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The Effect of High Intensity Ultrasound on the Quality and Shelf Life of Tilapia (*Oreochromis niloticus*) Muscle

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Abstract: It has been documented that the shelf life of fishery products is extremely reduced due to microbial development and its endogenous biochemistry. For this reason, food technologists around the world are researching how to reduce the main processes that lead to spoilage. Recently, high-intensity ultrasound (HIU) has had different applications in the food industry because the cavitation effect can inhibit or reduce microbial development as well as cause conformational changes in muscle enzymes. Therefore, in this study, HIU was applied for 30, 60, and 90 min to the tilapia (*Oreochromis niloticus*) fillet, and subsequently, it was stored on ice for 20 days. During this period, samples were taken every 5 days (day 0, 5, 10, 15, and 20), and moisture content, pH, total volatile base (TVB-N), non-protein nitrogen (NPN), texture, electrophoresis, color, and microbiological analyses (mesophiles and psychrophiles) were determined. No significant changes ($p \geq 0.05$) were observed in the moisture content, pH, and the L* parameter, while a significant decrease ($p < 0.05$) in TVB-N (from 29.67 to 15.09), NPN (from 0.39 to 0.27%), and texture (from 4.88 to 2.69 N) were found. On the other hand, an increase ($p < 0.05$) in a* (from 2.02 to 4.27) and b* (from 10.66 to 12.45) parameters, as well as total mesophile count (from 2.48 to 6.52 log CFU/g) were detected due to the application of ultrasound. The results suggest that the application of this treatment represents a viable alternative to increase the shelf life and quality of tilapia fillets stored on ice.

Keywords: tilapia fish; ultrasound; shelf life; seafood quality

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1. Introduction

One of the main problems facing the food sector in Mexico and in the wide world is post-harvest losses. It is estimated that around a third of food production for human consumption is wasted worldwide. This is equivalent to approximately 1.3 billion tons per year, while in Mexico, the percentage of losses is around 37%, aquatic products being one of the most sensitive, of which approximately 50% are wasted. Due to their inherent characteristics, fishing products are highly perishable and can be wasted throughout the entire food chain, from capture to final consumption in homes [1].

The main causes of food losses in underdeveloped countries are mainly related to economic and technical limitations, where the implementation of technological infrastructure is little or none. Therefore, the introduction of new technologies is necessary to extend the shelf life of the main aquatic species, thus reducing losses and improving the quality of the product offered to the consumer [2]. Over the years, different technologies have been used to increase the shelf life of fishery products and food in general. Examples of this are canned products, dried products, salted products, products packaged in modified atmospheres, frozen products, etc. However, in recent years, the consumption of fresh seafood

has increased. This requires the implementation of non-thermal technologies without the addition of additives. In this sense, one of the most-used processes has been the application of "superchilling" [3], alone or in combination with essential oils, achieving the reduction of the bacterial load and a slight increase in shelf life [4]. Another methodology that has been widely used is packaging in modified atmospheres [5] or even a combination of modified atmospheres and "superchilling" [6]. Other treatments have used the application of edible coatings, whose antibacterial action has managed to slightly increase the shelf life [7], or plastic films with the purpose of delaying lipid oxidation [8]. Likewise, the application of emerging technologies has recently been used in the conservation of fishery products.

Based on the above, the use of emerging technologies, such as high-intensity ultrasound (HIU), could be a viable alternative to reduce post-capture losses [9] of representative species from Mexico. This technology positively affects the two main causes of deterioration in fishing products: endogenous enzymatic action and microbial development. Ultrasonic waves can destroy the cell membrane of microorganisms, causing their death [10]. Likewise, it affects the structural conformation of proteins, which can then affect the endogenous enzymatic action [11]. In an investigation carried out by Pedros-Garrido et al. [12], it was found that HIU (30 kHz) affected the microbiological and quality parameters of several fish species (salmon, mackerel, pollock, and hake). These researchers observed a decrease between 1.5 and 0.5 CFU/g in mesophiles compared to control treatments (samples without HIU application). Similarly, a significant decrease in thiobarbituric acid-reactive species (TBARS) was found. On the other hand, Yi-Ming et al. [13], studying mackerel, found an inactivation of 0.72, 0.62, and 0.5 CFU/g for *L. innocua*, *E. coli*, and *P. fluorescens*, respectively, while Yang et al. [14] reported that ultrasound can improve the preservation of sea basal (*Lateolabrax japonicus*), inhibiting the growth of microorganisms in fillet and, moreover, maintaining the flavor quality. Therefore, the present research studied the effect of HIU on the quality and shelf life of tilapia fillets during their storage on ice for 20 days.

2. Materials and Methods

2.1. Raw Material

Specimens of *Oreochromis niloticus* were collected in an aquaculture farm located in San Pedro de la Cueva, Sonora, with an average weight and size of 421.8 g and 21.3 cm, respectively. The organisms were stored on ice using a cooler and transferred to the laboratory, where they were gutted, filleted, and washed with distilled water.

2.2. Ultrasound Application and Ice Storage

The tilapia fillets were placed in a two-liter beaker. Subsequently, these were sonicated with 0, 30, 60, and 90 min at 70% amplitude using a Branson Digital Sonifier SFX 550 (Branson Ultrasonics Corporation, Danbury, CT, USA), operating at 20 kHz and equipped with a 1.27 cm diameter titanium probe. During the process, samples were maintained in ice bath, and temperature did not exceed 10 °C. Then, sonicated fillet was stored in ice using a hermetic cooler. To determine the HIU effect on microbial, biochemical, and textural changes, samples were taken every 5 days (0, 5, 10, 15, and 20 d).

2.3. Moisture Content

The tilapia muscle was cut into small pieces. Subsequently, two grams of sample were placed in an oven (Model: 1320, VWR Scientific Products, Cornelius, OR, USA) for 8 h [15]. The moisture content was estimated in relation to the initial and final weight of the sample.

2.4. pH

This determination was made directly in the tilapia muscle [1]. The measurement of pH was conducted using a Hanna HI 90140 penetration pH meter (Hanna Instruments, Inc., Woonsocket, RI, USA). Equipment was calibrated daily with commercial standard solutions.

2.5. Total Volatile Base Nitrogen (TVB-N)

Determination of TVB-N involves the quantification of low-molecular-weight nitrogen compounds. For this, 5 g of tilapia muscle were mixed with 300 mL of distilled water. Then, 2 g of magnesium oxide and 25 mL of commercial oil were added as defoamers. The mix was heated to the boiling point and allowed to distill for 25 min. The distilled liquid was recovered in an Erlenmeyer flask with 15 mL of 2% boric acid, which was titrated with a solution of 0.05 N H_2SO_4. The TVB-N was expressed as mg of N/100 g sample [15].

2.6. Non-Protein Nitrogen

NPN in fish is mainly composed of free amino acids, peptides, TMA, nitrogenous bases, and urea. For its quantification, 10 g of tilapia muscle was mixed with 50 mL of 10% trichloroacetic acid (TCA). After that, the homogenated was centrifuged at 2000× *g* at 4 °C for 15 min in a refrigerated centrifuge (Sorvall Biofuge Stratos, Thermo Scientific, Hanau, Germany). Then, nitrogen content was determined in the supernatant, according to the methodology described by Woyewoda et al. [15].

2.7. Texture

The texture of the fillet is one of the main sensory attributes when purchasing fish; it can be measured subjectively (by touching the fish) or objectively (using a texturometer). In this study, texture was determined through shear stress using a Warner–Bratzler blade in a texturometer Shimadzu (Model EZ TEST EZ-S, Shimadzu Corp., Canby, OR, USA). Standardized size of 1 × 1 × 2 cm was used and maintained at 25 °C during the analysis, and the necessary force (N) to shear the muscle was recorded. The speed was set at 20 cm/min, and the shearing force was applied transversely to the orientation of muscle fibers [16].

2.8. Color

Color is one of the attributes that can determine the acceptance or rejection of a food. Changes in color can be determined both subjectively (using the senses) and objectively (using a colorimeter). In this study, color changes in tilapia fillets was determined on the external side (fillet close to the skin) by tri-stimulus colorimetry using a Minolta CR300 colorimeter (Minolta Co., New York, NY, USA). The measurements were carried out on the surface of the muscle [1].

2.9. Microbiological Analysis

The physical, chemical, and biochemical indicators used to determine the quality or deterioration of the fish must be accompanied by the total microbial count because its quantification helps to determine how many days the fish can be stored, either refrigerated or during ice storage. To study the effect of HIU on microbial inhibition, the total plate count of mesophiles and psychrophiles were determined. For this, 10 g of tilapia fillet was taken aseptically and homogenized in 40 mL of peptone solution (1 g/L). Serial dilutions of homogenates were made, and total viable count was determined with the pour plate method at 37 ± 2 °C for 48 h using plate count agar. Total viable count (TVC) was quantified according to the methodology described by NOM-092-SSA1-1994 [17].

2.10. Statistical Analysis

Descriptive statistics (mean and standard deviation), one-way analysis of variance, and multiple comparison by Tukey were applied using a significance level of 5%. For the analytical work, three specimens (n = 3) were sampled at days 0, 5, 10, 15, and 20. All analytical determinations were performed by triplicate. Data were analyzed using Jump 5.0.1 (SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. pH

The pH of fish muscle is usually close to 7; however, the pH of the postmortem muscle can vary, and it depends on the way the fish is caught, the season, diet, stress levels, among other factors [6]. Figure 1a shows the behavior of pH in tilapia fillet. An initial value of 6.36 was found, like that reported by Liu et al. [18], who found an initial value of 6.40 in tilapia muscle (*Oreochromis niloticus*). As can be seen, the control treatment showed a slight increase in pH ($p < 0.05$) at the end of storage. This increase could be associated with the accumulation of low-molecular-weight nitrogenous compounds due to endogenous biochemistry or bacterial action in the fillet. On the other hand, for fillets subjected to ultrasound, the pH did not increase during storage ($p \geq 0.05$), which means that the formation of alkaline compounds was not sufficient to generate an increase in pH muscle [19].

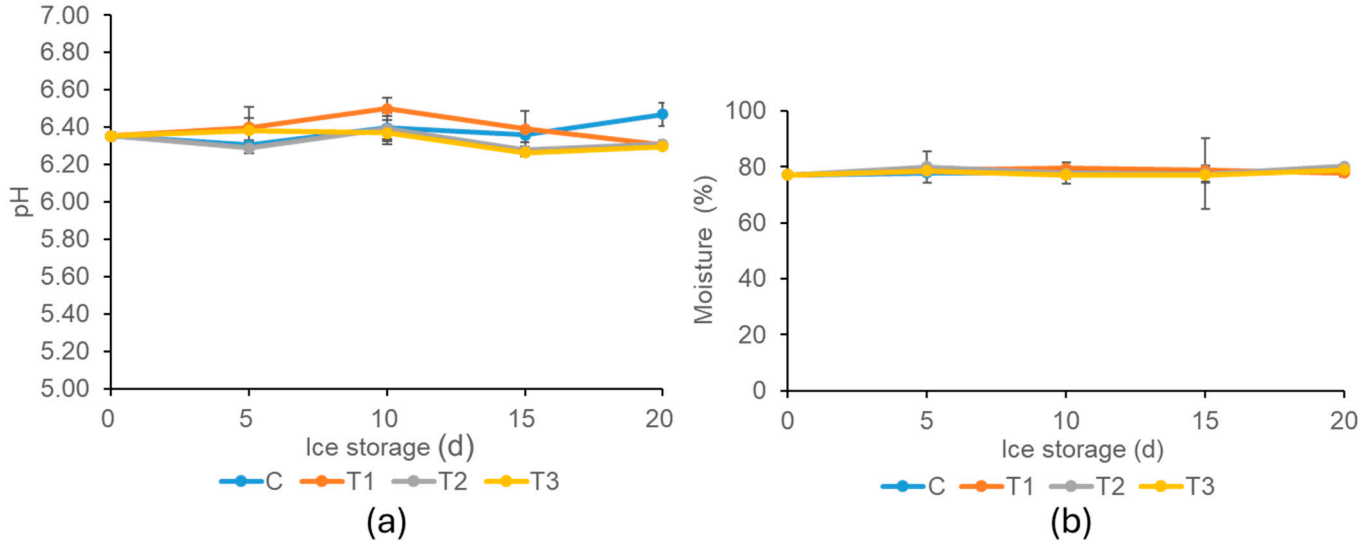

Figure 1. The behavior of pH (**a**) and humidity (**b**) in tilapia fillets (*Oreochromis niloticus*) in ice storage. C (control); T1 (30 min of HIU); T2 (60 min of HIU); T3 (90 min of HIU). The values are the average of n = 3 ± standard deviation.

3.2. Moisture Content

It is known that moisture content in food has a great influence on its stability and conservation. Likewise, slight variations in the water content can cause important changes in texture and color [20]. Figure 1b shows the results obtained for the moisture content during ice storage. As can be seen, there was a slight increase from 77.2 up to 80.2%, but this behavior was not significant ($p \geq 0.05$). It is common to find an increase in moisture because the muscle can absorb water during the ice melting [21]. It is known that moisture content is directly associated with pH since variations in pH affect the hydration capacity of proteins. In this sense, since there are no changes in pH during storage, it is expected that there will be no changes in moisture content either. On the other hand, the results show that the application of HIU does not affect the pH or moisture content in tilapia fillets. This could mean that ultrasound treatment is not damaging the cellular integrity of the tilapia fillets [22]. However, more studies are needed to conclude on the effect in the cellular integrity.

3.3. Total Volatile Base N (TVB-N)

The determination of TVB-N includes measurement of trimethylamine (produced by bacterial spoilage), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino acids and nucleotide catabolites), and

other nitrogenous compounds associated with the deterioration of fishery products [23]. Figure 2a shows the behavior of TVB-N in tilapia fillet. An initial value of 29.67 mg of N/100 g can be observed; considering that maximum limit is 30 mg of N/100 g of muscle, the obtained value in our study is high. However, levels greater than 30 mg of N/100 g do not always indicate deterioration. In this sense, high initial TVB-N values have also been reported for this species (*Oreochromis niloticus*). In a study carried out by Gutiérrez-Guzman et al. [24], a value of TVB-N of 28.75 g of N/100 g of muscle was reported. In another study by Castillo-Yañez et al. [25], working with sierra muscle (*Scomberomorus sierra*), an initial value of 23.7 mg of N/100 g was found. Therefore, the high initial content may be related to environmental factors as well as the endogenous biochemistry of these organisms.

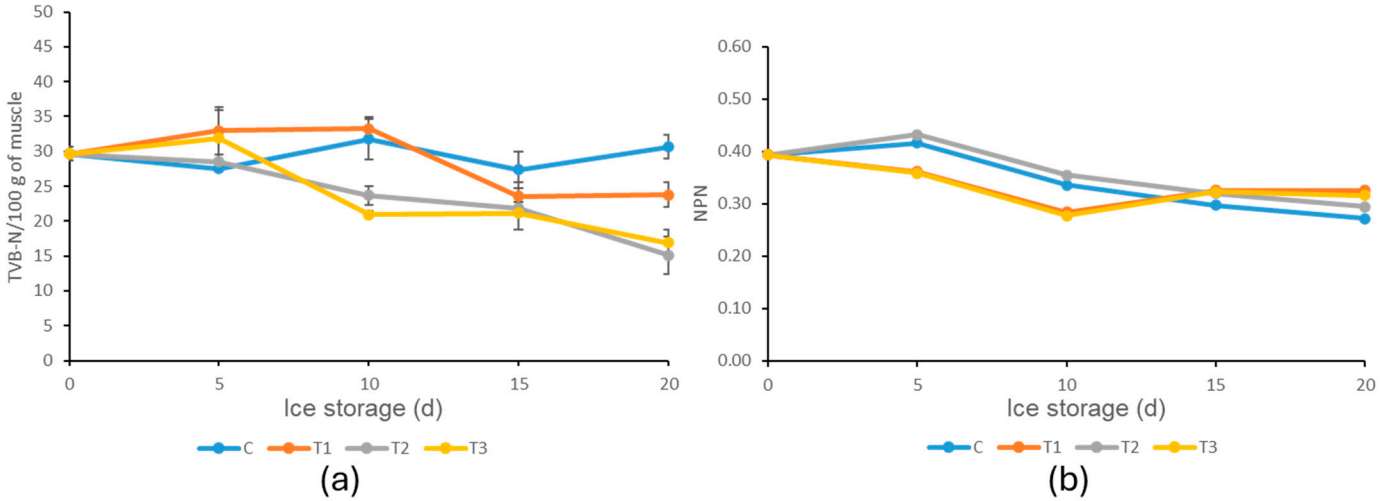

Figure 2. The behavior of TVB-N (**a**) and NNP (**b**) in tilapia fillets (*Oreochromis niloticus*) on ice storage. C (control); T1 (30 min of HIU); T2 (60 min of HIU); T3 (90 min of HIU). The values are the average of n = 3 ± standard deviation.

In our study, the control treatment did not show significant changes ($p \geq 0.05$) during ice storage. A similar behavior has been reported in other studies [24]; it shows that TVB-N is not a good indicator to predict the shelf life of this species. On the other hand, fillets treated with HIU showed a slight decrease in TVB-N. This decrease is not a usual behavior during the ice storage of fish. However, it has already been observed in other species. Sae-leaw et al. [26] reported a decrease in TVB-N in Pacific white shrimp when subjected to ultrasound and epigallocatechin gallate (EGCG) treatment. The lower content of TVB-N in the ultrasound-treated samples could be due to the inhibitory effect of microorganisms as well as a possible decrease in endogenous enzymatic activity. This could be a result of the ultrasound effect on the inhibition of microbial growth in addition to the possible changes that HIU is able to cause in tilapia muscle enzymes. This leads to a decrease in the formation of low-molecular-weight nitrogen compounds by microbial or endogenous action [27].

3.4. Non-Protein Nitrogen (NPN)

NPN is composed of free amino acids, peptides, amines, amine oxides, guanidine compounds, nucleosides, nucleotides, and urea, principally [28]. Figure 2b shows the behavior of NPN in tilapia fillets (*Oreochromis niloticus*) stored on ice for 20 days. An initial value of 0.39% was found; subsequently, NPN decreases from day 10 up to the end storage. As can be seen, a significant decrease ($p < 0.05$) in NPN was observed during storage, with the control treatment showing the lowest value. However, no significant differences were found between treatments on day 20. A decrease in NPN content has been reported for black skipjack muscle stored in ice for 24 d with 0.66 and 0.54% for the initial and final values, respectively [29]. In another study, Pacheco-Aguilar et al. [30] reported an initial

value of 460 mg/100 g of muscle, decreasing at the end of storage on ice to 325 mg/100 g of Monterrey Sardine (*Sardinops sagax*) muscle. Therefore, its reduction may be associated with bacterial proliferation since it has been reported that the bacterial load can use certain low-molecular-weight nitrogenous compounds for its development [31].

3.5. Texture

Texture is a very important sensory property of fish muscle. This can be influenced by several factors, such as species, size, fat content, among others, as well as by the conservation method [32]. Figure 3 shows texture changes during storage, finding that shear stress decreased significantly ($p < 0.05$) in all treatments, showing final values of 2.69 N (C), 3.03 N (T1), 4.01 N (T2), and 3.25 N (T3). This decrease is expected for all aquatic products stored in ice and can be associated with the endogenous proteolytic action on the structural proteins of the muscle, such as myofibrillar and connective tissue proteins [33,34]. In a study conducted by Lan et al. [19] with sea bass fillets, HIU was applied in combination with slightly acidic electrolyzed water (SAEW), and the authors found a drastic decrease in texture in the first two days of storage on ice. Subsequently, treatments that received HIU and the combination of HIU with SAEW (US + SAEW) showed a less pronounced texture reduction during the rest of storage compared to control fillets (without HIU). Similar results have been reported by Jayasooriya et al. [35], who applied ultrasound to bovine semitendinosus and longissimus muscles. These researchers found that the application of HIU reduced the decrease in texture during storage.

It is known that a reduction in texture during the ice storage of aquatic species is due to endogenous proteolytic action on myofibrillar proteins and connective tissue, cathepsins B, L, and collagenases being the main agents responsible [34]. On the other hand, the effect of ultrasound on texture muscle has been little studied. However, due to the results obtained in this study and those already reported in other investigations, it is likely that HIU is playing an important role in the denaturation of proteases, causing the tenderization of the fillet to occur more slowly. However, more research is necessary to establish the effect of HIU on the endogenous proteolytic activity of tilapia muscle.

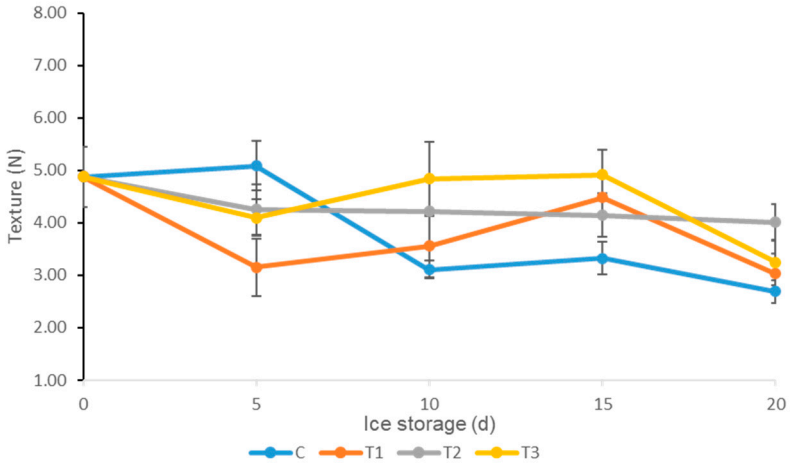

Figure 3. The behavior of texture in a tilapia fillet (*Oreochromis niloticus*) in ice storage. C (control); T1 (30 min of HIU); T2 (60 min of HIU); T3 (90 min of HIU). The values are the average of n = 3 ± standard deviation.

3.6. Color

Appearance, particularly color, is one of the key factors involved in the acceptance of fish products. Figure 4 shows the color changes depending on the parameters L* (luminosity), a* (red—green), and b* (yellow—blue) on tilapia muscle (*Oreochromis niloticus*) stored on ice for 20 days. The behavior of the L* parameter (Figure 4a) showed a non-significant increase ($p \geq 0.05$), with an initial value of 55.77 and final values of 57.36 (C),

56.01 (T1), 56.96 (T2), and 58.28 (T3). Comparable results have been reported by Coronado and Moreno [36], who stored tilapia muscle (*Oreochromis niloticus*) on ice, obtaining an initial and final value of 54 and 58.1, respectively. As can be seen, the behavior of this parameter was not affected by the application of HIU. Figure 4b shows parameter a*, with an initial value of 2.02 and final values of 4.27 (C), 3.60 (T1), 1.97 (T2), and 3.02 (T3). A significant increase ($p < 0.05$) was detected regardless the storage time. These results were lower than those reported by Coronado and Moreno [36], who found initial and final values of 10.6 and 8.10, respectively, for tilapia muscle (*Oreochromis niloticus*). These discrepancies may be due to the side selected to take the readings. The results of parameter b* are shown in Figure 4c. An initial value of 10.66 was obtained, while final values of 15.12 (C), 13.29 (T1), 12.45 (T2), and 12.92 (T3) ($p < 0.05$) were found. A slight increase at the end of storage indicates that the tilapia fillet tends towards a yellowish hue, a characteristic color of a fillet that is no longer fresh. However, this change was barely perceptible sensorially. Finally, Figure 4d,e show photographs of the fillets on days 0 and 20. On day 0 (Figure 4d), the fillet had a pinkish-red color, while on day 20 (Figure 4e), the control fillet showed a browner hue compared to the fillets subjected to ultrasound. These observations agree with what was detected through tristimulus colorimetry.

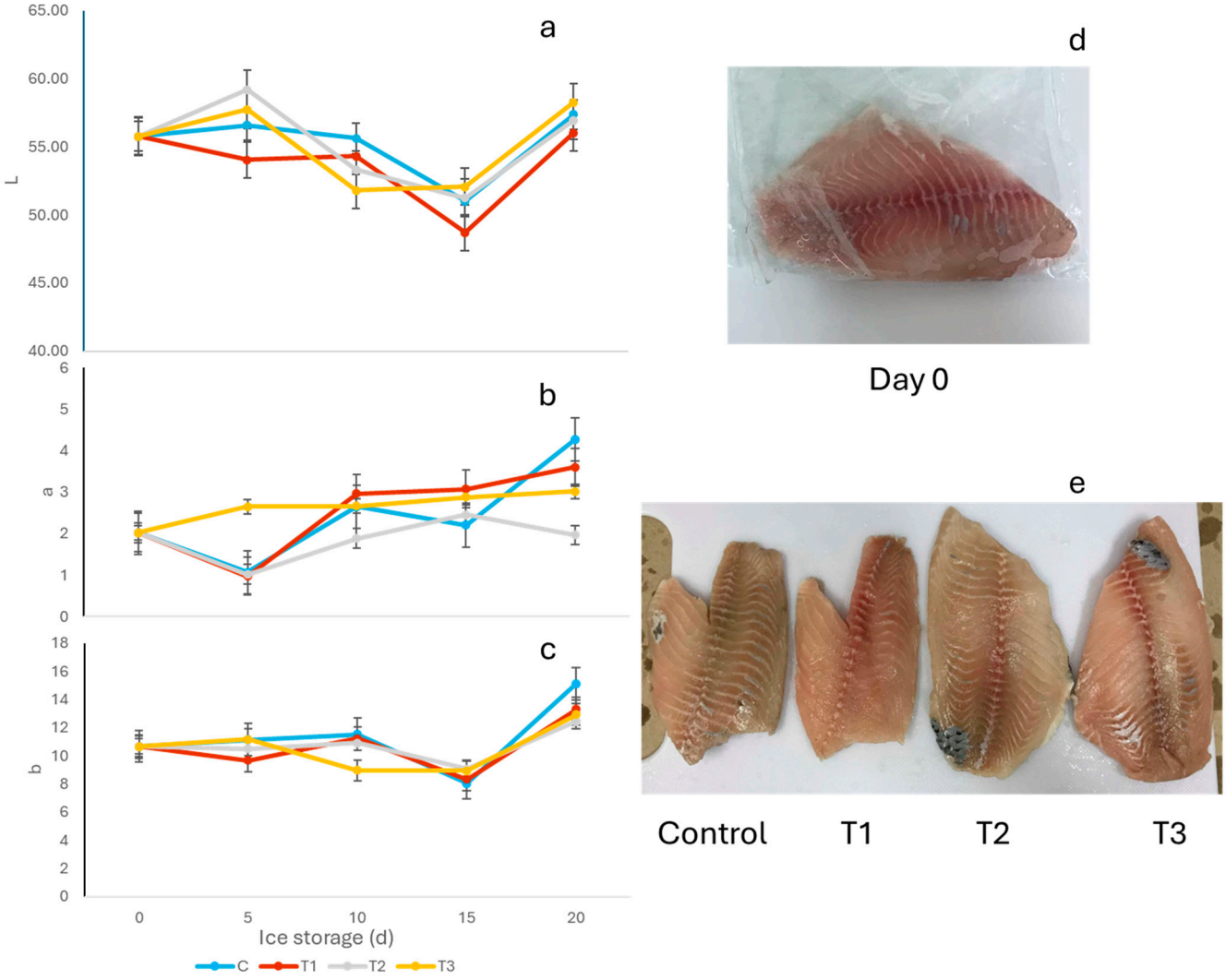

Figure 4. The behavior of parameter L* (**a**), a* (**b**), b* (**c**), color at 0 day (**d**), and color at 20 day (**e**) of tilapia fillets (*Oreochromis niloticus*) in ice storage. C (control); T1 (30 min of HIU); T2 (60 min of HIU); T3 (90 min of HIU). The values are the average of n = 3 ± standard deviation.

3.7. Microbiological Analysis

Microbial growth is one of the main causes responsible for the deterioration of aquatic products. Therefore, it is one of the main factors that determines the quality and shelf life of fish [37]. The international standard sets a maximum permitted value of 6 Log CFU/g of mesophilic microorganisms as satisfactory for fresh fish and from 6 to 7 Log as acceptable. Figure 5 shows the results of the total count of mesophiles in tilapia muscle stored in ice for 20 days. At the beginning of storage, a microbial load of 2.48 ± 0.11 log CFU/g was detected. This value is like that reported by Coronado and Moreno [36], who found an initial value of 3.2 log CFU/g in tilapia (*Oreochromis niloticus*), and is lower than that reported by Márquez-Ríos et al. [37] in loricariidae (4.2 log CFU/g).

This initial bacterial load is the result of handling during harvesting, transportation, gutting, and filleting since it is assumed that the bacterial load of a live fish is close to zero. The bacterial count increased significantly ($p < 0.05$) during storage, reaching values of 6.52 (C), 5.78 (T1), 5.47 (T2), and 5.97 (T3) log CFU/g at the end of storage. Therefore, the control treatment has an acceptable shelf life, while for fillets subjected to ultrasound (T1, T2, and T3), the satisfactory shelf life was extended up to the end of storage. These results are similar to those already reported by Liu et al. [38] for bay scallops (*Argopecten irradians*) during storage at 4 °C. They found reductions of 3.0, 13.7, 29.5, 32.7, and 29.8% at the end of storage (6 days) when applying HIU at 150, 250, 350, 450, and 550 W, respectively. The reduction in microbial load because of ultrasound application could be attributed to cavitation phenomenon, which generates microregions of high pressure and temperature, and can lead to the formation of free radicals, which are very reactive and can produce the oxidation of the phospholipids that form the cell membranes of microorganisms and, consequently, the inactivation of bacterial cells [39].

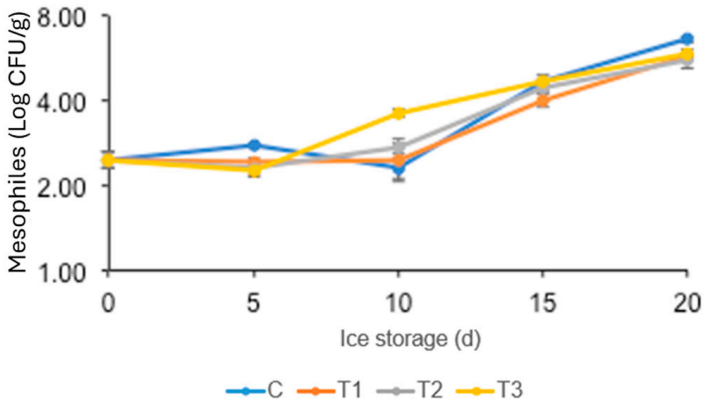

Figure 5. Behavior of mesophiles in tilapia fillets (*Oreochromis niloticus*) on ice storage. C (control); T1 (30 min of HIU); T2 (60 min of HIU); T3 (90 min of HIU).

4. Conclusions

The application of ultrasound showed a favorable effect on tilapia fillets (*Oreochromis niloticus*) stored on ice. Its application kept TVB-N and NPN without increases and delayed microbial development, thus significantly increasing its shelf life. On the other hand, it helped to maintain the muscle structure and coloration of the fillet. It means that when ultrasound was applied to fillets, texture and color parameter L had minimal changes during storage, providing a better physical appearance at the end storage. The results suggest that the application of this treatment represents a viable alternative to increase the shelf life and quality of tilapia fillets stored in ice. On the other hand, more research is required on the effect that HIU could have on endogenous enzymatic activity to explain in greater detail its effect on proteolytic activity as well as changes in coloration of the tilapia fillet.

Author Contributions: Data curation, G.M.S.-J.; Investigation, A.U.-T.; Methodology, V.M.O.-H., S.R.-C. and N.M.-C.; Writing—original draft, W.T.-A.; Writing—review and editing, E.M.-R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data from this study can be requested from the corresponding author by sending an e-mail to enrique.marquez@unison.mx.

Conflicts of Interest: The authors declare no conflicts of interest.

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