



**THE EFFECT OF DIFFERENT ULTRASOUND POWERS TREATMENT  
ON SOME QUALITY PARAMETERS OF SARDINES (*SARDINA PILCHARDUS*)  
PACKED IN VACUUM PACKAGING**

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**ABSTRACT**

In this study, the effect of different ultrasound powers treatment (200 W/L, 300 W/L, 500 W/L) on the chemical, microbiological and physical quality parameters of sardine fillets (*Sardina pilchardus* W.,1972) packed in vacuum packaging were investigated. The determination of quality parameters were studied to be performed by using pH, TVB-N, TBAR's analyses and various microbiological analyses and by evaluating texture properties and water activity changes in 0, 2, 4, 6, 8, 10, 12, and 14th day of storage. Consequently, it was observed to use of 200 W/L ultrasound delayed microbial spoilage and gave better results compared to the other groups in terms of hardness of product ( $P < 0.05$ ). The fact that use of ultrasound technology in seafood didn't effect negatively connective tissue of product and had positive effect on microbial load show that this technology can be developed and used in seafood.

**Keywords:** Ultrasound technology, sardine fish, vacuum packaging, TBARs, TVB-N.

**FARKLI GÜÇTE ULTRASES UYGULAMALARININ VAKUM PAKETLENEN  
SARDALYA BALIKLARININ (*Sardina pilchardus*) BAZI KALİTE  
PARAMETRELERİ ÜZERİNE ETKİSİ**

**ÖZ**

Bu çalışmada farklı güçte ultrases uygulamalarının (200 W/L, 300 W/L, 500 W/L) vakum ambalajlanmış sardalya balığı filetolarının (*Sardina pilchardus* W.,1972) kimyasal, mikrobiyel ve fiziksel kalite parametreleri üzerine etkisi araştırılmıştır. 0, 2, 4, 6, 8, 10, 12. ve 14. günlerde pH, TVB-N, TBAR's analizleri ile mikrobiyolojik analizler yapılmış ve su aktivitesi ile tekstür özelliklerinin değişimleri de değerlendirilmeye çalışılmıştır. Elde edilen sonuçlardan 200 W/L yoğunlukta ultrases kullanımının mikrobiyal bozulmayı geciktirdiği ve ürünlerin sertlik özelliği açısından diğer gruplara kıyasla daha iyi sonuçlar verdiği gözlemlenmiştir ( $P < 0.05$ ). Ultrases teknolojisinin su ürünlerinin bağ dokusunu olumsuz yönde etkilemediği ve mikrobiyel yük üzerine olumlu etkisinin olduğu belirlenmiştir. Bu nedenle, ultrases teknolojisinin su ürünlerinde geliştirilerek kullanılabileceği sonucuna varılmıştır.

**Keywords:** Ultrases teknolojisi, sardalya balığı, vakum paketlenme, TBARs, TVB-N

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## INTRODUCTION

Increased consumer interest in a proper lifestyle in nutrition and attention to the quality of foods it contributed to them consumer's towards to seafood (Cortesi et al., 2009). Seafood meat is a great food in terms of protein quality and nutritional value (Göğüş and Kolsarıcı, 1992). However, seafoods are among the products that can deteriorate rapidly after death due to their rich nutrient content and high pH level and the high amount of water they contain (Li et al., 2011). The processing techniques have to be applied in these products to prevent spoilage and to consume them for a longer period. Methods such as drying, salting, smoking, cooling, freezing are used in the preservation of seafood from past to present (Hall, 2012).

Today, researchers are researching on new technologies that products can maintain properties for a longer period, do not harm the taste and nutritional properties of the products, and do not use heat treatment. These technologies, which do not use heat treatment (non-thermal); high pressure application, irradiation technology, pulsed electric field, pulsed white light technology, ultraviolet application and ultrasound technology (Ohlsson and Bengtsson, 2002). High intensity ultrasound offers an alternative to traditional methods of food preservation, and is considered as a green and promising emerging technology. Ultrasound is an acoustic energy; it is a non-invasive, non-ionized, and non-polluting form of mechanical energy. It is considered an emerging method with a great potential to control, improve, and accelerate processes without damaging the quality of food (Alarcon-Rojo et al., 2019).

High intensity ultrasound waves can be used alone and in combination with other preservation methods to prevent microbial growth in foods and to ensure inactivation of enzymes (Ulusoy and Karakaya, 2011). There are many studies investigating the effect of ultrasound on the structure of products such as vegetables, fruits and meat products (Aday et al., 2013; Anese et al., 2013; Lagnika et al., 2013; Kordowska-Wiater and Stasiak, 2011; Cárcel et al., 2007; Pohlman et al.,

1997). While there are a limited number of studies on processed seafood (Ayvaz et al., 2019), any study has not been found investigating the effect of this technology on the quality of raw seafood in the literature. In this study, it is aimed to investigate the effect of ultrasound technology on quality parameters of vacuum packed sardine fillets (*Sardina pilchardus* W., 1972). In this context, microbiological, physical and chemical analyzes were carried out to determine the changes in the structure of the product.

## MATERIALS AND METHODS

### Fish and packaging material

In this study, sardines fish (*Sardina pilchardus* W., 1972) used as material was obtained from Çanakkale fish market in March 2015. They were approximately  $15.96 \pm 0.52$  cm length  $29.03 \pm 1.14$  g weigh. The internal organs and their blood of the fish were removed then filleted. 70 g sardine fillet in vacuum packaging material that 50  $\mu\text{m}$  thickness and 160  $\text{cm}^3/\text{m}^2/24\text{h}$  oxygen permeability using was vacuumed. Approximately 7 kg of sardine fillet were used in the study.

### Application of ultrasound process

For the ultrasound treatments (Hielscher/UIP1000hd, Germany), vacuum packaged sardine fillets were immersed in 1000 ml glass beaker which was filled with distilled water for per treatment to form an acoustic cavitation by vibrating the molecules. Vacuum packaged sardine were treated with 20 kHz ultrasound at powers of 200 W/L, 300 W/L, and 500 W/L for 2 min.

Four different experimental groups were designed formed in this study and treatments can be summarized as follows:

-Control: Control group; Raw sardine fish fillets are vacuum packed.

-200-U: Raw sardine fish fillets were vacuum packed and applied ultrasound (20 kHz, 200 W/L, 2 min).

-300-U: Raw sardine fish fillets were vacuum packed and applied ultrasound (20 kHz, 300 W/L, 2 min).

-500-U: Raw sardine fish fillets were vacuum packed and applied ultrasound (20 kHz, 500 W/L, 2 min).

### Measurement of water activity

The water activities of sardine fillet were measured with a water activity meter (Aqua Lab Series/4 TE-USA) at 25 °C. After the samples were homogenized and placed in the measuring chamber of the device, when the moisture content was equilibrated, the water activity value was read.

### Lipid oxidation

Lipid oxidation, measured as Thiobarbituric acid reactive substances (TBARS) values, was determined according to Kılıç ve Richards (2003).

### Total volatile base nitrogen (TVB-N)

A vapour distillation method was used for total volatile bases nitrogen (TVB-N) estimation (Anonymous, 1988). The results were expressed as mg TVB-N/100g.

### pH value

The pH values were recorded by using a pH meter (Hanna/Hi 8314-USA) after homogenization of each 10 g fish muscle sample in 100 ml distilled water (Gökalp et al., 2001).

### Microbiological analysis

Total mesophilic aerobic bacteria (TMAB), yeast-mould and *Enterobacteriaceae* analyses were made for microbiological analysis, 10 g sardine fillet sample was aseptically placed in a stomacher bag containing 90 mL of physiological saline water and homogenized for 30 sec in a stomacher (Seward stomacher/400 Circulator-England). Plate Count Agar (PCA, Merck) was used for TMAB count and incubated at 30 °C for 48 h. For the *Enterobacteriaceae* count, Violet Red Bile Agar (VRB, Merck) was used and incubated at 37 °C for 24 h. For the yeast and mould count, Potato Dextrose Agar (PDA, Merck) was used and incubated at 30 °C for 72 h. Standard microbiological methods were applied in the analyses. Results were expressed as colony forming units per gram (CFU/g) (Harrigan, 1998; Anonymous, 2000; Öztürk and Gündüz, 2018a; Öztürk and Gündüz, 2018b). The microbiological analyses were performed in duplicate.

### Texture profile analysis (TPA)

Texture is a kinesthetic property of food (Szczeniak, 2002). Texture profile analysis was performed to determine hardness, springiness, cohesiveness, adhesiveness, and chewiness of sardine fillets (Aday et al., 2013). Samples were analyzed with TA-XT Plus texture analyzer (Stable Micro Systems Ltd., UK) using a 10 mm diameter cylinder plunger probe (SMS-P/10 CYL Delrin). Analyses were performed under the following conditions:

Test speed : 0.80 mm/s  
Strain : 55.00 %  
Time : 5.00 sn  
Trigger force : 3.0 g

Acquired data's were processed with Texture Exponent 32 by using PC software (Caner et al., 2008).

### Statistical analysis

The experimental data were subjected to One-way analysis of variance (ANOVA). The means comparison was performed by Duncan Multiple Comparison with the level of significant set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Physicochemical analysis results

TVB-N is a method used to determine the quality and amount of spoilage of fish meat during storage (Kılınc, 1998). TVB-N is one of the most important deterioration criteria in seafood. In terms of TVB-N, seafood products are evaluated as "very good" up to 25 mg/100 g, "good" up to 30 mg/100 g, "marketable" up to 35 mg/100 g and "spoiled" for more than 35 mg/100 g (Varlık et al., 1993). Changes in the TVB-N values of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 1a. TVB-N was 20.22 mg N/100 g in fresh sardine fillets. The TVB-N increased in all groups during storage ( $P < 0.05$ ), and all groups exceeded the consumable limit of TVB-N (35 mg N/100 g) on the 6th day of storage. Similar to this study, Gökoğlu et al. (1998) and Özden (1995) stated that TVB-N in sardine fish exceeded the consumption limit of 35 mg/100 g on the 6th day of storage.

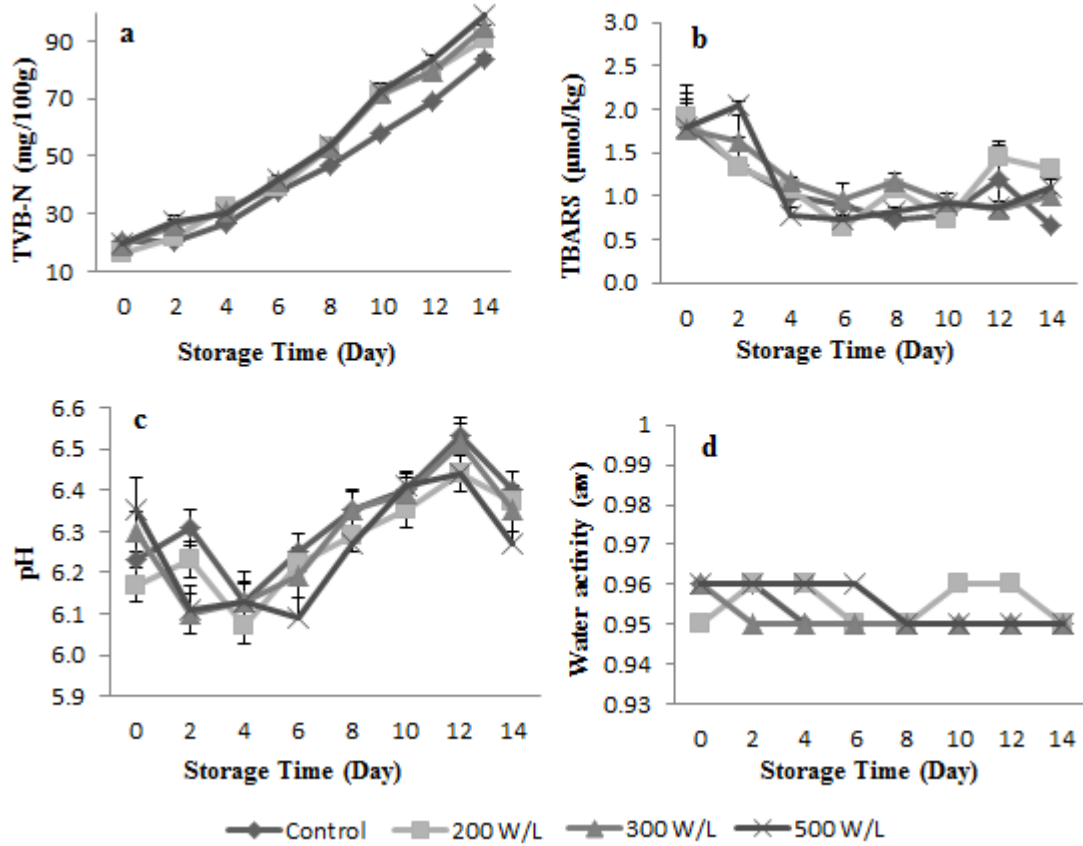


Figure 1. Effect of different ultrasound powers treatment on chemical parameters [TVB-N (a), TBARS values (b), pH (c) and Water activity (d)] of the sardine fillet stored at +4 °C during storage.

However, the results of our study showed that the use of ultrasound increases the amount of TVB-N. Similar results were obtained from Ayvaz et al. (2019). Researchers have reported that this is related to the easier breakdown of compounds found in muscles by the effect of cavitation. TBARS are an indicator of lipid oxidation. Changes in the TBARS values of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 1b. TBARS showed fluctuations between 0.64-2.04 µmol/kg in all groups. Kenar et al. (2010) investigated the effects of natural antioxidants in vacuum packed sardine fish fillets. The researchers observed fluctuations in TBARS in the control and antioxidant groups.

The researchers stated that the highest TBARS value was 2.4 mg malonaldehyde kg<sup>-1</sup> in all groups. At the end of the study, they stated that the decrease in TBARS values could be related to the removal of oxygen from the package. Kenar et al. (2010) as determined in their study, fluctuations were observed in TBARS values of all groups in this study. The highest TBARS determined in our study were 2.04 µmol/kg. Kenar et al. (2010) as stated in their study, it is thought that removal of oxygen from the packages in vacuum packed packages prevents oxidation.

Changes in the pH values of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented

in Figure 1c. At the start of storage, while the lowest pH was observed in 200-U group (6.17), the highest pH was observed in 500-U group (6.35) ( $P > 0.05$ ). On the 14th day of storage, the lowest pH was observed in 500-U group (6.27), the highest pH was observed in control group (6.40) ( $P > 0.05$ ). The data obtained in the pH measurement were in parallel with the other analyzes.

The start of deterioration, the occurrence of chemical changes and the increase of microbial activities caused an increase in the pH value on the 6th day of storage and following storage days. Also, it was observed that increase in pH value occurred during storage due to microbial degradation.

The pH value required for fresh fish is between 6.0-6.5. The consumption limit value for raw fish meat is between pH 6.8 and 7.0. But, the pH value alone is not adequate for the determination of the product quality and must be supported by other analyses (Varlık et al., 1993).

El Marrakchi et al. (1990) found that pH value of sardine fish stored in ice was 5.83, 6.36 and 6.57 on day 0, 9., and 18th day of storage, respectively. The researchers stated that the pH measurement was not effective in determining the amount of deterioration of sardine fish. In the study, an increase in pH was observed during storage however, El Marrakchi et al. (1990) and Varlık et al. (1993) as stated that it is considered that the measurement of pH alone is not a criterion that determines deterioration in fish.

Changes in the water activity of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 1d.

At the beginning of storage, the water activity value was determined as 0.95 in 200-U group, while the water activity value was measured as 0.96 in the other groups. Water activity values between the groups varied between 0.95 and 0.96. It was observed that the application of ultrasound had no effect on the water activity of sardine fish

( $P > 0.05$ ). Similar results were obtained from Ayvaz et al. (2019) that applied ultrasound to marinated anchovy.

### Microbiological analysis results

Changes in the total mesophilic aerobic bacteria (TMAB) counts of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 2a. In result of the study, it was observed that there was an increase in the TMAB during storage. In terms of TMAB, in Control and 500-U group, the consumption limit value 6 log CFU/g was exceeded at 10th day of storage. While limit value was exceeded in 300-U at 12th day of storage, this value was exceeded in 200-U at 14th day of storage. The 200-U group appears to be more effective in improving microbial quality than other groups. Kordowska-Wiater and Stasiak (2011) also stated that US applications have an effect on microbiological load. Pohlman et al. (1997) 20 kHz, 1.55 W/L intensity ultrasound process applied to vacuum packaged meat samples. The researchers reported that ultrasound was effective in reducing total bacterial load in meat samples, but there was no statistically significant difference between the control group and the ultrasound group. They also observed a decrease in the number of microorganisms in the samples after the ultrasound procedure and this decrease continued until the 5th day of storage. Choulira et al. (2010) observed a decrease in the total number of bacteria and the number of psychophilic bacteria when applied ultrasound treatment on untreated, heat treated, and pasteurized milk samples.

Changes in the *Enterobacteriaceae* counts of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 2b. It was observed that the number of *Enterobacteriaceae* was increased during storage. In terms of *Enterobacteriaceae*, in 500-U group, 5 log CFU/g was exceeded at 4th day of storage. While this value was exceeded in control and 300-U at 6th day of storage, this value was exceeded in 200-U at 8th day of storage.

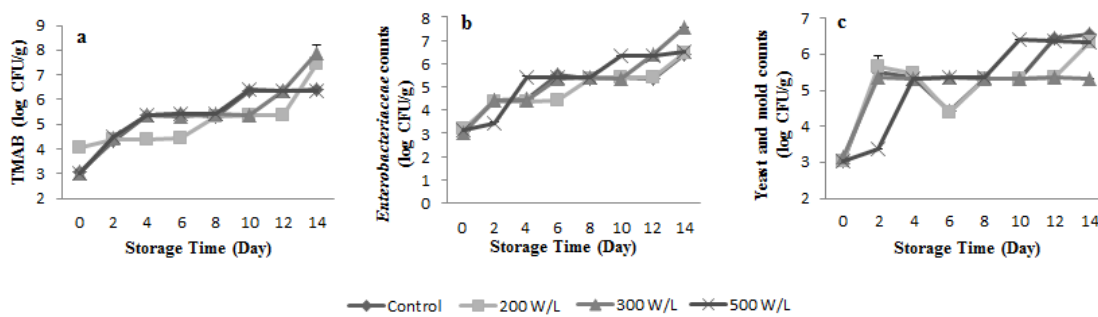


Figure 2. Effect of different ultrasound powers treatment on TMAB count (a), *Enterobacteriaceae* counts (b) and yeast and molds counts (c) of the sardine fillet stored at +4 °C during storage.

The number of *Enterobacteriaceae* was similar to the results in TMAB, and the application of 200 W/L ultrasound delayed the development of *Enterobacteriaceae* bacteria compared to other groups. Changes in the yeast and mould counts of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 2c. The number of yeasts and molds is an important quality indicator for products that in open marketed, in contact with air prior to packaging and contamination of packaging material. The number of yeasts and molds in all groups varied between 3.03 and 6.56 log CFU/g.

### Texture profile analysis (TPA)

The texture includes quality characteristics closely related to the mechanical and structural properties of a food. One of the most important criteria in the acceptance of a product by the consumer is its textural properties (Szczesniak, 2002). Changes in the texture parameters (hardness, chewiness, cohesiveness, springiness, adhesiveness) of sardine fillets that applied different ultrasound powers treatment during the storage period at +4 °C are presented in Figure 3a, 3b, 3c, 3d, 3e, respectively.

The hardness, which is the maximum force measured during the first compaction, is also defined as the force that must be exerted to achieve a certain deformation in the structure of the foods (Szczesniak, 2002).

One of the most important textural properties for seafood is the hardness of the product. Decreases in hardness values were observed from 2th day of storage. The data obtained from TVB-N analysis showed that the deterioration started on the 6th day; hardness values in the 200-U group were still high on the 6th, 8th and 10th days of storage. It was observed that microbial deterioration occurred later in the 200-U group compared to the other groups. It is thought that, this situation cause delayed softening of muscles, leading to the later occurrence of chemical and enzymatic activities in the muscles. Lyng et al. (1997) and Pohlman et al. (1997) reported that ultrasound application on red meat had not effect on hardness. Zayde et al., (2019) stated that short-term ultrasound application increases the hardness of fish. Differences between studies may be caused by frequency, intensity, time of application and temperature factors (Zayde et al., 2019).

Chewiness is defined as the energy required breaking down a solid food until it is ready to swallow. Also, the chewiness value of the food, which is a feature related to the number and duration of chewing in the mouth (Szczesniak, 2002). This value increased in all groups on the second day and decreased in later storage days. The chewiness value decreased in connection with the decrease in hardness. It was observed that the highest chewiness value was in 200-U group on the 6th, 8th and 10th days of storage.

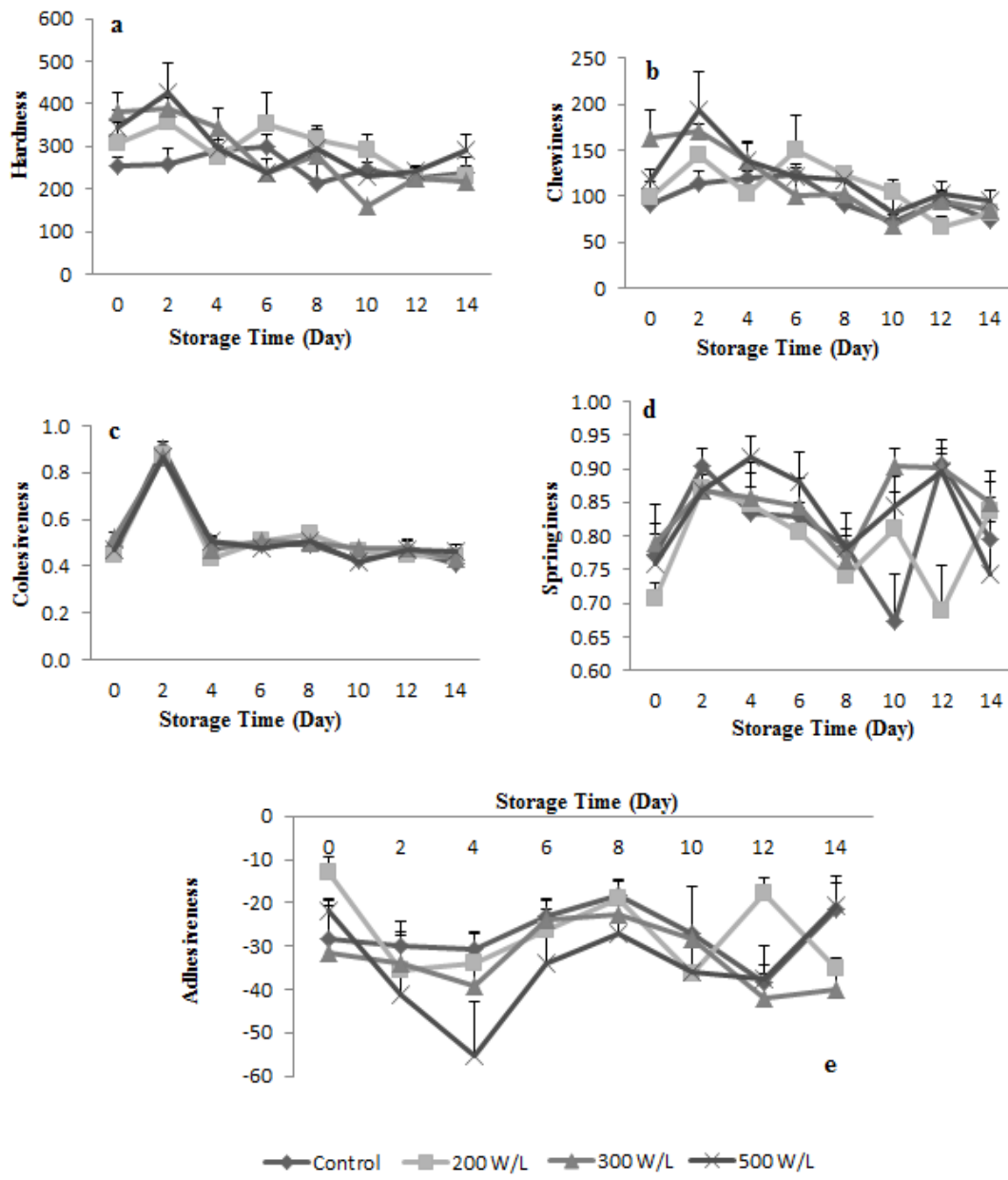


Figure 3. Effect of different ultrasound powers treatment on texture parameters [hardness (a), chewiness (b), cohesiveness (c), springiness (d), adhesiveness (e)] of the sardine fillet stored at +4 °C during storage.

In general, low powers ultrasound treatment decreased the chewiness value, but the chewiness value increased with increasing power. Similar results were also reported by Zayde et al. (2019). The cohesiveness shows the strength of the internal bonds that form the structure of the food.

It is the ratio of the positive force observed in the second compression to the positive force observed in the first compression in the texture profile analysis (Szczesniak, 2002).

The cohesiveness value, which gives information about the deformation of the product and the strength of the internal bonds, was determined in the group of 0.52 to 500-U highest on 0. day of storage and statistically significant difference was found between the 200-U and 500-U groups ( $P < 0.05$ ). However, there was no statistically significant difference between the groups on the other days ( $P > 0.05$ ). The fact that there is a significant difference in the cohesiveness of sardine fish between the control group and the ultrasound group shows us that the ultrasound process does not adversely affect the bond strength.

Springiness is defined as the rate of return to the state prior to deformation after the deforming force on the food is removed. In the texture profile analysis, it corresponds to the time interval between the end of the first compression and then the beginning of the second compression (Szczeniak, 2002). The application of ultrasound did not have a statistically significant effect on the springiness of sardine fish ( $P > 0.05$ ). In terms of days, there is not statistically significant difference between the groups. It has been determined that ultrasound technology has no effect on the springiness, which is the speed of rotation before the deformation applied to the products. Unlike our study, Aday et al. (2013) stated that high powers ultrasound treatment on strawberries reduced springiness value compared to low powers ultrasound treatment.

The adhesiveness value is defined as the work required overcoming the gravitational force between the surface of the foods and the surface it contacts (tooth, tongue, palate or prop). It is the negative area observed in the first compression in the texture profile analysis (Szczeniak, 2002).

At the start of storage, while the lowest adhesiveness value was observed in 200-U group, the highest adhesiveness value was observed in 300-U group. At the end of storage, while the lowest adhesiveness value was observed in 500-U group, the highest adhesiveness value was observed in 300-U group. Fluctuations were observed during storage at the adhesiveness value.

## CONCLUSIONS

In this study, it is aimed to investigate the effect of different ultrasound powers treatment (200 W/L, 300 W/L, 500 W/L) on quality properties of vacuum packed sardine fillets (*Sardina pilchardus* W., 1972). During the storage period, microbiological, chemical and physical analyzes were carried out to determine the changes in the structure of the product. It was observed from the results that the use of 200 W/L ultrasound in vacuum packed sardine fish fillets delayed microbial deterioration and gave better results in terms of hardness properties of the products compared to other groups. The determination that ultrasound technology does not adversely affect the connective tissue of sardine fish shows that this technology can be applied in seafood. Using different processing times, the effect of less than 200 W/L ultrasound power treatments on microbial growth and fish quality should be investigated with new studies.

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