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Property and Microstructural Changes in Skipjack Tuna (*Katsuwonus pelamis*) Chunks during Ultrasound-assisted Osmotic Dehydration

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Abstract: *This study investigates the physicochemical and microstructural changes in skipjack tuna subjected to ultrasound-assisted osmotic dehydration (US-OD). Results revealed that US-OD significantly reduced moisture content, with the greatest reduction observed at 28 kHz for 30 minutes. True density and ash content increased with longer ultrasound application, indicating higher salt infusion. While pH decreased after brining due to protein denaturation, ultrasound frequency and duration had no significant effect on it. Firmness increased with longer application time, attributed to salt-induced protein aggregation. Color lightness decreased due to water loss, but changes were not statistically influenced by ultrasound parameters. Scanning electron microscopy showed that higher frequency and longer duration caused more extensive muscle fiber disruption, increased myofibrillar gaps, and collagen denaturation. These findings suggest that US-OD effectively enhances mass transfer in tuna, with ultrasound frequency and application time playing key roles in determining the extent of structural and compositional changes in the product.*

Keywords: ultrasound; osmotic dehydration; skipjack tuna; microstructural changes; ultrasound brining

1. INTRODUCTION

Growing consumer knowledge of the nutritional advantages of seafood, especially its high-quality protein and omega-3 fatty acid content, has led to a recent boom in demand for premium, minimally processed seafood products worldwide [1]. Ultrasound is one of the emerging non-thermal food processing technologies [2] that has attracted plenty of interest since it can improve the physicochemical and functional qualities of seafood while maintaining its nutritional value and sensory appeal [3]. High-intensity ultrasound (HIU), typically operating at frequencies between 20 and 100 kHz, induces important mechanical effects like cavitation and sponge effect which can modify protein structure and improve mass transfer during processing [4]. In the context of seafood, these effects have been investigated for applications such as marination, tenderization, drying, freezing, thawing, and microbial decontamination [5].

The alteration of the functional characteristics of seafood products is one of the most significant areas where ultrasound has been demonstrated to be helpful. For the processing and ultimate quality of seafood products, functional characteristics such water-holding capacity (WHC), firmness, pH, and gelation behavior are essential [6]. According to several studies, ultrasonication can break down protein chains and disturb the architecture of muscle fibers, thereby increasing the exposure of reactive areas and enhancing functional behavior [7]. For example, it has been demonstrated that ultrasound-assisted processing improves the emulsifying stability of fish protein isolates, increases protein solubility and WHC in surimi [8], and makes it easier for gel formation in a variety of fish species [5]. Although the amount of research on the application of ultrasound in seafood processing is increasing, there is still a clear lack of information regarding how it affects valuable marine species like tuna.

Due to its nutritional value and economic significance, tuna especially species like *Thunnus albacares* (yellowfin), *Thunnus thynnus* (bluefin), and *Katsuwonus pelamis* (skipjack) is a prime target for functional augmentation through innovative processing technologies [9]. However, only a small number of research have examined the effects of ultrasound on the muscle proteins, functional traits, and textural integrity of tuna, even though ultrasound has been studied in white-fleshed fish like cod, and tilapia [5]. Anatomically, the tuna muscle differs structurally and biochemically from many commonly studied fish species. Myoglobin, connective tissue, and heat-stable proteins are more abundant in tuna muscle, which affects how the muscle behaves during processing and how it reacts to technological interventions [10] like ultrasound. Hence, conclusions drawn from research on other fish might not apply directly to tuna. For instance, because of its higher fat content and distinct sarcoplasmic protein composition, tuna may have different effects from ultrasound on protein denaturation temperatures, salt distribution, WHC, and chewiness [11]. Furthermore, tuna is often used in high-end food products such as sashimi, canned fillets, ready-to-eat meals, and dried delicacy such as *katsuobushi* where textural and sensory attributes are paramount. In processes like brining and osmotic dehydration, the operations are time-consuming, and distribution of solutes may be non-uniform [12, 13]. Ultrasound application in these situations needs to be properly calibrated to prevent negative consequences like excessive protein denaturation or the formation of an off-flavor due to lipid oxidation [4]. Thus far, few studies have thoroughly examined the effects of ultrasonic parameters such frequency, amplitude, and treatment duration on the functional properties of processed tuna products. Optimizing industrial applications of

ultrasound in tuna processing is additionally hampered by the scarceness of studies specifically on the fish. The lack of a comprehensive understanding of the molecular effects of ultrasound on tuna muscle may make processors hesitant to apply this technology on a commercial scale [5]. Thus, specialized studies are required to assess how ultrasound affects the physicochemical and functional characteristics of tuna muscle proteins, as well as the interaction between biochemical alterations and ultrasound settings [4]. To address this gap, the current work aims to investigate the property changes of skipjack tuna when subjected to ultrasound-assisted osmotic dehydration (US-OD) which is an important pre-treatment procedure for other food processing operations like drying and cooking. This study will evaluate the moisture content, true density, ash content, pH, firmness and color of ultrasonicated tuna, as well as the changes in microstructure as affected by ultrasound frequency and application duration.

2. MATERIALS AND METHODS

2.1 Sample preparation

The fresh whole skipjack tuna was purchased directly from the General Santos City Fishport market and immediately transported in iceboxes to Mindanao State University–General Santos City Postharvest Laboratory. At the experimental station, the fish was washed with running water to remove any salt or dirt that had accumulated on its surface. The head, fins, and tail were then chopped off, and the intestines, gills, and other internal organs were removed. Running water was used to wash the fish once more until all its blood had been removed. The fish was then sliced lengthwise into two sections and the bones were removed to fillet the meat. Afterwards, the tuna meat was manually chopped using sharp scalpel into 10x20x20 mm chunks. The entire preparation process needed to be finished in five minutes to minimize biochemical changes in the fresh meat [14]. While the experiment has not yet begun, samples were kept in the refrigerator for a short time at 5-10°C. Intermediate residence time should be less than 30min. To eliminate extra surface moisture, the samples were wiped with adsorbent paper just prior to ultrasound-assisted osmotic dehydration (US-OD) pretreatment.

2.2 Ultrasound-assisted osmotic dehydration

For treatment of samples, fabricated ultrasonic equipment was used. The equipment is water-bath type with three main components: generator, transducers, and tank. The generator provides energy to the system, and it has several buttons for selection of ultrasound frequencies which are preset at 20kHz, 28kHz, and 40kHz. The generator powered the transducers which convert the electric power to ultrasound at appropriate frequency. The transducers are located at the bottom of the tank which transfers the ultrasound power to the medium or the brine solution. The ultrasound bath has a capacity of 10L. A 20% brine solution (20g salt/100g water phase) was prepared using regular food-grade table salt (NaCl) for osmotic pretreatment. First, brine solution was added to the ultrasound bath, and it was warmed up to the desired frequency. The tank was then filled with a single layer of tuna chunks. The proportion of tuna samples to solution was at least 1:20 since this ratio is large enough to ignore concentration fluctuations during osmosis [15]. Due to possible heat production of ultrasound waves, ice cubes may be added to the water bath continuously to keep a constant temperature. A thermocouple thermometer was used to monitor water temperature in the brine solution. The tuna chunks soaked in 20% brine solution was subjected to US-OD with two factors: ultrasound frequency (20, 28 and 40 kHz), and application time (10, 20 and 30 min). After treatment, samples were taken out of the ultrasound bath and were washed quickly in a pan of distilled water to remove excess solution and salt adhering to the surface. Afterwards, they were gently dabbed with adsorbent paper to eliminate surface moisture. Pressing and squeezing of samples was avoided. Samples were then subjected to different property testing procedures.

2.3 Moisture Content Determination

The official AOAC oven drying procedure (AOAC 925.09) was used to determine the moisture content of tuna chunks. The oven used was Biobase drying oven model BOV-V136F with 1740W power supply, 220V, 60Hz. In this method, 100g tuna sample was chopped very finely and mixed thoroughly. Empty dish and lid was initially oven dried at 105°C for 30min, then transferred to the desiccator to cool for 30min. Weights of the empty dish and lid was measured up to 3 decimal places. About 5g of tuna sample was taken and placed in the dish, and then weighed. Partially covered by its lid, the dish with the sample was subjected to oven drying for 16hrs or overnight at 105°C. After drying, the dish with the lid was transferred to the desiccator for cooling for about 45min and then reweighed.

The moisture content (MC) is computed as:

$$MC\ (\%) = \frac{W_{\text{before oven drying}} - W_{\text{after oven drying}}}{W_{\text{before oven drying}}}$$

2.4 Ash Content Determination

For ash content, the dried samples from oven drying was transferred into the furnace. The equipment used was Vulcan A-130 muffle furnace, 200-240V, 50/60Jz, 1060W. After 2 hrs inside the furnace at 600^{O}C, the samples were taken out and the weights are measured. Ash content in fresh basis is computed as:

$$Ash\ (\%) = \frac{W_{ash}}{W_{\text{before oven drying}}}$$

2.5 True Density Measurement

The true density of tuna samples was measured using a modified pycnometric method [16]. A test tube with known volume was filled with small portions of the minced tuna so that free spaces were removed. The meat was smoothed at the ends of the tube using a glass plate. The filled test tube was weighed on an analytical scale (precision 0.001 g). The true density was obtained by dividing the weight of a sample by the volume of the test tube.

$$\text{True Density } (g/cm^3) = \frac{W\ (g)}{V\ (cm^3)}$$

2.6 pH Measurement

The determination of pH by Wang et al. [14] was followed with minor modifications. The 4.0-g tuna sample was shredded and put into a beaker which was initially filled with 70-80mL distilled water. The mixture was extracted using water bath with constant stirring at

80°C for 20min. The solution was be poured into a 100-mL volumetric flask, constant-volumed, and then centrifuged at 4,000rpm for 10min using ordinary centrifuge at room temperature. Filtration was done using common qualitative filter paper. For measuring the pH of the extract, a digital pH meter (Apera pH meter) with a spear-tip electrode was used. All pH measurements was first converted to [H+] before calculating the mean [17] in order to have valid symmetrical confidence limits. These values was then reconverted to pH units for further analysis. Conversion of pH to [H+] is given as:

$$pH = - \log[H+]$$

2.7 Color Measurement

For color determination, the CAPSURE portable spectrocolorimeter was used. A white standard plate was used for calibration. The color analysis was based on CIEL*a*b scale. At least five points on the sample surface were taken from each tuna chunk for color measurement.

2.8 Firmness Measurement

Since they are still relatively soft, the firmness parameters of tuna samples were evaluated through compression test using a digital force gauge (ShenCe 500N) with circular anvil. The tuna chunk was subjected to a single compression-test at an anvil speed of 40mm/min and the maximum force was recorded.

2.9 Microstructure Analysis

The microstructure analysis was done using scanning electron microscopy (SEM) following the procedures described by González-González et al. [18]. The laboratory analysis was conducted at MSU-IIT Center for Sustainable Polymers using JEOL JSM-IT200 SEM. Tuna chunks were initially dried using a laboratory even at 60°C until moisture content was reduced to 15%. Representative sample from each treatment was cut into half. The bottom part was used for the test with the broken surface upward. The dried samples were directly coated with gold for 10min in an ionizer for observation in a scanning electron microscope operated at 10 kV and 200x magnification.

2.10 Statistical Analysis

The collected data were analyzed using two-way ANOVA analysis and Least Significance Difference (LSD) through IBM SPSS Statistics 30.0.0 and graphically generated using Design Expert.

3. RESULTS AND DISCUSSIONS

3.1 Changes in Moisture Content

The moisture content (MC) of skipjack tuna had slightly decreased after US-OD. From the experiments, the MC of fresh skipjack tuna is roughly 73.9% which is close to the findings (69.0% to 72.60%) by a study of Balogun and Talabi [19]. The values in Table 1 are less than the MC of fresh sample which indicates that osmotic dehydration can relatively lower down the MC of processed products. The highest reduction in MC is observed in the 28kHz–30min US-OD where samples had a final moisture content of 70.6%. On the other hand, the least reduction in the moisture content was for the 40kHz-10min US-OD (71.7%). The results suggest that a lower ultrasound frequency and a longer application time can further decrease the MC of the treated samples. This observation can be appreciably seen in the 3D surface plots in Figure 1.

Table 1. Mean moisture content (%) of ultrasonicated brined skipjack tuna

Ultrasound Freq (kHz)	Time (min)			Grand Mean
	10	20	30	
20	71.5	71.2	70.7	71.1^{cd}
28	70.9	70.8	70.6	70.7^{c}
40	71.7	71.4	70.8	71.3^{d}
Grand mean	71.4^{a}	71.1^{a}	70.7^{b}	

*means with different letters are significantly at 5% using LSD

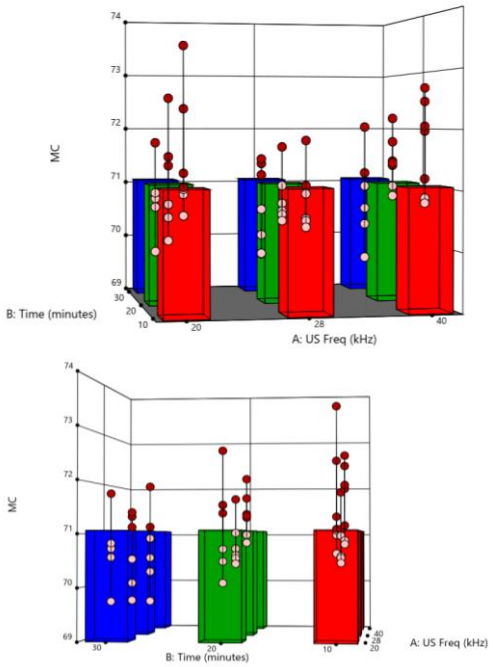


Figure 1. 3D surface graphs of MC in tuna as affected by ultrasound frequency (top) and time (bottom)

Based on ANOVA, there is indeed a significant difference in the MC of samples as affected by both factors. In terms of ultrasound frequency, the 40 kHz can be said to induce the least removal of moisture in the treated samples. Its performance is not significantly different from 20 kHz, but significantly different from 28 kHz. This is because higher-frequency ultrasound produces smaller, less destructive microbubbles, while lower-frequency ultrasound generates fewer but larger, energy-dense microbubbles that cause greater mechanical damage [20].

Moreover, the peak reduction in MC is observed at 28 kHz rather than 20 kHz, likely due to more effective microchannel formation and a stronger 'sponge effect' that promotes release of internal moisture [21]. At 20 kHz, dominant cavitation primarily erodes the surface, removing only external moisture. Although 40 kHz may form more efficient microchannels, increased salt infusion retains moisture, leading to less overall removal.

In terms of application time, the means for 10 and 20 mins are not significantly different from each other but differs significantly from 30 mins. Longer duration of ultrasound applications can therefore intensify the effects of ultrasound, leading to more extraction of water out of the product.

3.2 Changes in True Density

The true density of fresh tuna was computed to be 1.4207 g/cm^3 and this value is lower than those found in Table 2. Thus, it can be said that brining increases the density of the products because this process adds materials (the NaCl) into the tuna samples. From the ANOVA results, there is no significant difference among the means as affected by ultrasound frequency. This is obvious as the values of true density among the three ultrasound frequencies are too close and are not widely scattered.

Table 2. Mean true density (g/cm^3) of ultrasonicated brined skipjack tuna

Ultrasound Freq ^{ns} (kHz)	Time (min)			Grand Mean
	10	20	30	
20	1.424	1.437	1.443	1.434
28	1.437	1.439	1.447	1.441
40	1.431	1.438	1.442	1.437
Grand mean	1.431^{a}	1.438^{ab}	1.444^{b}	

ns means not significantly different at 5%
*means with different letters are significantly at 5% using LSD

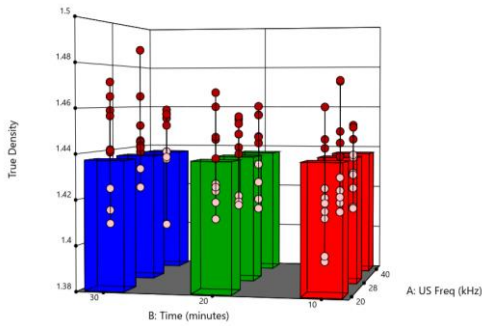


Figure 2. 3D surface graphs of true density of tuna as affected by ultrasound frequency and time

On the other hand, the application time of ultrasound technology resulted in significantly different means of true density. The highest density was recorded for values at 30 mins (Figure 2) which implies that longer application time of ultrasound can result in denser products. There is greater diffusion of salt into the tuna chunks at longer ultrasound duration due to the formation of more efficient microchannels which can generate products with higher density [11].

3.3 Changes in Ash Content

The ash content of fresh skipjack tuna is 1.42% which is close to the findings (1.53% to 2.42%) by Balogun & Talabi [19]. Comparing this to the values in Table 3, it can be surmised that the salt infused into the tuna chunks during brining increased the ash content of the products. The ANOVA results revealed that the mean ash content among the brined tuna is significantly different as affected by both factors. In terms of ultrasound frequency, all three treatments differ significantly from each other, with the 28 kHz registering the highest mean ash content (4.42%) while the 40 kHz having the lowest mean of 3.62%. This result suggests that while higher ultrasound frequencies promote microchannel formation, they may also facilitate easier transport of both moisture and salt [22], therefore reducing retention of these materials during the brining process.

Table 3. Ash content (%) of ultrasonicated brined skipjack tuna

Ultrasound Freq (kHz)	Time (min)			Grand Mean
	10	20	30	
20	3.26	3.86	4.54	3.89^{d}
28	4.05	4.31	4.91	4.42^{e}
40	2.90	3.71	4.24	3.62^{f}
Grand mean	3.40^{a}	3.96^{b}	4.56^{c}	

*means with different letters are significantly at 5% using LSD

The effect of ultrasound application time is once again highlighted with the results of ash content in the brined skipjack tuna. Longer application time resulted in greater uptake of salt by meat, thereby resulting in higher ash content.

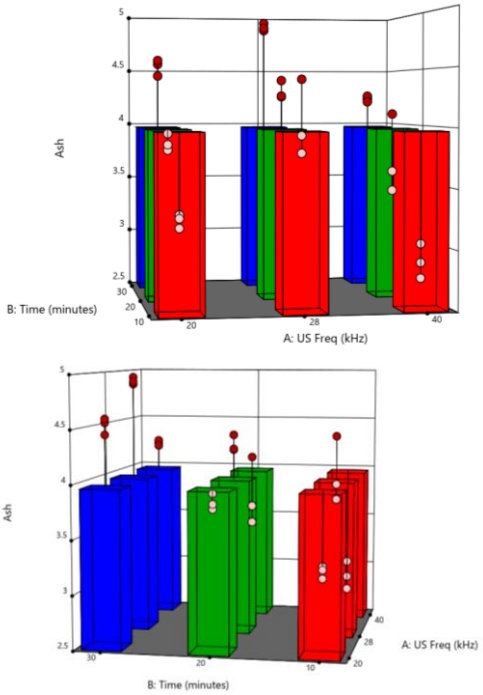


Figure 3. 3D surface graphs of ash in tuna as affected by ultrasound frequency (top) and time (bottom)

The differences in the influences of ultrasound frequency and application time is highlighted in the 3D surface graphs in Figure 3. Highest values are observed at 28 kHz US frequency and 30 min application time, validating the results of ANOVA.

3.4 Changes in pH

The pH of fresh skipjack tuna is approximately 5.68 which is within the range of 5.2 and 6.1, the pH of fresh big eye and skipjack tuna [23]. After brining, the pH value generally decreased (Table 4). This is primarily due to protein denaturation caused by the presence of salts which disrupts the structure of muscle proteins, thereby releasing ions that increases the acidity of meat [24]. Salting also causes the dissociation of amino acids and small peptides, leading to a decrease in pH [25].
Statistically, the means in Table 4 are not significantly different, and hence, both the ultrasound frequency and application time did not induce much effect on the pH of the brined tuna. This can be further observed in Figure 4 where the bar graphs are almost at the same level. In this case, the change in the pH of tuna chunks is due to the salt in the brine solution and not directly by the mechanical effects of ultrasound.

Table 4. pH of ultrasonicated brined skipjack tuna

Ultrasound Freq ^{ns} (kHz)	Time ^{ns} (min)			Grand Mean
	10	20	30	
20	5.58	5.59	5.62	5.60
28	5.61	5.62	5.64	5.62
40	5.61	5.62	5.64	5.62
Grand mean	5.60	5.61	5.63	

ns means not significantly different at 5%

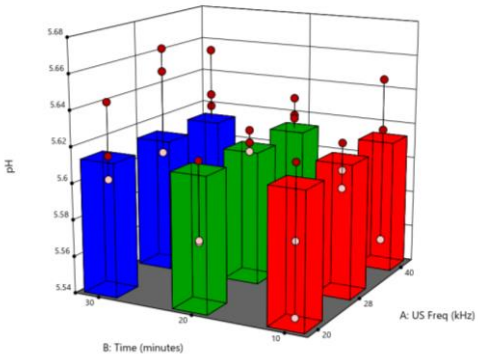


Figure 4. 3D surface graphs of pH in tuna as affected by ultrasound frequency and time

3.5 Changes in Firmness

The firmness values (Table 5) of the brined skipjack tuna are all greater than that for fresh sample which was roughly 9.33N. The increase in sample firmness is primarily due to salt integration into the meat; higher salt content correlates with greater hardness. Additionally, salt-induced protein denaturation, which causes alteration of the natural shape and structure of protein, leads to aggregation, resulting in a tougher, rubbery texture [26].

In Figure 5, there is obvious height differences among the surface plots, with the 30 mins application time showing the tallest bars. Among the two factors, only the application time was found to induce significant changes in the firmness of the samples. This means that though the mean values among the three ultrasound frequencies may appear to be not close to one another, the differences are not significant through ANOVA. Thus, the ultrasound frequencies tested have similar effects to the strength of the brined tuna. As to application time, its effect on the quality of the products is again given emphasis. This mean that longer ultrasonication during the brining process results in greater salt content in the product, thereby increasing its firmness.

Table 5. Firmness (N) of brined skipjack tuna

Ultrasound Freq ^{ns} (kHz)	Time (min)			Grand Mean
	10	20	30	
20	14.44	16.77	18.34	16.52
28	14.75	16.83	18.87	16.82
40	14.88	17.16	18.18	16.74
Grand mean	14.69^{a}	16.92^{b}	18.46^{c}	

ns means not significantly different at 5%
*means with different letters are significantly at 5% using LSD

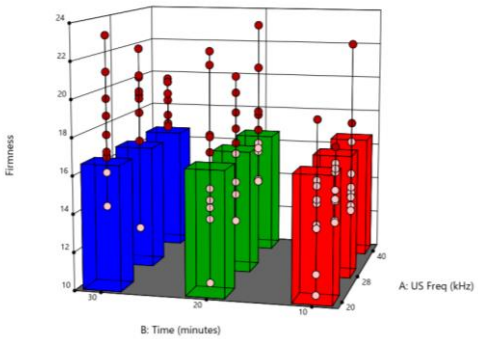


Figure 5. 3D surface graphs on firmness of tuna as affected by ultrasound frequency and time

3.6 Changes in Color

Color is the first sensory property that consumers usually evaluate in food products. In this study, the color values of L*, which corresponds to brightness or lightness, were measured in the brined tuna. It ranges from 0 (black) to 100 (white), and hence, higher values indicate lighter color in the material.

Table 6. Mean color (L*) of ultrasonicated brined skipjack tuna

Ultrasound Freq ^{ns} (kHz)	Time ^{ns} (min)			Grand Mean
	10	20	30	
20	44.71	45.17	48.88	46.25
28	45.84	47.50	49.37	47.57
40	45.80	46.53	47.13	46.49
Grand mean	45.45	46.40	48.46	

ns means not significantly different at 5%

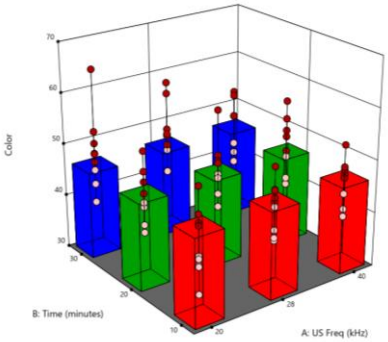
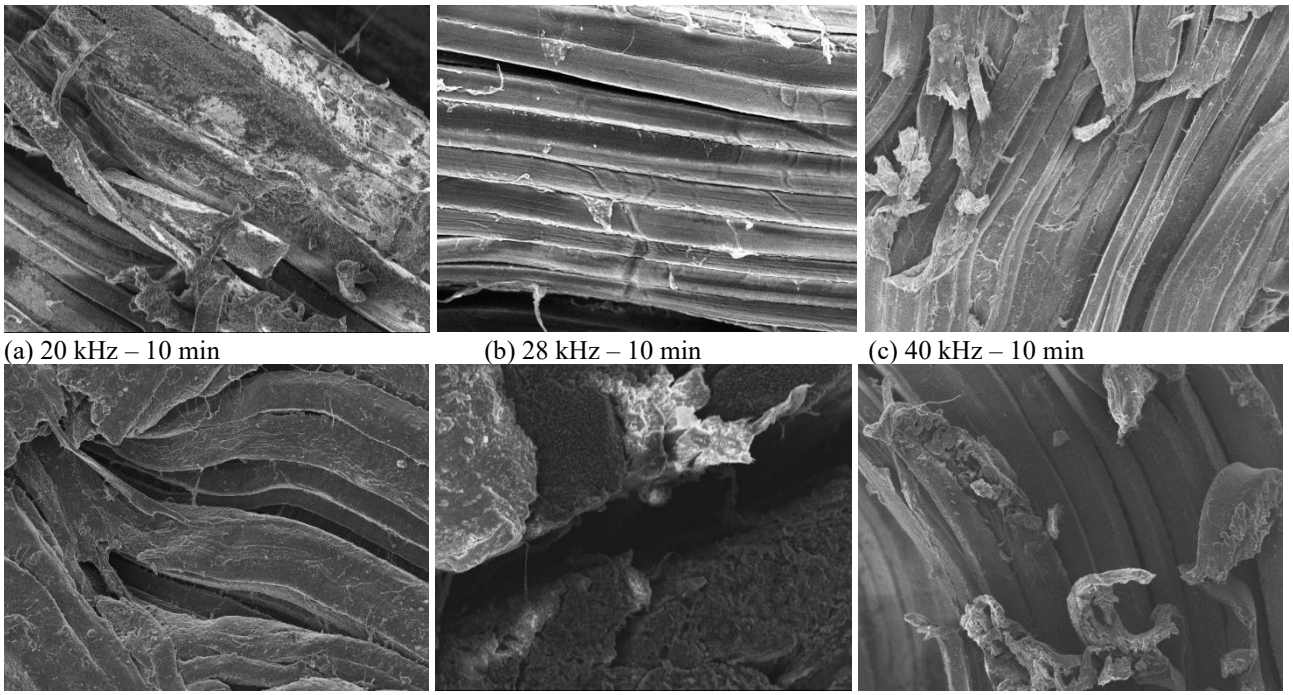


Figure 6. 3D surface graphs on color of tuna as affected by ultrasound frequency and time

The color in terms of L* of fresh tuna was initially computed as 53.30. Upon US-OD, the color of tuna was reduced to the range of 44.71-49.37 (Table 6). The decrease in lightness can be attributed to partial dehydration due to osmosis in which the loss of water can reduce light scattering and transparency [27]. Darkening was also observed in other salted fish products like sardines [25]. Results of ANOVA revealed no significant differences in the mean L* values of brined tuna as affected by both factors. Thus, though actual color values may differ, the differences are not statistically significant (Figure 6). This implies that ultrasound may not actually affect color values in processed tuna. This is supported by the absence of sufficient literature where ultrasound was found to induce changes in color. In this case, the reduction in the lightness of the tuna products was brought about by the brining process and not by the ultrasound treatment.



(a) 20 kHz – 10 min (b) 28 kHz – 10 min (c) 40 kHz – 10 min

(d) 20 kHz – 30 min (b) 28 kHz – 30 min (c) 40 kHz – 30 min

Figure 7. Tuna muscle fibers at different US-OD conditions at 200x magnification

3.7 Microstructural Changes

Representative samples were taken for microstructural analysis through SEM. Figure 7 shows the muscle fibers of ultrasonicated tuna at 200x magnification. Among the ultrasound frequencies, the sample at 20 kHz has the most intact muscle tissues. The muscle fibers are still connected to each other and the spaces between them are narrow. At 28 kHz, the spaces become broader, and the widening of the myofibers may indicate degradation of myofibrils within them, as well as enlargement of myofibrillar gaps. The myofibers become more prominent at 40 kHz as they get more detached and separated. Similarly, larger gaps can be seen in muscles treated with ultrasound at longer duration. The fibers have generally greater separation at 30 min than at 10 mins application time. Indeed, the application of ultrasound leads to instability of protein structures (Sanches et al., 2021), solubilization of myofibrillar proteins (Jin et al., 2023), and degradation of myosin heavy chains (Wang et al., 2021), all resulting to reduced aggregation of muscle proteins (Bian et al., 2022), extensive swelling of myofibers (Shi et al., 2022), greater myofibrillar gaps (Marino et al., 2023), and larger pores between muscle fibers (Xion et al., 2020). Cavitation bubbles from ultrasound also opens and denatures collagen (Marino et al., 2023), leading to loosening of myofibers since collagen is the main connective tissue in muscle. Therefore, intensifying ultrasound through higher frequency and longer duration leads to greater degradation of endogenous proteins and wider intramuscular spaces.

4. CONCLUSION

Based on the quality measurement, application time appeared to be the factor to cause greater changes in moisture content, ash content, density, and firmness of brined tuna. Longer ultrasonication time resulted in more salt being integrated into the product, resulting in significant changes in some functional properties of food. US frequency was also found to significantly affect some quality parameters like moisture and ash content. Specifically, the 28 kHz was found to have good synergy between cavitation and sponge effects, leading to better diffusion of salts into the meat. Both the diffusion kinetic models and microstructural analysis confirmed a more uniform distribution of salt in the meat at this frequency. Ultrasound was not found to statistically alter pH and color, and the changes in these properties were solely attributed to the effects of salts in the meat product during brining.

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6. DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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