PAB Pesquisa Agropecuária Brasileira 60 years

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Food Technology/ Original Article

High-intensity ultrasound in refrigerated tilapia fillets: emerging preservation technology

Abstract – The objective of this work was to evaluate the effects of high intensity ultrasound exposure at 37 kHz and 100 W of effective power at different durations on the nutritional, physicochemical, and microbiological attributes of tilapia fillets during refrigerated storage. The application time of ultrasound influences both the nutritional attributes and physicochemical aspects of tilapia fillets. Additionally, the 10 min treatment makes the tilapia fillets more acidic. Conversely, extending the treatment to 20 min induced greater physical changes, such as a yellowish-red coloration on the fillets, lower water retention capacity, and lower moisture and ash percentages, which can compromise the nutritional quality of the fish and increase lipid oxidation. Although the count of psychrotrophic aerobes increased during storage, it remained within the safe threshold according to international standards. The application of an ultrasonic frequency of 37 kHz at an effective power of 100W for 10 min demonstrated innovative potential for preserving tilapia fillets, mitigating nutritional losses, and maintaining good technological characteristics.

Index terms: *Oreochromis niloticus*, fish, innovative techniques, sonication

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Received
August 11, 2025

Accepted
February 23, 20264

How to cite
OLIVEIRA FILHO, P.R.C.; AMARAL, R.P.C.; SILVA, E.D.C. da; MELO, C.W.C.; SHINOHARA, N.K.S. High-intensity ultrasound in refrigerated tilapia fillets: emerging preservation technology. **Pesquisa Agropecuária Brasileira**, v.61, e04216, 2026. DOI: https://doi.org/10.1590/S1678-3921.pab2026.v61.04216.

Ultrassom de alta intensidade em filés de tilápia refrigerados: tecnologia emergente de preservação

Resumo – O objetivo deste trabalho foi avaliar os efeitos do ultrassom de alta intensidade em 37 kHz, e 100 W de potência efetiva, nos aspectos nutricionais, físico-químicos e microbiológicos de filés de tilápia mantidos sob refrigeração. O tempo de aplicação do ultrassom influencia os atributos nutricionais e os aspectos físico-químicos dos filés de tilápia. A exposição ao ultrassom por 10 minutos mantém maior umidade nos filés, menor dureza e elasticidade muscular sem comprometer a capacidade de retenção de água ou o percentual de proteína. Além disso, o tratamento por 10 minutos torna os filés de tilápia mais ácidos. No entanto, estender o tratamento para 20 minutos induziu maiores alterações físicas, como a coloração vermelha-amarelada dos filés, menor capacidade de retenção de água e menores percentuais de umidade e cinzas, o que pode comprometer a qualidade nutricional do peixe e aumentar a oxidação lipídica. A contagem de aeróbios psicrotróficos aumentou durante o armazenamento, embora continue em quantidade segura de acordo com os padrões internacionais. A aplicação da frequência ultrassônica de 37 kHz na potência efetiva de 100 W por 10 min demonstrou potencial inovador para preservar filés de tilápia, mitigando perdas nutricionais e mantendo boas características tecnológicas.

Termos para indexação: *Oreochromis niloticus*, pescado, técnicas inovadoras, sonicação

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Introduction

Tilapia (*Oreochromis niloticus*) production in Brazil grew by 14.36% between 2023 and 2024, reaching 662,230 thousand tons, representing 68.36% of the total fish produced (Anuário…, 2025). This extensive farming is driven by several favorable characteristics, including rapid growth, tolerance to drastic changes in temperature, salinity, and dissolved oxygen concentration, as well as its ability to reproduce readily in captivity. Consequently, these factors facilitate a higher commercialization flow (Emam et al., 2025). However, similarly to most fish species, tilapia is highly perishable and requires preservation techniques to extend its shelf life.

There has been a growing scientific interest in non-thermal food preservation techniques, such as high hydrostatic pressure, pulsed electric field, and ultrasound (Pedrós-Garrido et al., 2017). Among these, ultrasound is one of the most promising options, since it is considered a green technology due to its potential to reduce processing and extraction times, energy consumption, and CO_2 emissions (González-González et al., 2017).

Ultrasound uses low-frequency, high-intensity sound waves, ranging from 20 to 100 kHz, that are transmitted through a liquid medium (Inguglia et al., 2017). These waves create alternating regions of compression and expansion, which induces localized increases in pressure and temperature through the formation of gas bubbles (Kang et al., 2016; Inguglia et al., 2017). In food, these bubbles generate a series of physical, chemical (Pedrós-Garrido et al., 2017), and microbiological (Liu et al., 2023) effects that can have various applications in food technology processes such as salting, freezing, thawing, marinating, emulsification, meat tenderization, and the reduction of microbial contamination.

Previous studies have evaluated the application of ultrasound on tilapia. Santos et al. (2022) studied the shelf life of a cooked product made with mechanically separated tilapia meat subjected to ultrasound, reporting a shelf life of up to 21 days when refrigerated at 4°C. Similarly, Silva et al. (2022) studied the attributes of marinated tilapia fillets subjected to an ultrasonic bath. The ultrasound resulted in more acidic marinated fillets with lower water activity and no compromise to nutritional attributes. Furthermore, Ugalde-Torres et al. (2024) investigated the application of ultrasound on tilapia fillets during 20 days of refrigerated storage, finding that the treatment inhibited microbial development while maintaining the texture and lightness of the fillets. Although these studies have already evaluated the use of ultrasound in tilapia products, the use of distinct ultrasound exposure times, there are no known studies that use ultrasonic bath with a frequency of 37 kHz at 100W of power on fresh tilapia fillets using low-cost, easy-to-use equipment.

The objective of this work was to evaluate the effects of high intensity ultrasound exposure at 37 kHz and 100 W of effective power at different durations on the nutritional, physicochemical, and microbiological attributes of tilapia fillets during refrigerated storage.

Materials and Methods

A total of 27 tilapias were slaughtered and donated by the Estação de Aquicultura Professor Johey Koike of Universidade Federal Rural de Pernambuco (UFRPE). The fish have a mean weight of 611.3±137.0 g and mean length of 30.8±2.2 cm, and were filleted at the Fish Technology Laboratory of UFRPE Departamento de Pesca e Aquicultura, in the municipality of Recife, in the state of Pernambuco, resulting in nine fillets per treatment across three different treatments.

In the laboratory, each fillet was placed in a 50 µm low-density polyethylene Ziploc bag and subjected to an ultrasonic bath using an Elmasonic EASY 60H (Elma, Ruiselede, Belgium) with 5.75 L of capacity. This model features tank dimensions of 300 x 151 x 150 mm, basket dimensions of 255 x 115 x 75 mm, ultrasonic frequency of 37 kHz, and an effective ultrasonic power of 100W. Water maintained at 25±1°C served as the transmission medium. Treatments consisted of three exposure times: 0, as control treatment, 10 and 20 min. Subsequently, the fillets were removed from the bath, dried, placed on polystyrene trays covered with 11 µm PVC film, and refrigerated at 6±1°C for up to 14 days.

Analyses of proximate composition (moisture, protein, ash, and ether extract), water holding capacity (WHC), and instrumental texture profile (hardness, cohesiveness, and springiness) were conducted. Additionally, instrumental color, pH, microbiological psychrotrophic aerobes, and lipid oxidation were determined. All laboratory analyzes were performed after 0, 7, and 14 days of storage and conducted in triplicate.

The proximate composition of the fillets was determined according to the Association of Official Analytical Chemists (AOAC) methods. First, method 926.12 was used to perform moisture analysis by gravimetry in an air circulation oven at 105ºC until a constant weight was achieved (AOAC, 1996a). Following moisture analysis, the dried samples were stored frozen at -18ºC for 2 days before conducting the remaining analyses. Crude protein, ash, and ether extract were determined through methods 991.20 (AOAC, 1994), 900.02 (AOAC, 1996b), and 920.39 (AOAC, 1995), respectively. Specifically, crude protein content was calculated using a conversion factor of 6.25, ash content was determined using a muffle furnace at 550ºC for 5 h, and ether extract was quantified using petroleum ether in a Soxhlet extractor.

For the WHC analysis, 5 g samples were weighed in triplicate, placed on 125 mm qualitative filter paper, housed in Falcon tubes, and centrifuged at 3,500 rpm for 10 min. After centrifugation, the samples were carefully removed from the paper and weighed. The WHC was calculated according to Araújo et al. (2020) using the following equation:

$$\% \text{ WHC} = \frac{\text{Sample weight after centrifugation}}{\text{Sample weight before centrifugation}} \text{ X } 100$$

The instrumental texture profile was performed on fillets from each treatment using a CT3 Texture Analyser (AMETEK Brookfield, Middleboro, MA, United States of America). The fillets were compressed to 50% of their original thickness using a pre-test, test, and post-test speed of 2 mm s^{-1} at a temperature of 25°C, according to the methodology described by Bourne (2002). The measured variables were hardness in N, cohesiveness, and springiness in mm.

The instrumental color of the fillets was determined using a portable CR-400 colorimeter (Konica Minolta, Tokyo, Japan). The device was calibrated with a white standard before every analysis and operated with a xenon lamp, illuminant C (Y=92.78; x=0.3139; y=0.3200), observation angle of 40º, and measurement area of 8 mm diameter. For all treatments, three measurements were taken at different points on each of the three fillets. The color values were expressed according to the CIELab system: lightness (L*), red to green color intensity (a*), and yellow to blue color intensity (b*).

The pH was determined with the aid of a pH meter by immersing the electrode in a homogenate consisting of 10 g of tilapia fillet and 40 ml of distilled water (Macedo et al., 2021).

For the microbiological analysis, samples from each treatment were collected aseptically, weighed, and diluted in specific buffers according to Instrução Normativa 62 of the Ministério da Agricultura, Pecuária e Abastecimento (Brasil, 2003). Compact dry TC kits, rapid tests authorized by AOAC, ISO, and BAM, were used to total count the psychrotrophic aerobes. The results were expressed in Log CFU g^{-1}.

Lipid oxidation analysis was conducted using the thiobarbituric acid reactive substances (TBARS) method. To calculate the TBARS values, a straight line was obtained with tetramethoxypropane, and the results were expressed in mg malondialdehyde kg^{-1}, according to Vyncke (1970).

A completely randomized design was employed in a 3x3 factorial arrangement. The factors consisted of high-intensity ultrasound exposure for 0, 10, and 20 min, and the refrigerated storage duration of 0, 7, and 14 days.

The results were subjected to a two-way analysis of variance. The data were expressed as means ± standard deviation, and the means were compared using Tukey's post hoc test ($p<0.05$). When significant variation was observed for ultrasound exposure time or storage duration, polynomial regression analysis was performed. Additionally, Pearson's product-moment correlation analysis was also applied. All analyses were performed using SisEAPRO software, version 5.0 (Mendes, 2025).

Results and Discussion

The moisture analysis of tilapia fillets showed a significant interaction ($p<0.01$) between ultrasound application time and refrigerated storage duration. The control treatment without ultrasound application exhibited the lowest moisture percentage at day 0, which increased by day 7, and decreased again at day 14 (Table 1). In contrast, fillets treated with 10 min of ultrasound showed a higher moisture percentage at day 0, followed by a significant decrease at day 7 and remaining practically stable until day 14 of refrigerated storage. Tilapia fillets treated with 20 min of ultrasound remained unchanged in terms

of significant variation during the 14 days of storage. Overall, tilapia fillets without ultrasound treatment showed a lower moisture content than those treated with 10 or 20 min of ultrasound.

Ultrasound induces cavitation, generating localized high shear forces and microstreaming. These physical effects disrupt hydrogen bonds and hydrophobic interactions in proteins, thereby altering secondary structures (Jiang et al., 2025). This phenomenon may have caused a partial rupture of the myofibrils and an increase in interstitial spaces, facilitating greater water diffusion and retention between protein structures. Ugalde-Torres et al. (2024) evaluated the application of a 20 kHz probe ultrasound at 70% amplitude for 0, 30, 60, and 90 min, and observed practically the same percentage of moisture in the tilapia fillets, ranging from 77 to 80%, during 20 days of storage on ice. Despite the variation, the moisture values observed in the present study were consistent with those reported by Ugalde-Torres et al. (2024).

Protein content in the tilapia fillets showed a significant interaction ($p<0.01$) between the different ultrasound exposure times and the refrigerated storage periods (Table 1). Fillets treated with 20 minutes of ultrasound had the highest protein content at day 0, decreasing by day 7 and remaining stable until day 14 of storage. In contrast, the protein percentage was slightly lower in the control group and the 10 min of ultrasound treatment. Although there was no decrease ($p>0.05$) in the protein percentage over the 14 day storage period for these treatments, a study evaluating marinated tilapia fillets reported that the ultrasound did not affect the percentage of protein, maintaining values close to 23% (Silva et al., 2022). Notably, Silva et al. (2022) only evaluated the control and a 10 min application of ultrasound. A comparison between both studies reveals that while there was also no variation in the percentage of protein at day 0 in these treatments, variations occurred specifically at the 20 min of ultrasound application. This may indicate that variation in the percentage of proteins in both fresh and marinated tilapia fillets may occur with the application of ultrasound for more than 10 min.

The ash percentage in tilapia fillets showed a significant interaction ($p<0.05$) between ultrasound exposure time and refrigerated storage duration (Table 1). On day 0, the control fillets had the highest ash percentage, which decreased as the ultrasound exposure time increased. The microcurrents and turbulence generated by ultrasound increases the diffusion of mineral ions between cells and interstitial fluid. Furthermore, the damage to cell and sarcoplasmic membranes facilitate the migration of minerals to the free phase, which can then be released with the exudate. Since the ash content was measured in the whole tissue on a wet basis, any loss of mineral-rich fluid will reduce the proportion of ash retained in the solid sample. From a nutritional standpoint, this

Table 1. Means and standard deviation of moisture, protein, ash, and ether extract of Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7, and 14 days of refrigerated storage[(1)].

Proximate composition	Storage (days)	High-intensity ultrasound (min)		
		0	10	20
Moisture (%)	0	76.50±0.39aA	78.17±0.21cB	77.17±0.28bA**
	7	77.10±0.09aB	77.27±0.08aA	77.21±0.01aA
	14	76.56±0.08aA	77.06±0.13bA	77.26±0.06bA
Protein (%)	0	20.04±0.92aA	19.71±0.68aA	22.29±0.07bB**
	7	19.69±0.37abA	20.92±1.05aA	19.42±0.65bA
	14	19.05±0.54aA	19.63±0.14aA	20.00±0.31aA
Ash (%)	0	1.24±0.02bA	1.18±0.02abA	1.16±0.03aA*
	7	1.20±0.02aA	1.17±0.05aA	1.23±0.02aA
	14	1.19±0.02aA	1.15±0.04aA	1.16±0.01aA
Ether extract (%)	0	3.14±0.05bA	2.52±0.02aA	3.31±0.12cB**
	7	3.08±0.03bA	2.60±0.04aA	2.7 ±0.10aA
	14	3.00±0.04bA	2.82±0.10aB	2.73±0.08aA

[(1)]Means followed by different letters, lowercase in the lines and uppercase in the columns, differ significantly by Tukey's test at different high-intensity ultrasound application times and throughout the refrigerated storage period, respectively. *, and **, p-value < 0.05, < 0.01, respectively.

represents a potential disadvantage of ultrasound on fish meat, as it contains important minerals for human nutrition, such as calcium, phosphorus, selenium, iodine, and zinc. Nevertheless, research regarding the effect of ultrasound on the vitamin and mineral content of aquatic foods remains limited.

Among the limited literature, Sarwar et al. (2019) investigated alterations in mineral content of *Tenualosa ilisha* samples across various processing and preservation methods. Although mineral concentration decreased slightly, the difference was insignificant compared with thermal treatments. Furthermore, at 7 and 14 days of storage, no significant variation ($p>0.05$) between treatments were observed. Throughout the 14-day refrigerated storage period, the ash percentage remained stable after the 0, 10, and 20 min of ultrasound treatments. In contrast, Silva et al. (2022) observed different behavior in marinated tilapia fillets, where the control and 10 min of ultrasound treatments did not show significant changes in ash percentage, with values ranging from 2.29 to 2.99%. This may have occurred due to the variation in the type of product, whether it is marinated fish or fresh fillets, in which minerals can influence the efficiency of ultrasound. Additionally, the ash values reported by Silva et al. (2022) were higher than those in the present study. This may have occurred because the marinade naturally contains more minerals in its composition.

The ether extract percentage in tilapia fillets showed a significant interaction ($p<0.01$) between the distinct ultrasound exposure times and refrigerated storage duration (Table 1). On day 0, fillets subjected to 10 min of ultrasound had the lowest percentage of ether extract. However, this treatment showed an increase in the percentage of ether extract after 14 days of refrigerated storage. This phenomenon can be attributed to an ultrasound-induced structural reorganization, which initially facilitates the release of free lipids that tends to stabilize over time (Gao & Meng, 2022). In contrast, the fillets treated with 20 min of ultrasound showed the highest ($p<0.05$) percentage of ether extract at day 0. However, for this treatment, the percentage of ether extract decreased by day 7 and remained constant through day 14. This reduction may have occurred due to the natural processes of lipid oxidation and enzymatic activity during refrigerated storage.

WHC is a variable of great importance in fish quality assessment, as it directly influences the texture, yield, and juiciness of fillets (Chan et al., 2022). The WHC percentage of the fillets showed significant interaction ($p<0.05$) between the different ultrasound exposure times and refrigerated storage duration. On day 0, no significant differences ($p>0.05$) in WHC were observed between treatments (Table 2). However, after 7 days of storage, both the control and the 10 min ultrasound treatments showed higher ($p<0.05$) WHC values than the 20 min treatment. By day 14, the 10 min treatment achieved the highest WHC, followed by the control and 20 min groups (Table 2). Notably, the 20 min treatment was the only group that did not

Table 2. Means and standard deviation of water holding capacity, hardness, and springiness profiles of Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7, and 14 days of refrigerated storage[(1)].

Parameter	Storage (days)	High-intensity ultrasound (min)		
		0	10	20
WHC (%)	0	70.12±1.17aA	69.23±1.60aA	69.28±1.76aA*
	7	74.94±0.67bB	75.92±1.35bB	69.63±2.07aA
	14	73.87±3.23abB	76.23±0.64bB	72.30±1.34aA
Hardness (N)	0	0.98±0.15bA	0.54±0.09aA	0.58±0.10abA**
	7	0.99±0.19aA	0.69±0.13aAB	0.76±0.10aA
	14	1.54±0.10bB	1.12±0.50bB	0.63±0.12aA
Springiness (mm)	0	3.13±0.96aA	1.73±0.31aAB	1.87±0.64aA*
	7	3.33±1.00aA	3.00±0.52aB	2.03±0.97aA
	14	2.03±0.97aA	1.57±0.38aA	1.60±0.56aA

[(1)]Means followed by different letters, lowercase in the lines and uppercase in the columns, differ significantly by Tukey's test at different high-intensity ultrasound application times and throughout the refrigerated storage period, respectively. *, and **, p-value < 0.05, < 0.01, respectively.

show an increase ($p>0.05$) in the fillets WHC during the 14 days of storage. This behavior suggests that high-intensity ultrasound, when applied moderately for up to 10 minutes, may facilitate the reorganization or partial opening of the myofibrillar matrix, thereby enhancing the interaction between proteins and water (Zhang et al., 2023).

The hardness of tilapia fillets showed significant variation ($p<0.01$), as ultrasound exposure time increased. At day 0, increasing the exposure time to 10 min resulted in a decrease in fillet hardness, while the 20 min treatment yielded an intermediate value, suggesting a potential residual effect of the treatment (Table 2). A similar trend was observed in a study with tuna loin, in which hardness decreased as exposure time to ultrasound increased. This decrease could be observed after 3 min of application and remained stable up to 5 min of application (Fuentes et al., 2016). According to the same authors, high-intensity ultrasound can disrupt the integrity of muscle cells and promote enzymatic reactions, leading to a decrease in the fillets hardness. Conversely, a study on marinated tilapia fillets, found no significant variation in hardness, regardless of the ultrasound treatment used (Silva et al., 2022). One hypothesis is that marinated fillets and fresh tilapia have different textures. Therefore, marinated fillets would need more time to achieve changes in fillet hardness.

The present study observed a significant negative correlation ($p<0.05$) ($r = -0.631$) between hardness and moisture content of tilapia fillets. Essentially, fillets with higher moisture content tended to show lower hardness values. During refrigerated storage, a significant variation ($p<0.05$) in the hardness of tilapia fillets was observed (Table 2). In both the control and 10 min of ultrasound exposure treatments, the hardness of the tilapia fillets increased throughout the 14 days of storage. In contrast, the treatment that received 20 min of ultrasound showed no variation ($p>0.05$) in hardness across the 14 days of storage.

The cohesiveness of tilapia fillets did not show significant variation ($p>0.05$) between treatments, with a mean value of 0.38±0.08. A similar finding was also observed in marinated tilapia, where cohesiveness remained unaffected regardless of ultrasound application (Silva et al., 2022). However, in the study by Silva et al. (2022), marinated tilapia fillets were less cohesive, probably due to the action of acetic acid used in the marinating process, which decreased the cohesiveness of the fillets. Similarly, studies on tuna loins found no variation in cohesiveness following the ultrasound exposure for up to 5 min (Fuentes et al., 2016).

The springiness of tilapia fillets showed no significant variation ($p>0.05$) regardless of ultrasound exposure duration (Table 2). In addition, Silva et al. (2022) did not observe any significant difference in the springiness of between control and ultrasound-treated marinated tilapia fillets. Furthermore, these marinated fillets were less springy than the fresh fillets observed in the present study, showing that different products made from fish fillets have different springiness. In contrast, high-intensity ultrasound increased the springiness of tuna loin when applied for up to 5 min (Fuentes et al., 2016).

Pearson's correlation analysis, revealed a significant negative correlation between springiness ($p<0.05$) ($r = -0.398$) and moisture content. Essentially, the fillets with higher moisture levels exhibited lower springiness. Regarding the refrigerated storage period, significant variation ($p<0.05$) was observed only in the 10 min treatment. Specifically, at day 14, the fillets showed a reduction in springiness compared with the values recorded at day 7.

The L* values of tilapia fillets varied significantly ($p<0.01$) throughout the storage time. To optimize the visualization of these trends, a second-order polynomial regression was applied as the best-fit model (Figure 1). The L* values estimated by regression increased from 44.22 at day 0 to 53.88 at day 7 and decreased to 53.31 at day 14.

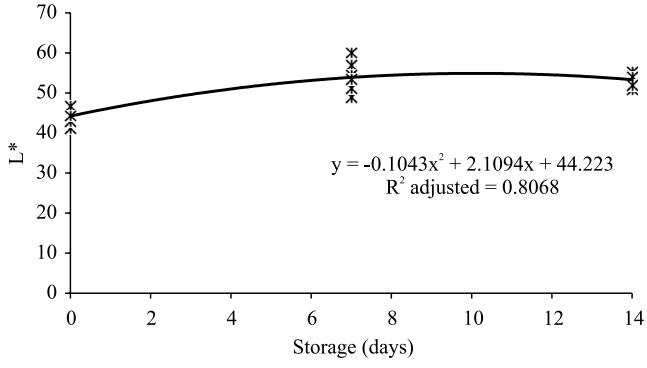

Figure 1. Lightness value (L*) of Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7 and 14 days of refrigerated storage.

Similarly, Ugalde-Torres et al. (2024) applied 20 kHz high-intensity ultrasound for durations up to 90 minutes followed by 20 days of ice storage, the study reported a increase in L* value from 55.77 to 58.28 during storage, although these differences were not significant across different ultrasound application periods. The L* values observed in the present study were similar to those observed in Ugalde-Torres et al. (2024). Similarly to the present study, Silva et al. (2022) observed no significant variation in L* between the control group (73.85) and ultrasound (73.29). Furthermore, Oliveira Filho et al. (2015), observed increasing *L values in tilapia stored on ice at 4°C for 26 days across various stunning methods. According to the authors, the reasons behind the color shifts that occur in fish muscle are still unclear, but it is believed that one of the primary causes is likely the oxidation of muscle myoglobin during storage.

The a* value of the fillets showed significant interaction ($p<0.05$) between the ultrasound exposure duration and refrigerated storage periods (Table 3). The a* value was higher in tilapia fillets subjected to 20 min of ultrasound at day 0. However, for this treatment, a significant decrease ($p<0.05$) was observed at day 14. The 10 min of ultrasound treatment resulted in lower a* values than the 20 min treatment with a similar decline in redness at day 14. Consequently, the differences between the 10 and 20 min treatments were evident only at the beginning of storage, as by day 14, the values became statistically similar.

A similar decrease in redness during refrigerated storage for up to 35 days was reported by Santos et al. (2022) for an emulsified mechanically separated meat (MSM) product from tilapia treated with a high-intensity ultrasound treatment. The authors attributed these color shifts to potential increases in lipid and protein oxidation, which causes changes in both myoglobin and WHC of the product. While both studies involve the same species, the comparison with MSM should be considered with caution over the data from the present study, as the muscle matrices are distinct. Therefore, the mechanisms of lipid and protein oxidation, as well as changes in myoglobin and WHC, may not occur in the same way. Conversely, Ugalde-Torres et al. (2024) observed an increase in red intensity in tilapia fillets subjected to ultrasound and stored on ice for 20 days, rising from 2.02 to values ranging from 3.02 to 4.27.

The b* value showed significant interaction ($p<0.05$) between ultrasound exposure time and refrigerated storage duration (Table 3). In the control treatment, b* values were lowest at day 0, increasing significantly ($p<0.05$) by day 7 and remained stable through day 14. The 20 min of ultrasound treatment resulted in the highest yellow intensity in the tilapia fillets. In this treatment, there was an increase in b* value over the first 7 days of storage and a reduction at 14 days. This reduction in the b* value may be attributed to pigment oxidation. Furthermore, b* values showed a significant positive correlation coefficient with L* values ($p<0.05$; $r = 0.867$), indicating that that as storage time of the fillets increased, the fillets became both brighter and more yellow.

These findings contrast with those of Ugalde-Torres et al. (2024), who reported initial b* values of 10.66 that increased to a range of 12–15 by the end of the storage period. Unlike the continuous increase observed in their study, the present study noted an increase only up to day 7 across all treatments. Similarly, Oliveira Filho et al. (2015) found a significant increase in the b* value of the fillets, suggesting that different physical treatments can influence changes in tilapia muscle coloration during refrigerated storage. In contrast, Silva et al. (2022) observed no variation in yellowness between marinated tilapia fillets that were treated with ultrasound (5.91) and the control group (6.06).

Table 3. Means and standard deviation of instrumental color of Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7, and 14 days of refrigerated storage[(1)].

Color	Storage (days)	High-intensity ultrasound times (minutes)	
		0	10
a*	0	2.11 ± 0.49aA	2.82 ± 0.79abAB
	7	2.06 ± 0.39aA	3.33 ± 0.99aB
	14	2.51 ± 0.67aA	1.16 ± 0.69aA
b*	0	-2.50 ± 0.26aA	-1.09 ± 0.92abA
	7	4.55 ± 1.81aB	5.20 ± 0.89aB
	14	4.09 ± 0.54aB	2.81 ± 0.43aB

[(1)]Means followed by different lowercase letters, in the lines, and uppercase letters, in the columns, differ significantly by Tukey's test at different high-intensity ultrasound application times and throughout the refrigerated storage period, respectively. *, and **, p-value < 0.05, < 0.01, respectively.

The pH values of the tilapia fillets showed a significant interaction ($p<0.01$) between the ultrasound exposure and refrigerated storage durations. For fillets subject to up to 10 minutes of ultrasound, the pH decreased during storage. In contrast, it remained constant for up to 7 days of storage in samples subjected to 20 min of ultrasound, although the pH value was significantly lower by day 14 (Table 4). Conversely, Ugalde-Torres et al. (2024) found initial pH of 6.36, which is notably lower than the values observed in the present study.

Ultrasound technology utilizes high-frequency sound waves, typically in the range of 20 kHz to 1MHz, to induce mechanical effects within the food matrix, such as cavitation, which can disrupt microbial cells and enzymes, leading to their inactivation (Russo et al., 2023). Although the count of psychrotrophic aerobic microorganisms did not show significant differences between treatments, significant variation ($p<0.01$) was observed during the storage period (Figure 2). The psychrotrophic aerobic count increased from < 2 Log CFU g^{-1} at day 0 to 3.43 Log CFU g^{-1} at day 7, and 4.83 Log CFU g^{-1} at day 14.

Current Brazilian legislation does not establish a limit for the presence of psychrotrophic aerobes in refrigerated fish fillets. However, according to international standards, the maximum acceptable threshold of psychrotrophic aerobes ranges from 6 to 7 Log CFU g^{-1} (Ugalde-Torres et al., 2024). This indicates that the tilapia fillets in this study remained suitable for consumption for the 14 days of refrigerated storage. Similarly, Ugalde-Torres et al. (2024) observed initial values of 2.5 Log CFU g^{-1}, reaching values close to 4 Log CFU g^{-1} at 15 days of refrigerated storage. The authors also observed no difference between treatments with high-intensity ultrasound and the control group.

Lipid oxidation is a primary phenomenon responsible for the deterioration of fish quality during storage. This process occurs through the degradation of unsaturated fatty acids, leading to the formation of secondary compounds such as aldehydes and ketones, which cause changes in the taste, odor, and color of the product (Secci & Parisi, 2016). The TBARS method showed a significant interaction ($p<0.05$) between ultrasound application and refrigerated storage periods (Table 5).

The control treatment and the treatment with 10 min of ultrasound exposure showed similar lipid oxidation behavior in the fillets, with no significant increase throughout the storage period (Table 5). However, the treatment with 20 min of ultrasound exposure showed an increase in lipid oxidation over the 14-day storage period resulting in a significantly higher accumulation of TBARS after 14 days of storage. This indicates that excessive exposure time to ultrasonic waves can catalyze lipid oxidation in tilapia fillets. According to Bai et al. (2023), the cavitational waves generated by ultrasound induce the rupture of water molecules, which generate hydroxyl radicals that accelerate the rate of lipid oxidation in fish muscle.

In a study with a MSM product subjected to 49 min of 500W ultrasound, TBARS levels significantly increased over 35 days of refrigerated storage, rising from 1.06 to 5.19 mg kg^{-1}. The product presented a rancid taste, loss of color, and altered texture. In

Table 4. Means and standard deviation of pH analysis of Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7, and 14 days of refrigerated storage[(1)].

	Storage (days)	High-intensity ultrasound times (min)		
		0	10	20
pH	0	6.96±0.11bB	6.61±0.09aC	6.54±0.03aB**
	7	6.37±0.11aA	6.46±0.01abB	6.59±0.03bB
	14	6.30±0.02bA	6.06±0.03aA	6.24±0.03bA

[(1)]Means followed by different letters, lowercase in the lines and uppercase in the columns, differ significantly by Tukey's test at different high-intensity ultrasound application times and throughout the refrigerated storage period, respectively. *, and **, p-value < 0.05, < 0.01, respectively.

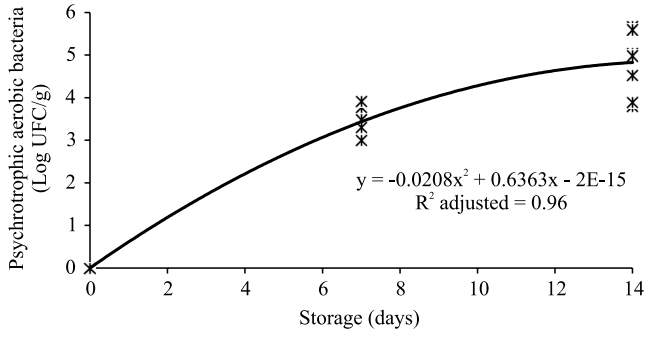

Figure 2. Results of the psychrotrophic aerobic bacteria count in Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7 and 14 days of refrigerated storage.

Table 5. Means and standard deviation of TBARS lipid oxidation analysis of Nile tilapia (*Oreochromis niloticus*) fillets subjected to 0, 10, and 20 min of high-intensity ultrasound and 0, 7, and 14 days of refrigerated storage[(1)].

	Storage (days)	High-intensity ultrasound time (minutes)		
		0	10	20
TBARS (mg malonaldehyde per kg)	0	1.48±0.13aA	1.29±0.09aA	1.26±0.08aA*
	7	1.25±0.04aA	1.52±0.14aA	1.36±0.08aAB
	14	1.23±0.02aA	1.43±0.19abA	1.68±0.42bB

[(1)]Means followed by different letters, lowercase in the lines and uppercase in the columns, differ significantly by Tukey's test at different high-intensity ultrasound application times and throughout the refrigerated storage period, respectively. *, and **, p-value < 0.05, < 0.01, respectively.

contrast, the results obtained in the present study were significantly lower. This discrepancy can be attributed to the difference in raw material, which was tilapia MSM, as well as the longer application time of high-intensity ultrasound and higher power.

Conclusions

1. The use of 10 min of ultrasound at an ultrasonic frequency of 37 kHz and 100W of effective power proved to be the most promising treatment, as it maintains higher Nile tilapia fillet moisture, and lower muscle hardness and elasticity.

2. No decrease in water retention capacity or protein percentage was observed in the 10 min of ultrasound treatment.

3. The 10 minutes of ultrasound treatment makes the Nile tilapia fillets more acidic, which is a positive factor for preservation.

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Chief editor: Edemar Corazza
Edited by: Madalena Rinaldi

Data availability statement

Data available upon request: research data are only available upon reasonable request to the corresponding author.

Declaration of use of AI technologies

No generative artificial intelligence (AI) was used in this study.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financing, in part, this study (Finance Code 001); to Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), for the Research Project Grant, Process: APQ-0956-5.06/24, and Pernambuco Productivity Grant, Process: BPP-0081-5.06/24; and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the granted scientific initiation scholarships.

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