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INFLUENCE OF ULTRASONIC BATH PRETREATMENTS ON THE QUALITY CHARACTERISTICS OF *Epinephelus diacanthus* FILLETS STORED UNDER REFRIGERATED CONDITIONS

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ABSTRACT

This study evaluated the effect of ultrasonic bath pre-treatments on the quality and shelf life of reef cod (*Epinephelus diacanthus*) fillets stored at 4 ± 1 °C. Fillets were subjected to five treatments: control (USC) and ultrasound exposure for 15 min (US1), 30 min (US2), 45 min (US3), and 60 min (US4). Microbiological, biochemical, and sensory attributes were assessed during refrigerated storage. Quality deterioration occurred in all samples; however, ultrasonic pre-treatment significantly delayed spoilage. The US2 treatment (30 min) showed the best preservation effect, with lower microbial counts (TPC: 5.12 ± 0.16 log CFU/g) and reduced spoilage indicators (TVB-N: 35.58 ± 0.52 mg/100 g; TMA-N: 14.90 ± 0.16 mg/100 g; pH: 7.21 ± 0.08). *Escherichia coli* was not detected in any sample. Sensory evaluation indicated that US2-treated fillets maintained higher acceptability throughout storage. Based on overall acceptability, US2 (30 min) samples remained acceptable for up to 20 days, while USC, US1, and US3 remained acceptable for 15 days and US4 for 10 days. The results demonstrate that a 30 min ultrasonic bath pre-treatment effectively suppresses microbial growth, delays spoilage, and extends the refrigerated shelf life of reef cod fillets.

Keywords : Ultrasonic Bath, Reef cod (*Epinephelus diacanthus*), refrigerated condition.

Introduction

Seafood is widely recognized as a rich source of high-quality protein, essential vitamins, minerals, and long-chain omega-3 fatty acids, which contribute significantly to human health and nutrition (Gopal, 2020; Hu and Chan, 2020). Regular seafood consumption has been associated with numerous health benefits, including improved cardiovascular function, enhanced cognitive development, and a reduced risk of chronic diseases owing to its anti-inflammatory properties (Gopal, 2020). Beyond its nutritional value, seafood plays a vital role in ensuring global food and nutritional security, particularly in developing nations where it serves as an affordable and accessible source

of animal protein (Viji *et al.*, 2018; Nielsen *et al.*, 2021).

The fisheries and seafood sector is an important component of the Indian economy, contributing substantially to food security, livelihood generation, and export earnings. India is one of the world's leading fish-producing nations, with a total fish production of approximately 197.75 lakh tonnes during 2024–25. In the same period, seafood exports reached 16.98 lakh tonnes, generating a value of ₹ 62,408.45 crores, underscoring the sector's economic significance and the increasing global demand for seafood products (MPEDA, 2025). These trends emphasize the need for effective preservation technologies to maintain seafood

quality, extend shelf life, and ensure product safety throughout storage and distribution.

Despite its benefits, seafood is highly perishable and susceptible to rapid deterioration due to microbial proliferation and enzymatic reactions, resulting in quality loss and significant post-harvest losses (Barange *et al.*, 2018; Ravishankar and Elavarasan, 2024). Refrigerated storage is commonly employed to retard spoilage and prolong shelf life; however, it does not fully suppress microbial growth, especially psychrotrophic microorganisms that remain active under low-temperature conditions. (Sampels, 2015; Nugrowibowo *et al.*, 2023). Therefore, there is a need for additional preservation methods to improve microbial stability and preserve product quality.

In recent years, non-thermal processing technologies have gained attention due to their ability to improve food safety while preserving nutritional and sensory attributes (Delorme *et al.*, 2020; Campus, 2010). Among these, ultrasonic pre-treatment has emerged as a promising technique in seafood processing. Ultrasound operates through acoustic cavitation, where the formation and collapse of microbubbles generate localized shear forces capable of disrupting microbial cell membranes and reducing microbial load (Beitia *et al.*, 2023). This technique has been reported to delay spoilage, improve physicochemical properties and extend the shelf life of fish products during refrigerated storage (Zhang *et al.*, 2023; Sireesha *et al.*, 2022).

Reef cod (*Epinephelus diacanthus*) is an economically important marine fish species widely consumed due to its good flesh quality and increasing market demand in India (CMFRI, 2022). However, its shelf life under refrigerated conditions is limited because of rapid microbial growth, which leads to quality deterioration during storage. Although ultrasonic treatment has been reported as an effective non-thermal method to improve the quality and extend the shelf life of fish, limited information is available on the effect of different treatment durations on microbial quality and storage stability.

Therefore, the present study was carried out to evaluate the effect of different durations of ultrasonic pre-treatment on the microbial, biochemical and sensory characteristics of reef cod (*Epinephelus diacanthus*) fillets during refrigerated storage.

Material and Methods

Raw material

Fresh reef cod (*Epinephelus diacanthus*) were procured from the Veraval landing center on the west

coast of Gujarat, where they were originally caught using trawl nets. A total of 10 kg of fish with an average length of 28.1 ± 0.35 cm and weight of 266.00 ± 9.85 g was used for the experiment. The fish were transported in insulated boxes under iced conditions ($0-2^{\circ}\text{C}$) to the Fish Processing Laboratory, College of Fisheries Science, KU, Veraval. Upon arrival, the fish were washed, weighed, descaled, beheaded, gutted and filleted. The fillets were then rewashed with chilled potable water to ensure cleanliness before further experimental use.

Ultrasonic

The ultrasonic treatment used in this study was a Digital Ultrasonic Cleaner (Model: KLDUC-50L, KINGLAB Instruments Private Limited, India) with a capacity of 50 L. The instrument operates using high-frequency sound waves in water, generating cavitation bubbles that assist in effective cleaning and removal of surface contaminants. It is equipped with digital controls for time and temperature, enabling precise and uniform ultrasonic treatment conditions.

Experimental Procedures

The experiment was conducted to evaluate the effect of different ultrasonic exposure times on fish fillets. Samples were divided into five treatments: control (USC) without ultrasonic treatment and US1, US2, US3 and US4 subjected to ultrasound for 15, 30, 45 and 60 minutes, respectively. The fillets were packed in 200-gauge LDPE pouches and treated accordingly. After treatment, all samples were stored under refrigerated conditions ($4 \pm 1^{\circ}\text{C}$). Quality parameters were analyzed at 5-day intervals to assess the impact of ultrasonic treatment on the quality and shelf life of the fillets.

Quality analysis

Standard methods were used to evaluate the microbiological, biochemical and sensory quality of reef cod (*Epinephelus diacanthus*) fillets. Microbiological analyses were performed using standard plate count techniques. Total plate count (TPC) was determined as described by Mailoa *et al.* (2017), *Escherichia coli* according to AOAC (2006) and psychrotrophic count as per Singh *et al.* (2023). Biochemical parameters, including total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N), were estimated following Beatty and Gibbons (1937), while pH was measured as per AOAC (2006). Sensory attributes were evaluated using a nine-point hedonic scale based on Peryam and Pilgrim (1957).

Statistical Analysis

Statistical analysis was carried out using data obtained from triplicate measurements at different storage intervals. Analysis of variance (ANOVA) was performed to determine significant differences among treatments as described by Snedecor and Cochran (2014). The analysis was conducted using IBM BASIC Windows (Release 1.13) to evaluate variations among treatments and to identify the most effective treatment.

Result and Discussion

Quality Characteristics of the Raw Material

Fresh reef cod, *Epinephelus diacanthus*, was utilized as the raw material for the study and its physical, biochemical, microbiological and sensory characteristics were analyzed to assess its composition and freshness.

Bacterial load was Measured in colony-forming units per gram (CFU/gm). Total plate count (TPC) is an important indicator of microbial quality in fish fillets. A TPC value exceeding 6 log CFU/g is generally considered indicative of spoilage and values above this limit render the product unsafe and unsuitable for human consumption due to potential health risks (FSSAI, 2017). The initial values ranged from 3.49 ± 0.16 to 3.55 ± 0.13 log CFU/g, indicating good initial quality. A significant ($p < 0.05$) increase in TPC was observed in all treatments during storage, reflecting progressive microbial growth; however, the rate of increase varied among treatments. The highest increase was recorded in US4, followed by US3, while US2 showed a comparatively slower rise, indicating better microbial stability. At the end of storage, TPC values reached 6.01 ± 0.32 , 5.56 ± 0.18 , 5.12 ± 0.16 , 6.86 ± 0.18 and 7.56 ± 0.23 log CFU/g for US(c), US1, US2, US3 and US4, respectively. Based on the acceptable limit of 6 log CFU/g (FSSAI, 2017), US4 exceeded the limit by day 10, US(c), US1 and US3 by day 15, whereas US2 remained within acceptable limits up to 20 days, indicating extended shelf life.

Table 1 : Raw material characteristics of fresh reef cod (*Epinephelus diacanthus*)

A. PHYSICAL CHARACTERISTICS		Mean $\pm$ S.D.
1	Total length (cm)	28.10 ± 0.35
2	Weight of fish (g)	266.00 ± 9.85
B. PROXIMATE COMPOSITION		
1	Moisture (%)	76.92 ± 0.55
2	Total Protein (%)	18.01 ± 0.05
3	Total Fat (%)	1.53 ± 0.04
4	Total Ash (%)	1.75 ± 0.93
C. CHEMICAL CHARACTERISTICS		
1	pH	6.8 ± 0.03
2	TMA-N (mg/100g)	1.38 ± 0.02
3	TVB-N (mg/100g)	10.20 ± 0.08

D. MICROBIOLOGICAL CHARACTERISTICS			
1	Total Plate Count (TPC) (log CFU/g)	3.54 ± 0.09	
2	Total Psychrotrophic Count (log CFU/g)	3.35 ± 0.08	
3	<i>Escherichia coli</i> (cfu/g)	N.D.	

The observed increase in TPC may be attributed to microbial proliferation during refrigerated storage. The comparatively lower microbial load in ultrasonic-treated samples, particularly US2, could be due to the cavitation effect, which disrupts microbial cell structures and inhibits bacterial growth. Similar findings were reported by Zhao *et al.* (2021), who observed reduced microbial load and extended shelf life in ultrasound-treated fish samples. These results indicate that an optimal duration of ultrasonic treatment can effectively enhance microbial stability and prolong shelf life.

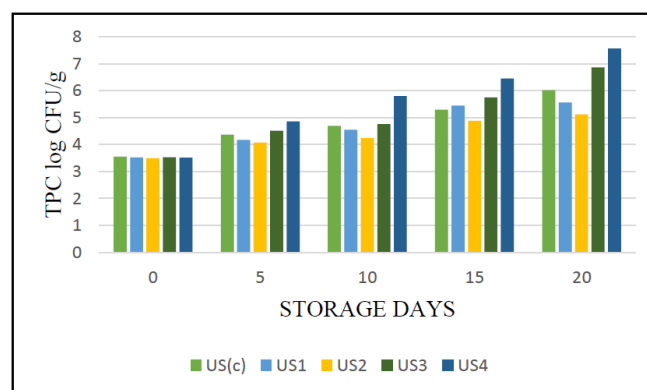


Fig. 1 : Change in Total Plate Count (TPC) log CFU/g of fresh reef cod (*Epinephelus diacanthus*) during refrigerated storage

The microbial load was measured in terms of colony-forming units per gram (CFU/g). Total psychrotrophic count is an important indicator of microbial quality, reflecting the growth of cold-tolerant microorganisms during refrigerated storage. The initial values ranged from 3.29 ± 0.13 to 3.35 ± 0.11 log CFU/g, indicating good initial quality. A significant ($p < 0.05$) increase in psychrotrophic count was observed in all treatments during storage, reflecting progressive microbial growth; however, the rate of increase varied among treatments. The highest increase was recorded in US4, followed by US3, while US2 showed a comparatively slower rise, indicating better microbial stability. At the end of storage, psychrotrophic counts reached 8.38 ± 0.21 , 7.96 ± 0.18 , 7.11 ± 0.19 , 9.02 ± 0.15 and 10.46 ± 0.15 log CFU/g for US(c), US1, US2, US3 and US4, respectively. Based on the acceptable limit of 7 log CFU/g (ICMSF, 1986), US4 exceeded the limit by day 10, US(c), US1 and US3 by day 15, whereas US2 remained within acceptable limits up to 20 days, indicating extended shelf life. The increase in psychrotrophic count may be attributed to the growth

of cold-tolerant bacteria during refrigerated storage. The comparatively lower counts in ultrasonic-treated samples, particularly US2, could be due to cavitation effects disrupting microbial cells and inhibiting their growth. Olgun and Diler (2025) reported that ultrasound treatment significantly reduced the growth of psychrotrophic bacteria in fish during refrigerated storage. Similarly, Shahrier *et al.* (2023) observed a gradual increase in total psychrotrophic count during refrigerated storage of tilapia fillets.

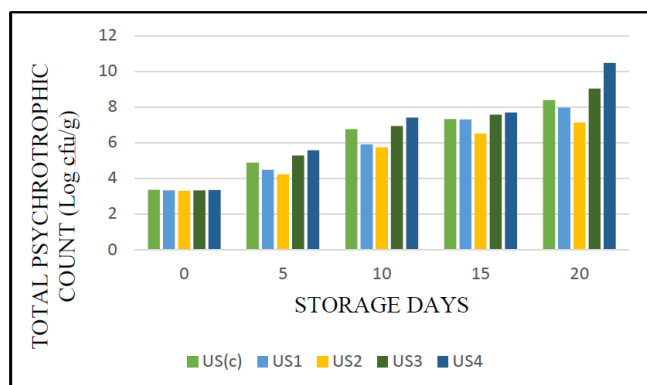


Fig. 2 : Change in total psychrotrophic count (log cfu/g) of fresh reef cod (*Epinephelus diacanthus*) during refrigerated storage

Escherichia coli (*E. coli*) was absent in all reef cod samples throughout the 20 days of refrigerated storage. The pH of fish fillets is an important indicator of freshness and spoilage during storage. An increase in pH during storage mainly results from microbial and biochemical activities and reflects the onset of spoilage, with pH values above 7.6 considered unacceptable (Nirmal & Benjakul, 2011). The initial pH values ranged from 6.11 ± 0.09 to 6.25 ± 0.10 , indicating the fresh condition of the samples. During refrigerated storage, a significant ($p < 0.05$) increase in pH was observed in all treatments, indicating progressive deterioration of fish quality. Among the treatments, US4 exhibited the most rapid increase in pH, followed by US3, whereas US2 maintained comparatively lower pH values throughout the storage period, suggesting better preservation efficiency. At the end of storage, the pH values increased to 7.81 ± 0.09 , 7.43 ± 0.09 , 7.21 ± 0.08 , 7.90 ± 0.08 and 8.18 ± 0.08 for US(c), US1, US2, US3 and US4, respectively. Based on the spoilage threshold ($pH > 7.6$) reported by Nirmal and Benjakul (2011), US4 exceeded the acceptable limit by day 10, followed by US(c), US1 and US3 on day 15, whereas US2 remained within the acceptable limit up to 20 days of storage. The increase in pH during storage may be attributed to the formation of alkaline compounds such as ammonia and other volatile basic substances produced through microbial and enzymatic degradation

of fish muscle proteins. The comparatively lower pH values observed in ultrasonic-treated samples, particularly US2, may indicate reduced microbial activity due to the cavitation effect of ultrasound, which can disrupt microbial cell membranes. In contrast, longer ultrasonic treatment durations (US3 and US4) may have accelerated biochemical reactions and tissue degradation, resulting in faster quality deterioration. Similar trends of increasing pH during refrigerated storage have also been reported by Zhao *et al.* (2021) reported similar findings, where ultrasound treatment slowed the rise in pH by reducing microbial load and delaying spoilage.

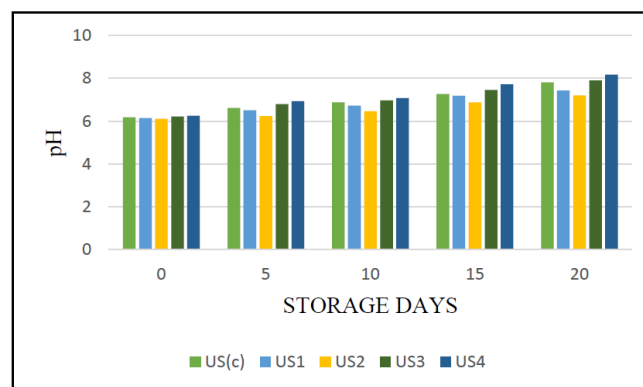


Fig. 3 : Change in pH of fresh reef cod (*Epinephelus diacanthus*) during refrigerated storage

Total volatile basic nitrogen (TVB-N) is a reliable index for evaluating the freshness of fish, with values exceeding 33–35 mg/100 g indicating spoilage (Gopakumar, 2002). The initial values (10.18 ± 0.15 to 10.22 ± 0.23 mg/100 g) suggested that all samples were fresh at the beginning of storage. A progressive and significant ($p < 0.05$) increase in TVB-N was observed in all treatments throughout the storage period, indicating continuous deterioration. The rate of increase differed among treatments, with US4 showing the most rapid rise, followed by US3, whereas US2 maintained comparatively lower values, reflecting better quality retention. By the end of storage, TVB-N values increased to 44.53 ± 0.93 , 40.00 ± 0.73 , 35.58 ± 0.52 , 46.25 ± 0.70 and 48.89 ± 0.67 mg/100 g for US(c), US1, US2, US3 and US4, respectively. Considering the permissible limit (33–35 mg/100 g), US4 samples reached spoilage by day 10, while US(c), US1 and US3 crossed the limit by day 15. In contrast, US2 remained within acceptable limits up to 20 days, indicating improved shelf life. The accumulation of TVB-N during storage is primarily associated with protein breakdown caused by microbial and enzymatic activities, resulting in the formation of ammonia and other volatile nitrogenous compounds. The relatively lower values observed in ultrasonic-treated samples, particularly US2, suggest that ultrasound treatment

may have suppressed microbial activity through cavitation effects. However, higher treatment durations (US3 and US4) might have accelerated biochemical reactions, leading to faster spoilage. Zhao *et al.* (2021) reported that ultrasound treatment significantly inhibited the formation of TVB-N during refrigerated storage. Similarly, Torres-Arreola *et al.* (2025) observed an increase in TVB-N during shrimp storage, indicating spoilage progression due to microbial activity. In contrast, Chang and Wong (2012) found that ultrasound treatment enhanced biochemical reaction rates in cobia sashimi, resulting in increased TVB-N values during storage.

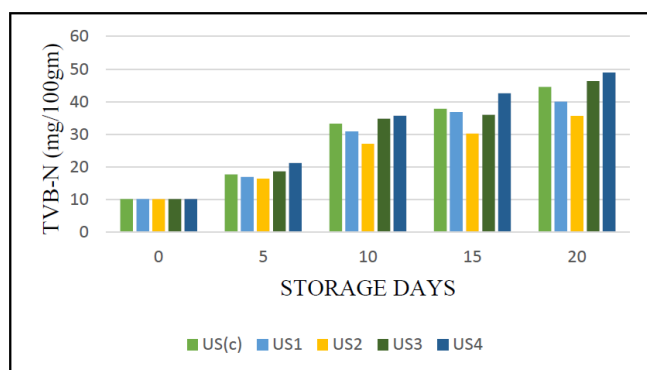


Fig. 4 : Change in Total Volatile Basic Nitrogen (TVB-N) (mg/100gm) of fresh reef cod (*Epinephelus diacanthus*) during refrigerated storage

Trimethylamine nitrogen (TMA-N) serves as a sensitive indicator of spoilage in marine fish, as it is produced through the microbial conversion of trimethylamine oxide during storage. The initial levels were low, ranging from 1.36 ± 0.05 to 1.40 ± 0.04 mg/100 g, confirming the freshness of the raw material. With the progression of refrigerated storage, a marked ($p < 0.05$) increase in TMA-N was observed across all treatments. However, the pattern of increase differed notably. Samples subjected to longer ultrasonic exposure (US3 and US4) exhibited a sharper rise in TMA-N, whereas US2 showed a more controlled increase throughout the storage period. At the final stage, TMA-N values were recorded as 16.74 ± 0.14 , 15.38 ± 0.13 , 14.90 ± 0.16 , 17.39 ± 0.18 and 19.71 ± 0.15 mg/100 g for US(c), US1, US2, US3 and US4, respectively. Considering the acceptability threshold of 10–12 mg/100 g (FSSAI, 2017), spoilage was reached earlier in US4 (day 10), followed by US(c), US1 and US3 (day 15), while US2 maintained acceptable levels up to 20 days. The accumulation of TMA-N reflects intensified microbial activity and reduction of TMAO into trimethylamine, which is responsible for characteristic fishy odours. The relatively lower values observed in US2 suggest that moderate ultrasonic treatment was effective in delaying

microbial metabolism. In contrast, prolonged exposure may have enhanced tissue disruption, facilitating enzymatic reactions and accelerating spoilage. Similar observations have been documented by Similarly, Ocaño-Higuera *et al.* (2009) observed a significant increase in TMA-N values during storage of fish muscle, indicating progressive spoilage. Chang and Wong (2012) noted that ultrasound treatment enhanced biochemical reaction rates in cobia sashimi, leading to an increase in TMA-N values during storage.

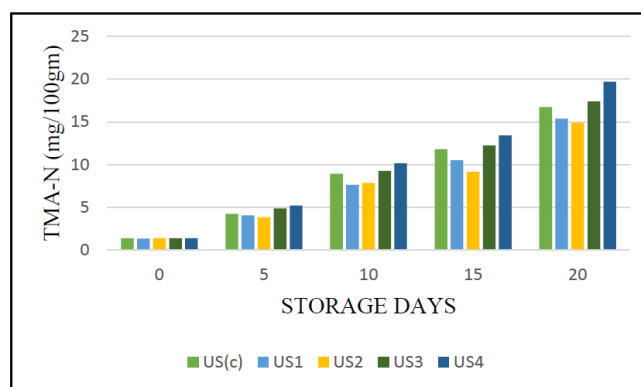


Fig. 5 : Change in Trimethylamine Nitrogen (TMA-N) (mg/100gm) of fresh reef cod (*Epinephelus diacanthus*) during refrigerated storage

Peroxide value (PV) serves as a key indicator of lipid oxidation in fish fillets, with increasing values reflecting the formation of primary oxidation products during storage. The initial PV ranged from 1.18 ± 0.05 to 1.23 ± 0.03 meq O_2 /kg across all treatments, indicating minimal lipid oxidation at the beginning. A significant ($p < 0.05$) increase in PV was observed in all treatments throughout storage, demonstrating progressive oxidative deterioration. However, the extent of increase differed among treatments. The highest rise was recorded in US4, followed by US3, whereas US2 exhibited a comparatively slower increase, suggesting better oxidative stability. At the end of storage, PV reached 13.88 ± 0.15 , 12.95 ± 0.12 , 10.01 ± 0.11 , 14.20 ± 0.10 and 15.73 ± 0.13 meq O_2 /kg for US(c), US1, US2, US3 and US4, respectively. Based on storage duration, US4 samples became unacceptable by day 10, while US3, US(c) and US1 reached the limit by day 15. In contrast, US2 remained within acceptable levels up to 20 days, indicating extended shelf life. The increase in PV during storage can be attributed to the oxidation of unsaturated lipids, leading to the formation of hydroperoxides. The relatively lower PV observed in ultrasonic-treated samples, particularly US2, may be due to reduced oxidative reactions and better preservation of lipid stability. Ocaño-Higuera *et al.* (2009) reported a rise in peroxide value in fish during refrigerated storage due to lipid

oxidation. Zhang *et al.* (2023) observed a continuous increase in peroxide value during cold storage of bay scallops, reflecting ongoing lipid oxidation, while ultrasound treatment was found to reduce the rate of this increase.

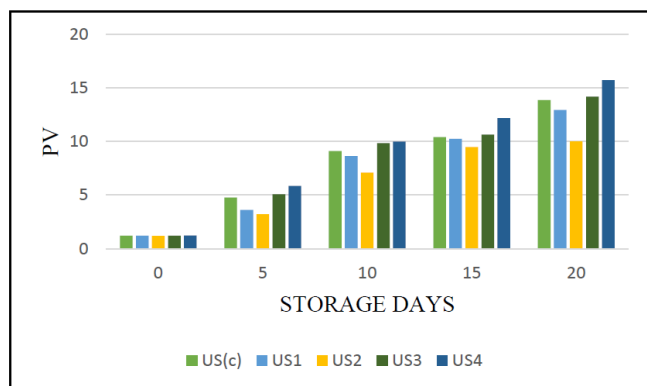


Fig. 6 : Change in PV of fresh reef cod (*Epinephelus diacanthus*) during refrigerated storage

Change in sensory characteristics during refrigeration storage

Sensory Evaluation

Changes in sensory scores were observed in all treatments during refrigerated storage of reef cod fillets. Nearly similar sensory scores were recorded at the initial stage of the experiment, indicating the freshness and good quality of the samples. During refrigerated storage, sensory scores gradually declined in all treatments for all evaluated sensory attributes. Among the treatments, US2 (30 min) maintained comparatively higher appearance scores throughout the storage period, whereas US3 and US4 showed a faster decline in sensory quality. Similar observations were reported by Zhang *et al.* (2023), who noted improved visual quality in ultrasound-treated fish during refrigerated storage.

A similar decreasing trend was observed in colour and odour scores during refrigerated storage. The decline in odour quality may be associated with the formation of volatile spoilage compounds such as ammonia, trimethylamine and other degradation products produced through microbial and enzymatic activities. Likewise, the reduction in colour quality during storage may be due to pigment degradation, oxidative changes and microbial activity. Similar findings were reported by Zhang *et al.* (2023) and Olgun and Diler (2025), who observed gradual deterioration in odour and colour quality of fish during refrigerated storage.

Texture scores also showed a progressive decline during storage due to microbial and enzymatic degradation of muscle proteins, resulting in softening

of the fillets. However, the comparatively better texture retention observed in US2-treated samples may be related to the inhibitory effect of ultrasound on microbial growth, thereby helping to preserve muscle structure. Similar results were reported by Zhang *et al.* (2023) and Olgun and Diler (2025), who stated that ultrasound treatment helped maintain structural stability and delayed texture deterioration during refrigerated storage.

Overall acceptability scores decreased continuously in all treatments throughout the storage period. The reduction in overall acceptability may be attributed to the deterioration of sensory attributes such as appearance, odour, colour and texture during storage. Based on the sensory rejection limit (score < 5), US4 samples became unacceptable by day 10, whereas US(c), US1 and US3 reached the rejection limit by day 15. In contrast, US2 remained acceptable up to 20 days of refrigerated storage, indicating improved sensory shelf life and preservation efficiency. Similar findings were reported by Olgun and Diler (2025) and Zhang *et al.* (2023), who demonstrated that ultrasound treatment could effectively extend the sensory shelf life of fish under refrigerated conditions.

Conclusion

Ultrasonic pre-treatment had a significant effect on the quality and shelf life of reef cod (*Epinephelus diacanthus*) fillets stored under refrigerated conditions ($4 \pm 1^\circ\text{C}$). Among the different treatment durations, 30 min (US2) was found to be the most effective in maintaining microbiological, biochemical and sensory quality of the fillets. The US2 treatment successfully extended the shelf life up to 20 days by reducing microbial growth and delaying spoilage. In contrast, the 60 min treatment (US4) showed comparatively inferior results, with rapid deterioration in quality parameters and a reduced shelf life of 10 days. The control and other intermediate treatments also exhibited lower stability compared to US2. Overall, ultrasonic pre-treatment for 30 min can be considered the optimal condition for enhancing the shelf life and preserving the quality of reef cod fillets during refrigerated storage.

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