

Quality changes of marinated vacuum-packed frozen saline-tolerant tilapia hybrid, UPV SpiN® (*Oreochromis spilurus* × *Oreochromis niloticus*) fillets

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Abstract

Maintaining the safety and quality of tilapia fillets during frozen storage is crucial for both consumers and fish processors. The present study investigates the effects of different rearing conditions (freshwater vs. brackish water) and marination on the microbiological, chemical, and sensory quality of vacuum-packed saline-tolerant tilapia hybrid, UPV SpiN® (*Oreochromis spilurus* × *O. niloticus*) fillets during frozen storage. Tilapia reared in freshwater and brackish water tanks were harvested, filleted, marinated, vacuum-packed, and frozen for 56 days. Results indicated that marination significantly reduced psychrophilic bacterial counts in both freshwater (2.10 log CFU/g) and brackish water (1.90 log CFU/g) fillets. All samples maintained acceptable levels of Total Volatile Basic Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) (<35 mg N/100 g) throughout frozen storage. Marination also significantly reduced the pH and moisture content of freshwater (4.94; 70.98%) and brackish water (4.93; 71.74%) fillets. Furthermore, irrespective of rearing conditions, marinated fillets exhibited superior sensory acceptability regarding appearance, texture, and taste, while minimizing off-odors and off-flavors. This study demonstrates that marination significantly improves the microbiological, chemical, and sensory characteristics of frozen tilapia fillets, regardless of the rearing environment. Overall, the combination of vacuum-packaging, marination, and freezing effectively preserves quality, suggesting a viable method to enhance product safety.

1. Introduction

Tilapia is a collective common name for cichlid species of the genera *Oreochromis*, *Sarotherodon*, and *Tilapia*, with *Oreochromis niloticus* and *O. mossambicus* as the two major globally cultured species (El-Sayed, 2020). Of the known genera, *Oreochromis* is one of the most saline-tolerant and economically important fish species in the Philippines (Guerrero, 2019). Favored for its highly adaptable traits, local tilapia production contributed 307,808.28 tons in 2023 and is currently increasing with efforts to reduce production costs while maintaining self-sufficiency (Bureau of Fisheries and Aquatic Resources (BFAR), 2022; Philippine Statistics Authority (PSA), 2024). At global scales, tilapia production is dominated by aquaculture, ranking fourth at 5.3 million tons, which helped offset food security problems brought by the rising population and consumption (Food and Agriculture Organization (FAO),

2024). The growing tilapia production also increased consumer purchasing power, which influenced current consumer preference shift from the traditional small-sized unprocessed to large-sized processed tilapia forms (El-Sayed, 2020; BFAR, 2022). Recently, tilapia received considerable attention in global trade and marketing, having an increasing demand for frozen and fresh forms, with Indonesia and China as major exporting countries (El-Sayed, 2020; FAO, 2024).

Tilapia fillets represent about 30–35% of the fish by weight, and the remaining are considered as processing wastes, which can undergo product transformation and valorization (Peñarubia *et al.*, 2023; FAO, 2024). Whole tilapia and fillets are susceptible to quality deterioration and spoilage, usually due to ambient storage temperature fluctuation, storage, transport, sale, and mishandling when intended for trade and distribution (Zhang *et al.*, 2021). Off-flavors are also a bottleneck to tilapia

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suppliers and processors as it affects consumer acceptability (Pongsetkul *et al.*, 2022). As a highly perishable aquatic product, value addition of tilapia fillets as ready-to-cook (RTC), eat (RTE), or serve (RTS) is important while providing diverse and convenient products for working households (Peñarubia *et al.*, 2023). Considering numerous processes for extending shelf stability of tilapia fillets, vacuum packaging, marination, and freezing are seen as hurdle technologies highly adaptable in the Philippines. The global market for the processing of tilapia, which mainly focused on frozen whole and fillet forms, accounted for most tilapia exports (El-Sayed, 2020). Marination is applied primarily to preserve and improve the flavor and texture of meat since marinades contain aroma enhancers, antimicrobials, acids, spices, salt, and sugar (Lopes *et al.*, 2022). Moreover, marinated fish are usually packed and stored, marketed as RTC or RTE with no thermal treatment, and sold as a high-value commodity (Lopes *et al.*, 2022).

Owing to the expanding demand for alternative protein sources, culture strategies are currently intensified, considering tilapia hybrids and strains with better growth performance at euryhaline conditions (Guerrero, 2019; El-Dakar *et al.*, 2024). Salinity tolerance of Nile tilapia, as well as other *Oreochromis* species are well-studied from conventional to molecular breeding techniques (Lumayno *et al.*, 2025). However, the influence of freshwater and brackish water rearing salinity conditions on the quality of tilapia fillets remains unclear. There are also a few studies investigating the effect of long-term salinity exposure on the sensorial quality of aquatic products. In Liang *et al.* (2008), *Penaeus vannamei* cultured in seawater (30 ppt) exhibited better umami taste, sweetness, and overall flavor than samples reared in low salinity water (0.5–1.5 ppt). Zhang *et al.* (2021) reported that salinity in aquaculture production influenced the physiology of crustaceans, where temporary rearing in brackish water (12 ppt) accounted for improved taste quality in the meat of adult male Chinese mitten crab (*Eriocheir sinensis*). Meanwhile, Kumari *et al.* (2021) found that varying salinities (0–20 ppt) in a biofloc system did not significantly influence the appearance, texture, and overall acceptability of raw and cooked (smoked) red tilapia (*Oreochromis* sp.). Recently, do Carmo *et al.* (2025) compared the physico-chemical, sensory, and microbiological properties of *O. niloticus* reared in freshwater and brackish water. However, there are still gaps in understanding the synergistic effects of varying salinity rearing conditions and marination technique on tilapia fillets during frozen storage. Some studies showed that there is significant variation in the nutritional and mineral composition between freshwater and brackish

water-reared *O. niloticus* (Olopade *et al.*, 2016). However, little is known about the influence of the combined effects of different rearing conditions and marination technique on the sensory and physico-chemical and microbiological quality of tilapia fillets during frozen storage. Evaluating the influence of rearing conditions and marination of vacuum-packed frozen storage of tilapia fillets may provide consumers and processors with alternative techniques for improving their safety and shelf stability.

2. Materials and methods

2.1 Rearing of saline-tolerant tilapia hybrid, UPV SpiN® (*Oreochromis spilurus* × *Oreochromis niloticus*)

The saline-tolerant tilapia samples utilized in this study were cultured at the Multi-Species Hatchery Complex, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines (N 10°38'20.291'' E 122°13'35.757'') (Dinaga *et al.*, 2025). The fish were cultured for a period of eight months in 1-ton concrete tanks under two distinct conditions: freshwater (FW) at 0 ppt and brackish water (BW) maintained at approximately 15 ppt. Rearing protocols followed the methodologies described by Huervana *et al.* (2022). The fish were fed a commercial diet to satiation, and water quality parameters were maintained within optimal ranges suitable for tilapia culture. During the first two months of rearing, daily water changes and siphoning were performed to ensure optimal water quality. Key parameters, including temperature (28.20±0.50°C) and salinity (14.50±0.55 ppt for brackish water; 0 ppt for freshwater), were monitored and recorded three times weekly. In subsequent months, water changes and siphoning were conducted every other day. Prior to the sampling of tilapia fillets, additional water quality parameters, such as dissolved oxygen (6.10±0.09 ppm) and ammonia (0.05±0.01 ppm), were recorded daily over a six-day period.

2.2 Collection and preparation of tilapia fillets

Saline-tolerant tilapia, ca. 50 kg from freshwater (FW) and brackish water (BW) groups, were harvested and packed with ice in polystyrene insulated boxes at 1:3 (fish, kg: ice, kg) ratio. Different ice boxes were prepared for FW and BW samples, which were then transported immediately to the Seafood Processing Laboratory (N 10°38'18.677'' E 122°13'47.856''). Upon arrival, tilapia samples were washed with freshwater, removed of any extraneous matter, descaled, degutted, and placed in ice at 1:3 (ice: fish, kg: kg). Cleaned FW and BW sample groups were filleted and placed again in ice, with each set divided further into two: unmarinated

and marinated. A total of four treatment sets were prepared in this study, namely: Treatment 1 (FW unmarinated, FW0), Treatment 2 (FW marinated, FW1), Treatment 3 (BW unmarinated, BW0), and Treatment 4 (BW marinated, BW1).

2.3 Morphometric measurement of tilapia and filleting yield

Measurement of the whole and filleted tilapia samples in terms of total length (cm), weight (g), and filleting yield (%) was conducted ($n = 10$ individuals). The total length of the whole tilapia sample was measured along the tip of the snout of the tilapia to the tip of the caudal fin, which was recorded using a Mitutoyo™ absolute solar digital caliper (at 0.00 cm). The total weight of the whole tilapia and fillet samples was measured using a top-loading balance (at 0.00 g).

$$\text{Filleting Yield (\%)} = (\text{tilapia fillet}_g / \text{whole tilapia}_g) \times 100$$

Meanwhile, the filleting yield (%) was calculated by dividing the weight (g) of tilapia fillets by their initial whole weight (g), multiplied by 100.

2.4 Marination of tilapia fillets

The preparation of the marinating solution was based on Simora *et al.* (2021). The freshly prepared marinade, comprised of white vinegar (35.0% v/v), commercial salt (15.0% w/v), white sugar (10.0% w/v), garlic powder (2.4% w/v), bay leaves (2.0% w/v), white pepper (2.0% w/v), and distilled water (33.6% v/v) was pre-chilled at $4 \pm 2^\circ\text{C}$. Marination was then performed, maintaining a 1:1.5 (fish, kg: marinade solution, L) ratio at $4 \pm 2^\circ\text{C}$ for 1 h with gentle mixing every 10-min interval. The tilapia fillets were then allowed to drain using a stainless-steel strainer for 20–25 min at $10 \pm 2^\circ\text{C}$.

2.5 Vacuum packaging and frozen storage

The marinated and unmarinated samples were directly vacuum-packed using polyamide plastic bags (8 cm x 11 cm size), labelled, and stored at -20°C . For each treatment, at least 35 fillet packs were prepared for a 56-day frozen period at -20°C . Seven fillet packs from each treatment were randomly taken and analyzed every 14 days for five frozen periods, from Day 0, 14, 28, 42, until Day 56.

2.6 Analyses

2.6.1 Total psychrophilic aerobic plate count

The microbiological examination based on total psychrophilic aerobic plate count of tilapia fillets during frozen storage was based on FDA BAM (Maturin and Peeler, 2001). Tilapia fillet samples (25 g) were aseptically weighed and homogenized and then placed in

sterile 225 mL 0.1% peptone water (pH 7.0 ± 0.1) to prepare a 10^{-1} diluent. Succeeding serial dilutions (from 10^{-2} to 10^{-5}) were then prepared, and an aliquot (1 mL) from each dilution was spread onto sterile nutrient agar (NA) plates. The NA plates were then inverted and incubated for 10 days at $4-7^\circ\text{C}$, allowing psychrophilic bacterial growth. The plates containing 25 to 250 colonies were then recorded and multiplied by their dilution factor; colony counts beyond 250 were recorded as too numerous to count (TNTC). All sampling and analyses were conducted in triplicate, and the total psychrophilic counts were expressed in log CFU per gram of the sample.

2.6.2 Chemical analyses: pH, moisture, total volatile basic nitrogen, and trimethylamine nitrogen

Frozen vacuum-packed tilapia meat samples from each treatment were thawed using cold water. For these analyses, tilapia samples were initially homogenized using a mortar and pestle. Samples were then analyzed for pH, moisture, TVBN and TMAN.

2.6.2.1 pH

In measuring pH, tilapia meat samples from each treatment (10 g) were homogenized with 20 mL neutralized distilled water within 1 min. The pH of each sample was then read using a digital benchtop pH meter (C-73X pHision, Japan) (Endoma *et al.*, 2022). All pH measurements in triplicate were carried out at room temperature ($25 \pm 2^\circ\text{C}$).

2.6.2.2 Moisture

The moisture contents of tilapia samples were measured using a Halogen Rapid Moisture Analyzer (Shimadzu MOC63u). Homogenized tilapia fillet samples from each treatment (2 g) were spread evenly in a clean aluminum pan. The moisture contents were automatically measured gravimetrically after subjecting the sample to 120°C for 15–20 min. All measurements were conducted in triplicate, and results were expressed as % moisture content.

2.6.2.3 Total volatile basic nitrogen and trimethylamine nitrogen

The total volatile basic nitrogen (TVBN) levels of tilapia fillet samples were determined using a Conway microdiffusion technique (Ng 1987). Homogenized tilapia fillet samples (2 g) were mixed with 8 mL 4% TCA. The sample was set aside for 30 min to completely react with TCA, with occasional mixing. The mixture was then centrifuged at 3000 rpm for 10 min, and the supernatant was collected and stored in glass vials at -20°C . Aliquots of each sample extract (1 mL) were used

to react with 1 mL saturated K_2CO_3 , allowing the diffusion of total volatile basic nitrogen components present in the sample to 1 mL 1% H_3BO_3 with bromocresol green and methyl red indicator for 1 h at $37^\circ C$. The inner ring was then titrated using standardized 0.02N HCl, and the volume was recorded to compute the TVBN levels. Separately, the trimethylamine nitrogen (TMAN) levels of tilapia fillets were also measured based on Ng (1987). In TMAN, 1 mL 10% neutralized formaldehyde was incorporated in the reacting mixture of 1 mL saturated K_2CO_3 and 1 mL of the sample

$$TVBN \text{ or } TMAN \left(\frac{mg}{100g} \text{ sample} \right) = (V_S - V_B) \times (M_{HCl} \times A_N) \times \left(\frac{(W_S \times \frac{M}{100}) + V_E}{W_S} \right) \times 100$$

extract. TCA (1 mL 4%) was used as a reference blank for determining both TVBN and TMAN levels in tilapia fillet samples. The TVBN and TMAN levels were calculated using the equation:

where $(V_S - V_B)$ is the titration volume difference (mL) between sample and blank, M_{HCl} is the molarity (mol/L) of standardized HCl used for titration, A_N is the formula weight of nitrogen (g/mol), W_S is the weight of the sample (g), M is the moisture content of the sample (e.g., 80%); and V_E is the 4% TCA volume (mL) used during extraction. All analyses for TVBN and TMAN were conducted in triplicate, and results were expressed in mg/100 g TVBN or TMAN.

2.6.2 Sensory evaluation of steam-cooked tilapia fillets

The sensory evaluation of tilapia fillets was conducted based on Simora *et al.* (2021), with modifications. Prior to analysis, consent and agreement of the sensory protocol, and screening of 10 semi-trained laboratory panelists were performed. People with allergies to fish and the ingredients in the marinating solution did not participate in the sensory evaluation. During preparation, tilapia fillets of equal size and thickness were used, covered with aluminum foil, and labelled randomly using 3-digit alpha-numeric codes. Tilapia fillet samples were then steam-cooked for 15–20 min and set to cool down prior to sensory evaluation, providing controlled, uniform cooking while preserving their sensory attributes. During evaluation, each laboratory panelist was simultaneously given tilapia fillet samples from four different treatments. Laboratory panelists were tasked to evaluate the sensory attributes of steam-cooked tilapia fillet samples based on appearance, odor, taste, texture, and overall acceptability ratings. Laboratory panelists evaluated the sensory and acceptability attributes on a 15-point line scale with a 1-point graduation and a median. Scores below 3 indicated that the sensory attribute is extremely not acceptable, between 3 and 6 indicated moderately unacceptable,

between 6 and 9 indicated neither acceptable nor unacceptable, between 9 and 12 indicated moderately acceptable, while scores between 12 and 15 indicated extremely acceptable. Moreover, a 5-point purchase intent score based on the overall impression of the panelists were recorded based on the criteria: 1) definitely would buy it, 2) probably would buy it, 3) might or might not buy it, 4) probably would not buy it, and 5) definitely would not buy it. Panelists' scores and written comments were then taken with utmost confidentiality. To avoid interferences in between sensory testing, plain crackers and water were provided to cleanse their palates.

2.6.4 Statistical analyses

All results in this study were presented as mean \pm standard error of the mean (SEM), and all analyses were conducted in triplicate, except for morphometric measurements, where 10 individuals were used. An independent sample t-test procedure was used to determine significant differences ($p < 0.05$) between freshwater and brackish water saline-tolerant tilapia samples in terms of length, weight, and filleting yield. Meanwhile, significant changes in the microbiological, sensory, and physico-chemical properties of tilapia fillets during frozen storage were determined using one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was then used for comparing significant grouping among treatments at $p < 0.05$. All statistical analyses were performed using IBM® SPSS® Statistics software version 27 (SPSS Inc., USA).

3. Results and discussion

3.1 Effects of salinity rearing condition on fish body weight, length, and filleting yield

The total length (cm), weight (g), and yield (%) of freshwater (0 ppt) and brackish water-reared (15 ppt) saline-tolerant tilapia samples are shown in Table 1. Results showed that between freshwater and brackish water-reared tilapia samples, no significant differences were found in terms of total length (21.50 cm), total weight (208.90–211.40 g), and filleting yield (27.23–28.51%) ($p > 0.05$). Our findings showed that different rearing salinity levels (0 and 15 ppt) did not negatively affect the morphological and filleting yield of tilapia samples. Although it is known that hypo- or hypersaline conditions are key external factors affecting the growth of aquatic animals, Mena-Herrera *et al.* (2002) observed that hybrid red tilapia (*Oreochromis mossambicus* \times *Oreochromis niloticus*) reared at 0 and 15 ppt also found to have no significant difference in terms of growth. However, at increased salinity levels (25, 35 ppt), growth rates may have been impaired (Mena-Herrera *et al.*,

Table 1. Length and weight of the saline-tolerant tilapia whole and fillets.

Treatment	Whole tilapia		Tilapia fillet	
	Length (cm)	Weight (g)	Weight (g)	Yield (%)
Freshwater (FW)	21.50±0.37 ^a	208.90±10.98 ^a	57.70±3.31 ^a	28.51±1.05 ^a
Brackish water (BW)	21.50±0.38 ^a	211.40±12.16 ^a	59.55±3.13 ^a	27.23±0.99 ^a

Values are presented as mean±SEM. Values with different superscripts in the same column are statistically significantly different at $p>0.05$ (Student's T-test).

2002). Kamal and Mair (2005) also corroborated this study, where weight gain (g/ fish) of *O. niloticus* between 0 and 15 ppt did not differ significantly, but observed decreasing weight gain at higher salinity levels (22.5 and 30 ppt). Recently, do Carmo *et al.* (2025) observed no significant differences in terms of total weight (g), filleting yield (%), and eviscerated fish yield (%) in *O. niloticus* reared in freshwater and brackish water. Our observation confirms *O. niloticus*' adaptive behavior at 0 and 15 ppt, which could present a sustainable strategy to rear saline-tolerant tilapia in regions limited by freshwater. Results were also similar to the reported findings of Nunes *et al.* (2023), where filleting yields (%) of male and female *O. niloticus* ranged 29.06–31.30%, showing no significant differences with each other as well. However, filleting yields (%) in this study were found to be lower than those of Thodesen *et al.* (2012) (30.1%) and Nguyen *et al.* (2010) (31.1–34.6%). While filleting yield is an important parameter in the fish processing industry, low filleting yields in this study might be due to the lower proportion of fish used, since larger fish typically have higher edible muscle or higher yield than smaller fish, as observed in Thodesen *et al.* (2012).

3.3 Changes in the psychrophilic aerobic plate count of frozen vacuum-packed tilapia fillets

Marination, vacuum packaging, and freezing are traditional yet effective hurdle processing technologies for fish and fishery products in the tropics. Their synergistic interaction helps improve sensory attributes and extends the shelf-life of tilapia fillets during frozen storage. Marinating is a method used to preserve raw fish, like tilapia, by soaking it in a mixture of vinegar, salt, sugar, garlic, bay leaves, and pepper, which helps prevent spoilage by inhibiting the growth of harmful bacteria (Arason *et al.*, 2014; Van Haute *et al.*, 2016). Vinegar helps prevent spoilage by lowering pH levels, which inhibits bacterial growth and enzymatic activity, lowers water activity, and enhances meat sensory attributes, ultimately extending its shelf life (Van Haute *et al.*, 2016). The changes in the psychrophilic APC of vacuum-packed marinated freshwater and brackish water tilapia fillets are shown in Figure 1. The low initial bacterial counts of vacuum-packed marinated freshwater and brackish water tilapia fillets, with levels below 10^1

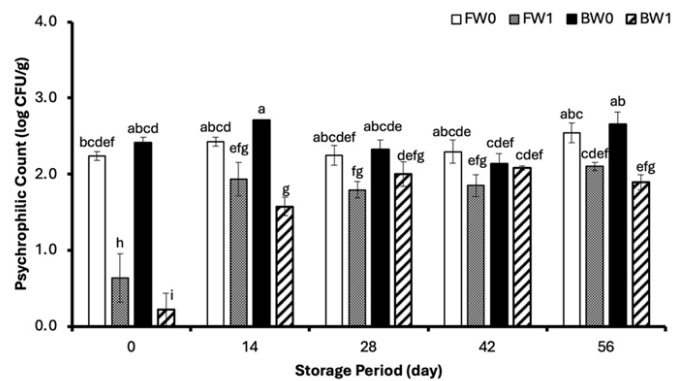


Figure 1. Changes on the psychrophilic counts (log CFU/g) of vacuum-packed freshwater and brackish water marinated saline-tolerant tilapia fillets during frozen storage (-20°C). Data is presented as mean±SEM. Bars with different superscripts are statistically significantly different between treatments throughout frozen storage period (days) ($p<0.05$, DMRT). FW0: Freshwater, unmarinated, FW1: Freshwater, marinated, BW0: Brackish water, unmarinated, BW1: Brackish water, marinated.

CFU/g for psychrophilic APC, indicated high fish quality and good manufacturing practices during product preparation. It has been reported that psychrophilic bacteria are not completely killed by marinating solution, vacuum packaging, and freezing and viable bacterial cells could still proliferate in tilapia fillet samples. Despite being frozen, psychrophilic bacteria could still proliferate, as observed in marinated freshwater and brackish water tilapia fillets. Meanwhile, unmarinated freshwater and brackish water tilapia fillets were shown to have high initial psychrophilic APC compared to marinated samples, which implies increased adaptation during long-term frozen storage. Another factor contributing to the increase in the microbial count would be the growth of specific spoilage organisms such as *Shewanella putrefaciens*, aside from the gram-negative psychrotrophic rods under refrigerated conditions, which could contribute to the increasing TVBN and TMAN levels in tilapia (Zhang *et al.*, 2022). In addition, Tsogas *et al.* (2019) reported that lactic acid bacteria at $>10^6$ CFU/g could contribute to the spoilage of vacuum-packed and light salted *Mugil cephalus* fillets during refrigerated and frozen storage. The result in this study agrees with the findings of Sallam *et al.* (2007), in which reduction rates of 1.55 and 1.70 log CFUs were achieved for marinated Pacific saury (*Cololabis saira*) fillets in 2% and 3% acetic acid combined with 12% NaCl,

respectively, in comparison with the control samples. Salt inhibits microbial growth by restricting the available water in the fish meat. Acetic acid, on the other hand, can penetrate through the tissues and cell membrane, where it can denature proteins. The application of vinegar is an effective acidulant that causes the depression of pH below the growth range of many bacteria (Simora *et al.*, 2021). Antimicrobial compounds in biological marinades (allicin, piperine, and polyphenols from herbs/spices) and chemical marinades (salt, vinegar, sugar) were effective against most gram-positive and -negative bacteria, which now transcends not only as a household additive but in many food industries. Moreover, results in this study conform to the Philippine National Standard for frozen tilapia, where the APC was lower than the 5×10^5 CFU/g limit (Bureau of Agriculture and Fisheries Standards (BAFS), 2008).

3.4 Changes in the total volatile basic nitrogen, trimethylamine nitrogen moisture, and pH of frozen vacuum-packed tilapia fillets

The chemical changes observed, demonstrating the quality of tilapia fillets during storage was shown in Figure 2. At Day 0, the TVBN levels (Figure 2A) of unmarinated (6.74–7.12 mg N/100 g) and marinated (6.50–6.62 mg N/100 g) tilapia fillets did not differ significantly from each other. However, as the frozen storage period progressed, TVBN levels increased until reaching 11.44–11.75 mg N/100 g for Day 56. Results indicated that marination technique and salinity rearing conditions (0 and 15 ppt) did not significantly affect TVBN levels of tilapia fillets. The initial TMAN levels

(Figure 2B) of all samples, which range from 2.62 to 3.01 mg N/100 g, did not show significant differences regardless of rearing condition and marination technique used. Tilapia, despite being a freshwater fish have high initial trimethylamine oxide (TMAO) levels for osmoregulation, which improved their capacity to tolerate a wide salinity range (Niizeki *et al.*, 2002). In addition, TMAO during freezing of fish could act as an antioxidant and a cryoprotectant while stabilizing enzymes and preventing protein denaturation during thermal denaturation (Anthoni *et al.*, 1990). This corroborated our results that while there are changes in TVBN and TMAN levels throughout the frozen storage period, no significant differences were observed between freshwater and brackish water, and between the marination technique used. The similar low TMAN levels in tilapia fillets could be attributed to the species-type since tilapia have low initial TMAO in their muscle tissue, and freezing at -20°C may have controlled the growth of TMAO-reducing microorganisms. However, the ingredients in the marinating solution did not significantly influence TMAN levels. Moreover, a similar pattern of increase in the TMAN level during refrigerated storage was also observed in marinated Pacific saury (Sallam *et al.*, 2007). Results also supported the findings in do Carmo *et al.* (2025), where TVBN values between freshwater (15.21 ± 3.10 mg N/100 g) and brackish water tilapia (19.88 ± 2.55 mg N/100g) did not differ significantly from each other. During the frozen storage (-18°C) of Nile tilapia fillets, the TVBN values also increased from 7.93 (Day 0) to 21.0 mg N/100 g (Day 150), due to the formation of ammonia and

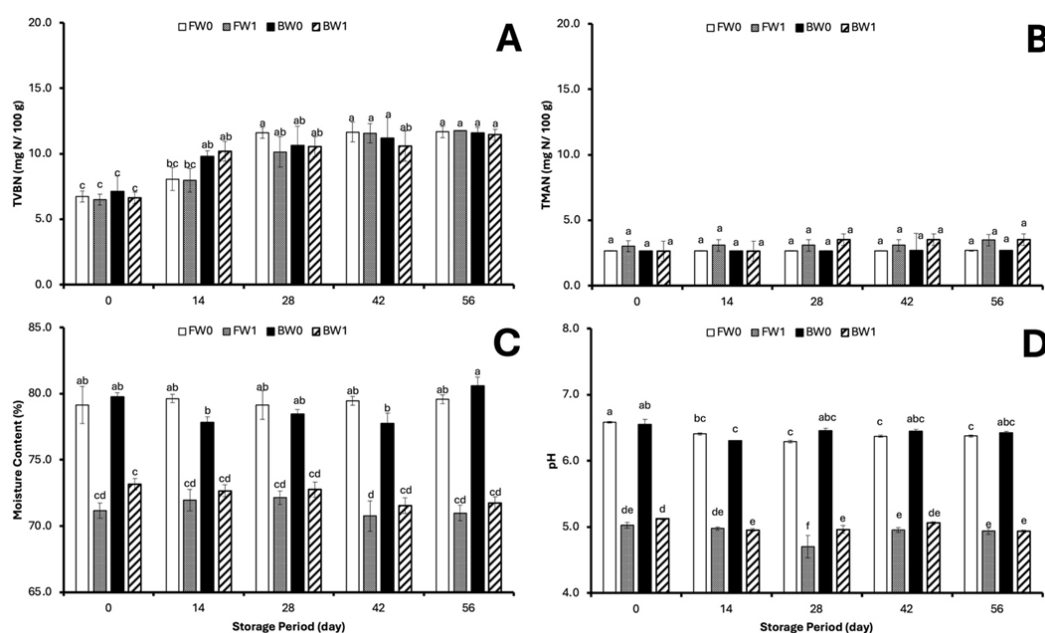


Figure 2. Changes in the chemical properties of vacuum-packed freshwater and brackish water marinated saline-tolerant tilapia fillets during frozen storage (-20°C): A – total volatile basic nitrogen, TVBN, B - trimethylamine nitrogen, TMAN, C – moisture content, and D – pH. Data is presented as mean \pm SEM. Bars with different superscripts are statistically significantly different between treatments throughout frozen storage period (days) ($p < 0.05$, DMRT). FW0: Freshwater, unmarinated, FW1: Freshwater, marinated, BW0: Brackish water, unmarinated, BW1: Brackish water, marinated.

other volatile amines as metabolic by-products of psychrophilic microorganisms (Subbaiah *et al.*, 2015). Based on BAFS (2008), good quality frozen tilapia has an acceptable TMAN limit of 5-10 mg N/100 g, while the acceptable TVBN limit was at 20–30 mg N/100 g. In this study, all samples showed low TVBN and TMAN levels despite being frozen for 56 days, maintaining below the maximum permissible TVBN level of 35 mg N/100 g in fish flesh specified by European Commission guidelines (Bekhit *et al.*, 2021). An increase in TVBN during the initial stages of storage between Day 0 and 14 may be initiated by autolytic degradation of nucleotides and free amino acids, while an increase at the later stages of storage is most likely caused by a combination of microbial and autolytic activities, as well as the complete microbial reduction of TMAO to TMA (Sallam *et al.*, 2007). The increasing trend in the TVBN values of the samples may be due to the increasing psychrophilic counts since TVBN is produced mainly by bacterial decomposition of fish flesh (Bekhit *et al.*, 2021). TMAO-reducing microorganisms involved the genera of bacteria typical of the marine environment (*Alteromonas*, *Photobacterium*, *Vibrio*, and *Shewanella putrefaciens*) but are also carried out by *Aeromonas* and intestinal bacteria of the Enterobacteriaceae. Under anaerobic conditions, these bacteria can use a variety of carbon sources as substrates in their TMAO-dependent anaerobic respiration, including formate and lactate. They could develop in vacuum-packed tilapia fillets during frozen storage. In seafood spoilage, TMAN particularly contributes to the characteristic ammonia-like off-odor and “fishy” off flavors (Bekhit *et al.*, 2021).

The moisture contents of tilapia fillets were shown in Figure 2C. The moisture contents at Day 0 of marinated tilapia fillets were found significantly lower (71.16–73.17%) than in unmarinated tilapia fillets (79.14–79.77%). Across the frozen storage period, significant differences in the moisture contents were observed between marinated and unmarinated groups. However, between freshwater and brackish water fillets, moisture contents did not differ significantly. It can be observed that the moisture content significantly decreased when the raw fillets were processed into different marinades. The decrease in moisture content in all tilapia fillets could be attributed to the replacement of water by organic salts from the marinades (Emire and Gebreamariam, 2010; Arason *et al.*, 2014). Results were found similar in do Carmo *et al.* (2025), where moisture contents of freshwater tilapia (76.62%) and brackish water tilapia fillets (77.24%) did not differ significantly from each other. Emire and Gebreamariam (2010) also corroborated the findings in this study, where no significant moisture changes can be observed between Day 0 and Day 60 (79.87–80.37%), but significantly

decreased at Day 75 (78.62%) until Day 90 (78.50%) during frozen storage ($-18\pm 2^{\circ}\text{C}$). Despite the reduction in moisture levels due to marination, the moisture contents of tilapia fillet samples were found within acceptable levels of 60–80% (Ahmed *et al.*, 2022). At Day 0, unmarinated tilapia fillets with higher moisture content showed a higher microbial load, making them more susceptible to spoilage. Moisture content of tilapia fillets is also a critical factor since it directly affects the yield of fillet and the texture and juiciness of meat (do Carmo *et al.*, 2025). Results in this study showed that all samples across the frozen period, between marination, freshwater, and brackish water treatments, were considered to have ideal moisture content levels.

The pH levels of tilapia fillet samples are shown in Figure 2D. Across the frozen storage period, significant pH changes were observed between marinated and unmarinated groups. High pH levels were found in unmarinated freshwater and brackish groups (pH 6.29–6.58) than in marinated freshwater and brackish groups (pH 4.70–5.12). A slight increase in pH was observed at the end of the storage period for both freshwater and marinated samples. Results were also in agreement with Arannilewa *et al.* (2006), where pH levels of frozen tilapia (*Sarotherodon galienus*) increased; reached nearly neutral (pH 6.90) after 60 days of frozen storage. The increasing pH levels of samples, especially the unmarinated tilapia fillets, could be associated with the increasing TVBN values, where basic nitrogenous components may be produced during frozen storage as a result of the increasing psychrophilic bacterial growth and its metabolic by-products (Arannilewa *et al.*, 2006). Unmarinated tilapia fillets fall within the expected post-mortem pH range (Xie *et al.*, 2023; do Carmo *et al.*, 2025). Afrin *et al.* (2023) also reported the initial pH of unmarinated Nile tilapia fillets at pH 6.36–6.39, indicating that the samples are fresh. Meanwhile, the pH reduction in some tilapia samples across the frozen storage period could be due to the presence of acetic acid in the marinade and the accumulation of lactic acid resulting from the degradation of glycogen in tilapia fillets (Afrin *et al.*, 2023). The gradual pH reduction in marinated and unmarinated tilapia fillets might be due to the effect of marination and the presence of phenolic compounds having antimicrobial properties while reducing the accumulation of alkaline compounds. Despite the increase in TVBN and TMAN levels, an increment in pH during the frozen storage, especially in unmarinated tilapia fillets, may be due to the formation of volatile basic nitrogenous compounds (Afrin *et al.*, 2023). The pH levels of frozen tilapia samples were also found at ideal levels based on the Philippine National Standard (PNS) for Frozen Tilapia (pH 6.20–6.90) (BAFS, 2008) and fall within limits of international

standards. This means that frozen tilapia samples, whether marinated or unmarinated, are still acceptable and have not undergone quality deterioration, given that pH values at Day 0 and Day 56 did not significantly differ between treatment groups. Arason *et al.* (2014) reported that the pH of marinades between pH 1.0 and 4.5 could prevent the growth of most food poisoning and spoilage-type bacteria. Salt plays a crucial role in the preservation of fish products, since it can remove water from the tissue and increase solute concentration, leading to lower water activity and pH and potentially impacts both the chemical and sensory attributes of fish (Arason *et al.*, 2014). Sugar may also effectively preserve fish, where it could lower water activity, thus potentially extending shelf life. Spices (i.e., garlic, bay leaves, pepper) have natural antimicrobial compounds that, while preventing proliferation of spoilage-causing microorganisms, could also minimize production of volatile basic nitrogenous components. Vacuum packaging could also extend the shelf-life of tilapia fillets as it effectively reduces air, lowering oxidative reactions in the product and preventing deterioration caused by aerobic bacteria (Kumar and Ganguly, 2014). The combined effects of marinades produce semi-preserved fish fillets, which could retard the bacterial and enzymatic action, resulting in a product with a characteristic flavor and an extended but limited shelf life (Simora *et al.*, 2021).

3.4 Changes in the sensory quality and purchase intent of steam-cooked tilapia fillets

The sensory quality of steam-cooked, marinated and unmarinated, freshwater and brackish water frozen tilapia fillets was shown in Figure 3. The appearance acceptability scores (Figure 3A) showed a declining trend during the frozen storage period. High appearance acceptability scores were observed between Day 0 and 14, and a significant reduction in scores was observed as frozen storage progressed. Despite having decreasing appearance scores, tilapia fillets still exhibited good acceptability scores, which did not reach the rejection point or to the extent that consumers will reject the fillets. The decline in visual appearance scores throughout the storage period was attributed to the increase in muscle gaping and muscle discoloration (i.e., yellowish and metallic). The gradual increase of these appearance-specific attributes on tilapia fillets could be influenced by time, storage conditions, and the used treatments, resulting in the decreasing trend in appearance acceptability throughout time. Freezing, particularly after rigor mortis, can increase gaping, while Jiang *et al.* (2023) suggested that light salting can reduce gaping by enhancing water-holding capacity and texture of fillets. Meanwhile, the yellow discoloration observed

in marinated tilapia fillets could be attributed to lipid oxidation, where vinegar and salt may increase oxidation, and Maillard reactions and microorganisms and their metabolic by-products may exacerbate muscle discoloration. Vacuum packaging also contributed to effectively preserving the appearance of fish fillets by minimizing exposure to oxygen and preventing dehydration and oxidation during storage (Kumar and Ganguly, 2014). Frozen storage of vacuum-packed fillets could also help maintain the appearance of fillets by reducing ice crystal formation while minimizing freezer burn (Sone *et al.*, 2020). Steam cooking, particularly at low temperatures, has also been shown to preserve the natural color and texture of fish fillets while minimizing excessive browning and surface discoloration (Wang *et al.*, 2018). Furthermore, results in this study reported similar findings where the color of cooked tilapia fillets raised in freshwater and brackish water did not differ significantly (Kumari *et al.*, 2021; do Carmo *et al.*, 2025). This also indicated that panelists could not significantly distinguish freshwater and brackish water tilapia fillets. In terms of odor acceptability (Figure 3B), all samples showed high odor acceptability, especially on Day 0 and Day 14. However, a gradual decrease in odor acceptability was observed from Day 28, 42, until Day 56. Despite the decrease in odor scores, all samples retained good acceptability scores and did not reach the rejection point. Odor scores between freshwater and brackish water cooked tilapia fillets did not differ significantly, as observed in previous studies (Kumari *et al.*, 2021; do Carmo *et al.*, 2025).

During frozen storage in this study, a significant reduction in odor acceptability scores was attributed to off-flavors, such as rancid, sour, and putrid odor detected by panelists. This process is influenced by the presence of certain volatile compounds and the evolution of free fatty acids, free amino acids, and nucleotides, as well as microbial activity during storage (Cheng *et al.*, 2024).

Steam cooking applied during sensory evaluation also preserves natural flavors and prevents burnt or overcooked odors, ensuring the fish retains a fresh and pleasant smell of tilapia fillets (Pongsetkul *et al.*, 2022). Meanwhile, marination significantly improved the taste acceptability scores of tilapia fillets (Figure 3C), especially during Day 0. Similar findings were also reported in previous studies where taste/ flavor acceptability scores of cooked tilapia fillets from freshwater and brackish water did not differ significantly (Kumari *et al.*, 2021; do Carmo *et al.*, 2025). While there is high market consideration for saline-bred tilapia, panelists could not discriminate between freshwater and brackish water-reared tilapia fillets. Results indicated that significantly high initial taste acceptability scores

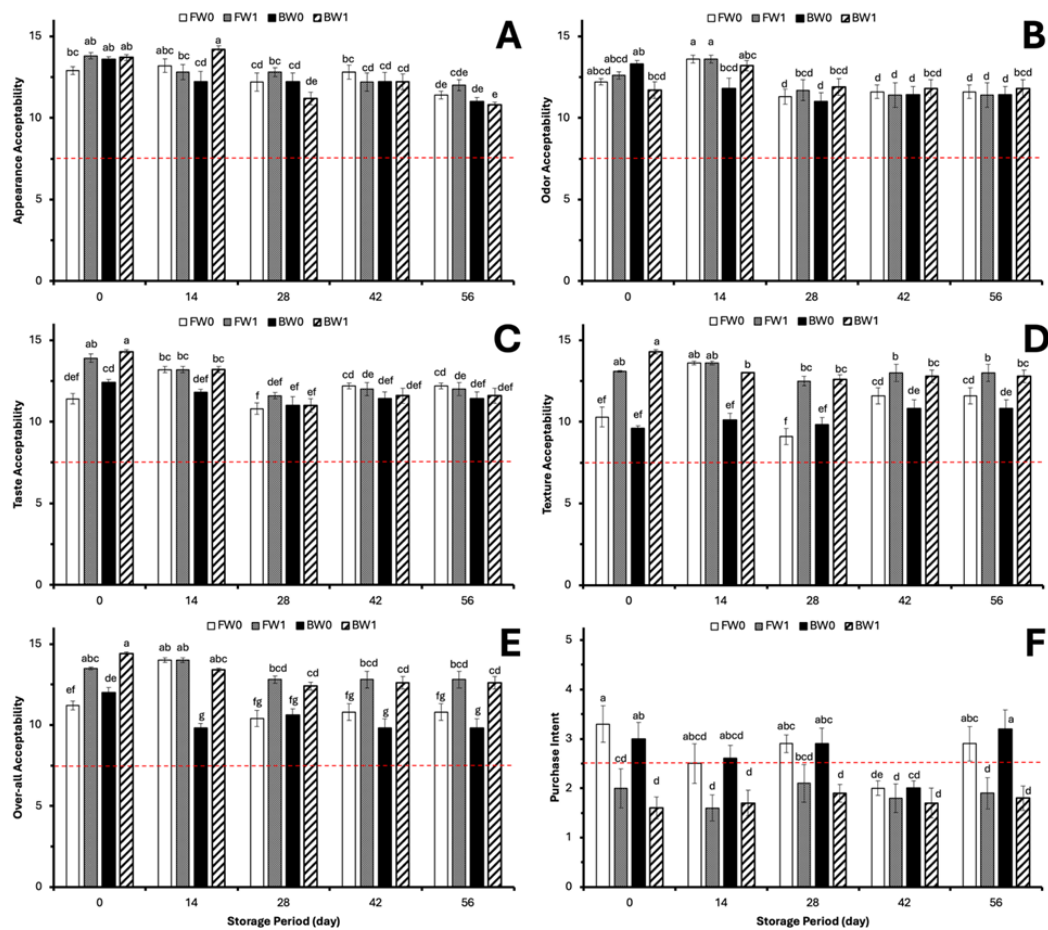


Figure 3. Changes in the sensory properties of vacuum-packed freshwater and brackish water marinated salme-tolerant tilapia fillets during frozen storage (-20°C): A – appearance acceptability, B – odor acceptability, C – taste acceptability, D – texture acceptability, E – overall acceptability and F – purchase intent. Data is presented as mean \pm SEM. Bars with different superscripts are statistically significantly different between treatments throughout frozen storage period (days)($p < 0.05$, DMRT). FW0: Freshwater, unmarinated, FW1: Freshwater, marinated, BW0: Brackish water, unmarinated, BW1: Brackish water, marinated. Red broken lines indicated rejection of the sensory attribute.

can be observed in marinated fillets; however, as frozen storage progresses, a decreasing trend was observed, but all samples still exhibited good taste acceptability scores and did not reach the rejection point. Based on the sensory evaluation, panelists reported that taste was influenced by saltiness and umami detected in steamed tilapia fillets. Prolonged freezing of tilapia fillets could lead to oxidation of unsaturated fatty acids, leading to high peroxide value and thiobarbituric acid reactive substances, resulting in off-flavors that could mask the desirable taste (Afrin *et al.*, 2023). Cheng *et al.* (2024) found that changes in volatile compounds, free fatty acids, free amino acids, and nucleotides, as well as the presence of spoilage microorganisms, could lead to undesirable flavors. Vacuum packaging and freezing have been found to effectively preserve the natural flavors of fish by preventing oxidation and microbial growth, which can lead to off flavors (Kumar and Ganguly, 2014). Meanwhile, the overall texture acceptability (Figure 3D) of tilapia fillets is influenced by firmness, mushiness, and flakiness detected by panelists. Based on sensory evaluation, marinated tilapia fillets exhibited firmer meat texture across the freezing

period. Meanwhile, similar findings were observed in previous studies where the texture of cooked tilapia fillets did not differ significantly between freshwater and brackish water-reared tilapia (Kumari *et al.*, 2021; do Carmo *et al.*, 2025). Although an acidic environment may cause protein denaturation, marinated tilapia fillets could stabilize meat pH and thus could prevent microbiological and autolytic processes that may induce degradation of myofibrillar proteins and muscle softening/ mushiness (Subbaiah *et al.*, 2015). Prolonged freezing reduces enzymatic activity, which prevents degradation of muscle proteins in tilapia fillets (Shi *et al.*, 2022), which is why minimal to no significant change was observed in all samples from Day 0 until Day 56. Based on our findings, texture generally dictates the overall acceptability (Figure 3E) of the tilapia fillets between freshwater and brackish water samples, and between marinated and unmarinated tilapia fillets across the frozen storage. Marinated tilapia fillets in this study had better meat texture and also showed significantly higher overall acceptability scores. In terms of purchase intent (Figure 3F), since tilapia fillets were steam-cooked, panelists preferred unmarinated samples,

expressing definite willingness to buy the product. Meanwhile, scores beyond 10 indicated that the product can be considered acceptable from a commercial sensory point of view. Results also indicated that panelists could not distinguish between freshwater and brackish water tilapia fillets. Kumari *et al.* (2021) also reported that between raw tilapia fillets reared at 0 and 15 ppt, no significant overall sensory changes (7.45–7.50) were observed. However, Kumari *et al.* (2021) reported that cooked freshwater tilapia fillets were found to have significantly higher overall sensory scores (8.00) than brackish water tilapia fillets (7.80). In Do Carmo *et al.* (2025), the overall acceptability of brackish water tilapia fillets was found to be significantly higher than that of freshwater tilapia fillets. Salinity has also been found to increase flesh water-holding capacity and muscle hardness, while also enhancing umami taste and flavor richness of largemouth bass *Micropterus salmoides* (Du *et al.*, 2022). Interestingly, Zhang (2021) reported that in crabs (*Eriocheir sinensis*), temporary rearing in brackish water has been found to enhance the taste quality of the meat, with increased umami flavor and higher levels of certain taste components. This unique flavor profile can be further enhanced through various cooking methods, which can improve the meat quality of tilapia fillets in terms of flavor and taste characteristics (Pongsetkul *et al.*, 2022).

4. Conclusion

This study showed that marination improves the quality of saline-tolerant tilapia fillets regardless of rearing conditions (freshwater and seawater). The combined effects of vacuum packaging, marination, and freezing minimized the psychrophilic APC, TVBN and TMAN, pH, and moisture, which maintained good quality sensory acceptability compared to other treatments. The findings in this study may provide consumers and fish processors with alternative strategies to extend the shelf-life of frozen tilapia fillets under hygienic conditions.

Conflict of interest

The authors declare no conflict of interest.

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