealed that ultrasound treatment preserved the acceptability of trout fillets in cold storage.

### 4.2. Physical changes

Meat color is one of the most important sensory characteristics for consumers and producers. Some reports suggest that ultrasound does not affect meat color because the heat generated is insufficient to denature proteins and pigments (Sikes et al., 2014). On the contrary, in the evaluation of the effect of ultrasound (22 W/cm$^2$) on meat, it was observed that the color changed to lighter, less red, and more yellow-orange (Pohlman et al., 1997).

Ultrasound applied to fillets of different fish species without storage showed no change in all color values for mackerel (*Scomber scombrus*) and Atlantic cod (*Gadus morhua*). However, changes in yellowness, blueness, and green or red color values were observed in hake (*Merluccius merluccius*) and Atlantic salmon (*Salmo salar*) fillets (Pedros-Garrido et al. 2017). After the ultrasound treatment applied to the vacuumed *Culter alburnus* fillets, irregular changes were observed in L$^*$ and a$^*$ colour values according to the power, while b$^*$ colour value was observed as a decrease in all treatment groups (Liu et al., 2023). In pork products, such as Italian salami, ultrasound treatment was evaluated positively as it had no effect on both pigments and metmyoglobin (Alves et al., 2018). Jayasooriya et al. (2007) found that although there was no change in the color parameters of *Semitendinosus* and *Longissimus* muscles subjected to ultrasound at different times, all color parameters ($L^*$, $a^*$, and $b^*$) increased during storage at 5 $^o$C. In evaluation of color change in trout fillets at 4±1 $^o$C; only $L^*$ values showed an increase during storage. Among other color parameters, "$a^*$" values at the end of storage and "$b^*$" values during storage showed irregular changes. The difference in the muscle structure of red meat shows that the effect of ultrasound application on color change is observable. Our results show that there is no storage-related difference in the treatment groups, and the existing color of light colored fish meat can be preserved.

Meat quality depends on aroma, flavor, appearance, tenderness, and juiciness. Consumer behavior has shown that tenderness is the most important palatability factor in determining meat quality (Smith et al., 1991). Numerous studies have been conducted to develop methods to improve meat tenderness. Among these, ultrasound application methods have been used at various sonication times (33 seconds to 90 minutes), frequencies (15 to 130 kHz) and intensities (1.89 to 64 W/cm$^2$). Most researchers agree that ultrasound improves meat tenderness (Peña-González et al., 2017; Wang et al., 2018; Zou et al., 2018).

Vacuum packed sardine fish (*Sardina pilchardus* W.) fillets subjected to ultrasound and stored at 4 $^\circ$C showed a decrease in chewing value and hardness (Gündüz et al., 2019). Li et al. (2015) determined that the hardness of the tissue decreased with an increase in the duration of ultrasound applied to chicken breast meat. Changes in texture parameters of raw shrimp (*Penaeus vannamei*) were observed to vary with increasing duration of ultrasound applied a$^\circ$C and 50 $^\circ$C (Li et al., 2011). Zou et al. (2018) found that the hardness, elasticity, and chewiness values of spiced beef with ultrasound power-assisted cooking decreased with increasing power and cooking time. The ultrasound treatment of *Culter alburnus* fillets without storage with different powers (100, 150, 200, 250, 300 W) showed that the 250 W and 300 W treatment groups exhibited the highest hardness and the 250 W group exhibited the greatest gumminess and chewiness (Liu et al., 2023). In the texture profile analysis of trout fillets, it was observed that hardness, elasticity and chewability parameters increased in the 30W/15 min. group, while stickiness decreased in all treatment groups. Wang et al. (2018) determined that hardness decreased and flexibility values increased with an increase in ultrasound power applied to grass carp (*Ctenopharyngodon idella*) samples without storage. When the texture parameters (hardness, stickiness, elasticity, and chewability) of trout fillets were analyzed with storage, it was observed that although each parameter changed during the process in the groups, it decreased at the end of storage. Results of this and other studies suggest that different temperatures during ultrasound application, application time, and muscle structure have a determining effect on the shaping of the texture profile.

**4.3. Sensory changes**

Sensory analysis is considered as the main and most important method for evaluating the freshness of fishery products (Huss, 1995). In addition, sensory methods are used to monitor changes in foods during storage.

Peña-González et al. (2017) concluded that ultrasound treatment of beef significantly reduced the ripening time while maintaining the sensory characteristics of the meat. Yeung and Huang (2017) applied similar ultrasound application conditions to trout fillets and pork fillets at different power and duration, and it was determined that the samples accepted in the general evaluation section were in the treatment groups with high power and duration. Peña-González et al. (2019) stated that after 14 days of storage of beef samples treated with ultrasound, the lipid oxidation increased, the shearing force decreased, and the processed meat was more tender and juicy. Our results showed that although the scores of sensory parameters of trout fillets decreased depending on storage, it did not affect the scores of general evaluation. Ultrasound-assisted marinating of beef showed a more homogeneous distribution of mass transfer and improved meat acceptability in sensory analysis (González-González et al., 2017). Ayvaz et al. (2019) found that ultrasound application had positive effects on the color and texture characteristics of marinated anchovy (*E. encrasicolus*) and was appreciated by panelists. Ultrasound application alone supports the general acceptance of sensory parameters in the cold storage of fresh trout.

Barretto et al. (2018) emphasized that ultrasound treatment improved taste, texture, and overall acceptance of the product in the sensory analysis of low-sodium cooked ham subjected to ultrasound. Moreover, Pinton et al. (2019) indicated that ultrasound treatment could efficiently increase the scores of sensorial parameters (such as color, texture, and overall acceptance) of reduced-phosphate emulsified meat products. It was showed that ultrasound treatment has positive effects on the preservation and improvement of sensory properties. Ultrasound treatment supports the acceptability of fish meat by consumers.

**4.4. Microbiological changes**

The mechanism by which ultrasound inactivates microorganisms is explained by the heat and pressure released by acoustic cavitation, which destroys the functional components and structure of the cell and disintegrates the cell (Piyasena et al., 2003).

Caraveo et al. (2015) determined that the number of TMAB and TPAB decreased depending on the application time in the microbiological analysis of beef stored at 4 °C after high-intensity ultrasound application. When the inactivation of *Listeria innocua* and mesophilic bacteria in milk by heat and ultrasonic treatment was compared, it was found that the inactivation rate was higher in ultrasonic treatment (Bermudez-Aguirre et al., 2010). Contrary to the previous studies, ultrasound application showed an increase in the number of TMAB in trout fillets, and only the 80W/15 min group was able to maintain its consumability until the 9$^{th}$ day.

Maximum reduction in bacterial groups (TMAB, TPAB and Enterobacteriaceae) was observed with ultrasound applied to fillets of different fish species and without storage, while differences were detected in the numbers of *Pseudomonas* sp. (Pedrós-Garrido et al., 2017). Chouliara et al. (2010) found a decrease of 1.0-2.1 log cfu/g in total bacteria and psychrophilic bacteria counts in ultrasound-treated raw milk, thermized milk, and pasteurized milk up to 6$^{th}$ day in cold storage. TPAB counts increased in trout fillets depending on storage, and the increase was determined to be 2.82-3.26 log cfu/g at the end of storage.

Kordowska-Wiater and Stasiak (2011) contaminated the surface of chicken wing skin with strains of gram-negative bacteria (*Salmonella enterica* subsp., *enterica* sv. *anatum*, *Escherichia coli*, *Proteus* sp., and *Pseudomonas fluorescens*) and applied ultrasound treatment in water and 1% aqueous lactic acid solution, concluding that the reduction in bacteria depended on the treatment time and type of liquid. *P. fluorescens* was found to be the most sensitive bacterium to "1% aqueous lactic acid solution + ultrasound" treatment. Herceg et al. (2012) found that gram-negative bacteria (*E. coli*) were more sensitive than gram-positive bacteria (*Staphylococcus aureus*) as a result of ultrasound application in raw milk. Different types of microorganisms have different membrane structures. For example, gram positive and gram negative bacteria do not show the same behavior towards ultrasonic waves due to their different cell and membrane structures (Piyasena et al., 2003). In our results showed that gram-negative bacteria Enterobacteriaceae and *Pseudomonas* spp. provided more microbial inactivation in trout fillets compared to other bacterial species.

It is revealed that the effect of ultrasound applications on microorganisms varies according to the material structure, processing method, bacterial species, packaging and storage status, and conditions (duration and temperature) other than the parameters of ultrasound (power, intensity, and duration). All these results provide the basis for further studies on the use of ultrasound for the processing and preservation of aquatic products.

## 5. CONCLUSION

Ultrasound waves did not affect all quality criteria for fish meat to the same extent. While changes were observed in moisture and pH values depending on storage, color and texture values remained more stable. In the sensory evaluation, it was observed that general acceptability came to the forefront in the ultrasound treated groups. It was determined that microbiological changes occurred differently according to bacterial species. In the ultrasound application groups where the power was 80% W, it was observed that the application was more effective by means of moisture, general evaluation, and Enterobacteriaceae and *Pseudomonas* spp. numbers. It was concluded that when the power and duration of ultrasound applications are optimized by repeating in different ways, it can be one of the methods that can be used to protect the safety and quality of fish meat.

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## FINANCE

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## CONFLICT OF INTEREST STATEMENT

The authors declare that there are no financial interests or personal relationships that may influence this study.

## AUTHOR CONTRIBUTIONS

Fiction: ÇO; Methodology: ÇO,AD; Conduct of the experiment: ÇO; Data analysis: ÇO, AD; Manuscript writing: ÇO; Editing: AD .All authors approved the final manuscript.

## ETHICAL APPROVAL STATEMENT

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

## DECLARATION OF DATA USABILITY

The data used in this study are available on the Figshare platform with the DOI address https://doi.org/10.6084/m9.figshare.11815566.v1.

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