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Physicochemical, microbiological and sensory quality changes in *Brama orcini* fillets during 90 days of frozen storage at -25 °C

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ABSTRACT

Freezing is one of the most effective methods for preserving fish quality, yet gradual deterioration can occur during storage. Extending the shelf life of underutilized bycatch species such as *Brama orcini* can enhance resource utilization and market potential. This study evaluated the effects of frozen storage at −25°C for 90 days on the physicochemical, microbiological, and sensory quality of skinless *B. orcini* fillets. Samples were assessed at 10-day intervals for drip loss (%), pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), total viable count (TVC), and sensory attributes of raw and steamed fillets. Drip loss increased slightly ($p > 0.05$) from 0.35% to 0.42%, while pH significantly rose from 5.27 to 6.36. TVB-N and TBARS increased significantly, reaching 25.97 mg N/100 g and 0.97 mg MDA/kg, respectively, both below spoilage thresholds. TVC remained well under the 10^5 CFU/g safety limit. Sensory evaluation revealed gradual declines in most attributes, with juiciness showing the most pronounced reduction in both raw and steamed samples. General acceptability decreased significantly but remained within the "like slightly" range by Day 90. Overall, *B. orcini* fillets maintained acceptable quality for up to 90 days at −25°C. These findings support the potential of freezing as a practical strategy to extend shelf life and improve market availability of this underutilized species.

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INTRODUCTION

Fish, consumed either fresh or frozen, is valued worldwide for its high nutritional content but is highly perishable, with quality deterioration beginning immediately after harvest due to enzymatic activity, microbial growth, and oxidative reactions, influenced by factors such as species, handling, and storage temperature (Karami *et al.,* 2013; Dawson *et al.,* 2018; Duarte *et al.,* 2020; Muzamail *et al.,* 2024).

Freezing, a widely used and cost-effective preservation method, inhibits microbial activity and slows spoilage reactions, allowing fish to retain nutritional and sensory qualities comparable to fresh products for months (Karami *et al.,* 2013; Wangtueai *et al.,* 2014; Hematyar *et al.,* 2017). However, quality degradation can still occur during frozen storage through protein denaturation, residual enzymatic activity, and lipid oxidation (Dawson *et al.,* 2018; Christophe *et al.,* 2024).

Quality changes in frozen fish are generally assessed through a combination of sensory, physicochemical, biochemical, and microbiological analyses (Duarte *et al.,* 2020). Common indicators include pH, which signals enzymatic breakdown of muscle proteins (Aberoumand, 2020; Phu *et al.,* 2022); total volatile basic nitrogen (TVB-N), reflecting protein degradation from microbial activity (Popelka *et al.,* 2016); thiobarbituric acid reactive substances (TBARS), which measure secondary products of lipid oxidation (Leygonie *et al.,* 2012; Phu *et al.,* 2022); and total viable count (TVC), indicating overall microbial load (Lakshmisha *et al.,* 2008). Sensory evaluation-encompassing appearance, color, odor, texture, and taste-remains a practical and consumer-relevant approach for determining product acceptability (Yasin *et al.,* 2018; Daita *et al.,* 2025).

Brama orcini, commonly known as bigbelly pomfret, is a marine epipelagic species widely distributed across tropical and subtropical Indo-Pacific waters (Bos and Gumanao, 2013; Motomura *et al.,* 2017). In Panay, Philippines, it is landed regularly as purse seine bycatch and is predominantly marketed fresh in local markets, thereby restricting its broader distribution (Babaran *et al.,* 2009; Bos and Gumanao, 2013). Taxonomically, *B. orcini* belongs to the order Scombriformes and family Bramidae, and its IUCN Red List status is currently "Not Evaluated" (Froese and Pauly, 2025). It is regarded as a locally important food fish in certain Philippine regions, including Panay and Southern Mindanao (Cantabaco *et al.,* 2015). Despite its potential as raw material for surimi (Endoma *et al.,* 2022), research on its preservation and processing is scarce. To the best of the researcher's knowledge, this is the first investigation to assess the frozen storage quality of *B. orcini*, a locally abundant tropical bycatch species. Effective frozen storage could enhance year-round availability, reduce post-harvest losses, and open markets for small-scale processors (Aberoumand, 2020; Zuanazzi *et al.,* 2020; Tahiluddin and Kadak, 2022).

B. orcini is a highly migratory tropical species (Cantabaco *et al.,* 2015) that is a locally abundant bycatch during peak seasons yet remains underexploited. Most research on freezing effects has focused on commercially important species, particularly temperate fish (Turan *et al.,* 2003; Popelka *et al.,* 2016; Dawson *et al.,* 2018); migratory marine species (Aubourg *et al.,* 2004; Lakshmisha *et al.,* 2008; Seki *et al.,* 2016); freshwater species (Liu *et al.,* 2010; Gandotra *et al.,* 2012; Karami *et al.,* 2013; Wangtueai *et al.,* 2014; Hematyar *et al.,* 2017; Zuanazzi *et al.,* 2020; Malik *et al.,* 2021); species commonly used for surimi production (Benjakul *et al.,* 2005); and various commercially sold fillets (Muzamail *et al.,* 2024).

Similar to the present study, Aberoumand *et al.* (2018) examined the physicochemical properties of fillets from seasonally abundant species such as *Sparidentex hasta* and *Pampus argenteus* in Iran, highlighting the importance of research on underutilized yet locally significant fish.

This study examined the physicochemical, microbiological, and sensory changes in skinless *B. orcini* fillets during 90 days of frozen storage at –25 °C, providing baseline data for optimal storage protocols.

Findings aim to support bycatch utilization, market expansion, and sustainable fisheries aligned with the United Nations Sustainable Development Goals—specifically SDG 2 (food security), SDG 12 (responsible consumption), and SDG 14 (sustainable marine resource use).

MATERIALS AND METHODS

Preparation of *B. orcini* fillets

The study was conducted in 2018 at the Post-harvest Laboratory, Institute of Fish Processing Technology, University of the Philippines Visayas, Miagao, Iloilo. Whole *B. orcini* (average body weight ~563 g) were purchased from a local wet market, washed, eviscerated, filleted, deboned, and skinned. A total of 7.2 kg skinless fillets was obtained. Paired fillets (average combined weight 240.99g ± 20.13g) were individually packed in low-density polyethylene (LDPE) bags, heat-sealed, and stored at –25 °C for 90 days. Each fish was processed within approximately 10 minutes at room temperature (28–29 °C), while the samples were held on crushed ice (0–4 °C) during handling to maintain chill conditions until freezing.

Quality evaluation of *B. orcini* fillets during frozen storage

Sampling was performed at Day 0 and at 10-day intervals until Day 90. All analyses were conducted in triplicate (n = 3) using three bags of paired fillets. From each bag, one fillet was halved for physicochemical analyses, the other half used for microbial testing, and the paired fillet reserved for sensory evaluation. All reagents used in the study were laboratory-grade.

Drip loss

Drip loss was determined following Wangtueai *et al.* (2014). Frozen samples were weighed, thawed under running water while sealed in LDPE bags, reweighed, and drip loss (%) calculated.

$$Drip\ loss(\%) = \frac{(weight\ of\ before\ thawing - weight\ of\ after\ thawing)}{weight\ of\ before\ thawing} X\ 100$$

pH

The pH was measured by homogenizing 10 g muscle with 20 mL distilled water for 1 min and reading with a calibrated pH meter.

Total volatile basic nitrogen

TVB-N content was analyzed by the Conway microdiffusion method (Conway and Byrne, 1933) using 2 g muscle homogenized with 8 mL 4% (w/v) trichloroacetic acid (TCA), filtered, and assayed.

Thiobarbituric acid reactive substances

Lipid oxidation was measured as described by Lemon (1975): 10 g sample was homogenized with 16 mL extracting solution, filtered, mixed with 5 mL 0.2 M TBA reagent, heated in boiling water for 40 min, cooled, and read at 530 nm with a calibrated spectrophotometer to quantify lipid oxidation.

Microbiological analysis

Microbial quality was assessed by total viable count (TVC). Twenty-five grams of sample was homogenized in 225 mL sterile peptone diluent (10^{-1} dilution), serially diluted, plated on nutrient agar using the spread plate method, and incubated at 35 °C for 24 h before enumeration (CFU/g).

Sensory evaluation

Sensory evaluation was performed by seven semi-trained panelists on thawed raw and steamed (unseasoned) fillets. Raw samples were evaluated for color, odor, firmness, and juiciness; steamed samples for color, odor, flavor, mouthfeel, and juiciness. General acceptability was rated for both. Attribute scores used a 5-point hedonic scale; general acceptability used a 9-point scale (Table 1).

Statistical analyses

Data were analyzed by one-way ANOVA to assess effects of storage duration on physicochemical (pH, drip loss, TBARS, TVB-N), microbiological (TVC), and sensory attributes, followed by Tukey's HSD ($p < 0.05$). Pearson's correlation coefficients were calculated among quality parameters and sensory scores ($p < 0.05$, 0.01, 0.001). Analyses were performed using JASP (Jeffrey's Amazing Statistics Program) v0.19.3.0 (University of Amsterdam, Netherlands). JASP is an accessible and user-friendly software package suitable for both basic and advanced statistical analyses, and has been recommended for use in research, including medical and biostatistics studies (Ashour, 2024).

Table 1. Sensory attributes and hedonic scales used for evaluating raw and steamed frozen *Brama orcini* fillets

Sample type	Attributes evaluated	Scale used	Scale description
Raw (Thawed)	Color, odor, firmness, juiciness	5-point hedonic scale	5 – Like very much
			4 – Like moderately
			3 – Like slightly
			2 – Neither like nor dislike
			1 – Dislike
Steamed	Color, odor, flavor, mouthfeel, juiciness	5-point hedonic scale	5 – Like very much
			4 – Like moderately
			3 – Like slightly
			2 – Neither like nor dislike
			1 – Dislike
Raw (Thawed) and Steamed	General acceptability	9-point hedonic scale	9 – Like extremely
			8 – Like very much
			7 – Like moderately
			6 – Like slightly
			5 – Neither like nor dislike
			4 – Dislike slightly
			3 – Dislike moderately
			2 – Dislike very much
			1 – Dislike extremely

RESULTS AND DISCUSSION

Physicochemical and microbiological changes

The variations in physicochemical and microbiological quality of *B. orcini* fillets during 90 days of frozen storage at –25 °C are presented in Fig. 1.

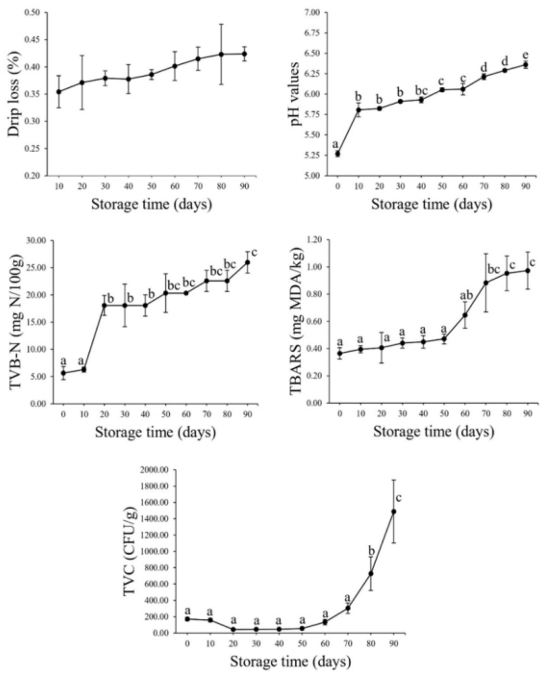

Fig. 1. Changes in physicochemical and microbiological quality parameters of *Brama orcini* fillets during frozen storage for 90 days. Values with similar superscripts are statistically not significantly different based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test (p<0.05). Vertical bars indicate the standard deviation of the mean (n=3).

Drip loss increased slightly from 0.35% (Day 0) to 0.42% (Day 90), with no significant differences ($p > 0.05$). This minor increase aligns with the known effects of freezing on fish muscle where ice crystal formation causes muscle cell membrane disruption, resulting in protein denaturation and reduced water-holding capacity, ultimately leading to thaw exudation and product dehydration (Duarte *et al.,* 2020; Christophe *et al.,* 2024). Similar patterns have been reported in Nile tilapia fillets (Zuanazzi *et al.,* 2020), common carp fillets (Hematyar *et al.,* 2017), Atlantic mackerel fillets (Cropotova *et al.,* 2019), and rainbow trout (Turan *et al.,* 2003). In this study, drip loss was strongly correlated with pH and TVB-N (r = +0.72, $p < 0.001$) (Table 2), suggesting a link between moisture loss and biochemical changes rather than microbial proliferation, as no significant association with TVC was found. Notably, the recorded drip loss values were lower than those reported by Zuanazzi *et al.* (2020), who observed >1% drip loss in Nile tilapia fillets after only two days at –18 °C.

The initial low post-mortem pH of 5.27 in *B. orcini* fillets is consistent with lactic acid accumulation from anaerobic glycogen breakdown post-harvest (Islami *et al.,* 2014). Such low values generally reflect good nutritional condition at harvest and potential for extended shelf life by slowing microbial proliferation (Kayim and Can, 2010; Aberoumand, 2020; Daita *et al.,* 2025). A comparable pH (5.76) was reported for *B. orcini* minced meat by Endoma *et al.* (2022). During frozen storage, pH rose significantly ($p < 0.05$) to 6.36 by Day 90, consistent with the accumulation of alkaline volatile bases from protein degradation and microbial metabolism (Gil and Barbosa, 2011). Despite this increase, values remained below the spoilage threshold of 7.9 (Aberoumand *et al.,* 2018).

Table 2. Correlation matrix among physicochemical and microbiological quality parameters of *B. orcini* fillets during frozen storage for 90 days

Variable	Drip loss	pH	TVBN	TBARS
pH	+0.87***			
TVB-N	+0.72***	+0.85***		
TBARS	+0.44*	+0.75***	+0.63***	
TVC	+0.24	+0.55**	+0.47**	+0.70***

Note: ***Correlation is significant at the 0.001 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlation matrix among sensory characteristics of raw (thawed) *Brama orcini* fillets during frozen storage for 90 days

Variable	Color	Odor	Firmness	Juiciness
Odor	+0.20			
Firmness	+0.24*	+0.32**		
Juiciness	+0.29*	+0.44***	+0.34**	
General acceptability	+0.30*	+0.51***	+0.47***	+0.72***

Note: ***Correlation is significant at the 0.001 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

TVB-N increased from 5.65 mg N/100 g to 25.97 mg N/100 g, with significant rises from Day 50 onward ($p < 0.05$). This trend reflects proteolysis and residual bacterial activity, consistent with previous findings in other species (Phu *et al.,* 2022). The final value remained within the generally accepted limit of 30–35 mg N/100 g (Huss, 1988; Connell, 1995).

TBARS rose gradually from 0.36 mg MDA/kg to 0.65 mg MDA/kg by Day 60, followed by significant increases, reaching 0.97 at Day 90 ($p < 0.05$). This pattern aligns with previous reports in tilapia fillets and Indian mackerel, where secondary oxidation progresses slowly under low-temperature conditions (Lakshmisha *et al.,* 2008; Liu *et al.,* 2010). The final TBARS value remained far below the rancidity threshold of 5–8 mg MDA/kg (Phu *et al.,* 2022), consistent with findings by Benjakul *et al.* (2005) who found that TBARS levels in tropical species-including threadfin bream, bigeye snapper, lizardfish, and croaker—stored at −18 °C for 15 weeks increased throughout storage but remained below 5 mg MDA/kg.

Microbiological analysis showed an initial TVC decline from 171.11 CFU/g to 43.33 CFU/g by Day 20, followed by an increase at Day 60 (133.33 CFU/g), reaching 1478.78 CFU/g at Day 90 ($p < 0.05$). The early reduction likely reflects the sensitivity of many microorganisms to freezing leading to inactivation (Xia *et al.,* 2025), while the subsequent rise likely resulted from the recovery and slow growth of psychotropic bacteria (Li *et al.,* 2023). Although only total viable counts were measured, it is important to note that the composition and abundance of microbial communities in raw fish can vary considerably depending on factors such as geographic origin, season, and post-harvest handling practices (Popelka *et al.,* 2016). Final TVC values remained well below the accepted limit of 10^5 CFU/g for fishery products (Huss, 1993), indicating microbiological safety at the end of the storage period.

Correlation analysis (Table 2) revealed strong positive associations between pH and TVB-N (r = +0.85, p < 0.001), TBARS (r = +0.75, p < 0.001), and TVC (r = +0.55, p < 0.01). TVB-N also correlated with TBARS (r = +0.63, p < 0.001), supporting the established link between lipid and protein oxidation with malondialdehyde from lipid oxidation reacting with proteins to form carbonyl compounds and promote denaturation, thereby accelerating quality deterioration (Benjakul *et al.,* 2005; Leygonie *et al.,* 2012).

The moderate correlation between TVB-N and TVC (r = +0.47, p < 0.01) and the strong correlation between TVC and TBARS (r = +0.70, p < 0.001) indicate that biochemical degradation, oxidation, and microbial activity progressed concurrently. These findings align with Dawson *et al.* (2018), who emphasized that both biological and chemical reactions—particularly enzymatic activity and lipid oxidation—have a significant impact on the long-term quality stability of frozen fish. Collectively, these interrelationships highlight the multifactorial nature of spoilage in *B. orcini* fillets under prolonged frozen storage.

Sensory quality changes

Sensory evaluation was conducted to determine sensory changes in the quality of *B. orcini* fillets during 90 days of frozen storage. For raw fillets, the organoleptic attributes evaluated included color, odor (fishy aroma), firmness (resistance to pressure and resilience), juiciness (perceived moisture based on visual and tactile cues), and general acceptability. For steamed fillets, the parameters assessed were color, odor, flavor (initial and aftertaste), mouthfeel (texture perception when eaten), and general acceptability. Representative images of the thawed raw fillets are shown in Fig. 2. Attributes were evaluated using the hedonic scales described in Table 1.

At Day 0, raw fillets were rated "like moderately" for color (4.43), odor (4.57), firmness (4.14), and juiciness (4.57), while general acceptability was "like very much" (8.29). Over the storage period, all parameters declined (Fig. 3). Color, odor, and firmness decreased to 3.71, 3.57, and 3.57, respectively, by Day 90, without significant differences (p > 0.05). Juiciness declined significantly (p < 0.05) from 4.57 to 3.00 ("like slightly"), and general acceptability also fell significantly from 8.29 to 6.00 (p < 0.05).

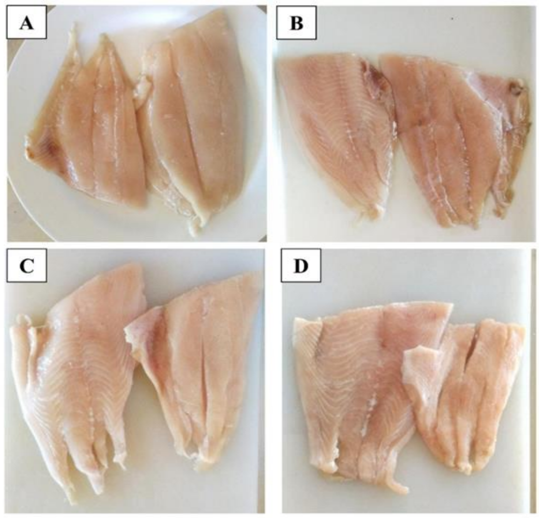

Fig. 2. Thawed *Brama orcini* fillet samples at various frozen storage duration: A) 0-day (fresh fillets), B) 30 days, C) 60 days, D) 90 days

This trend is consistent with reports of progressive declines in sensory properties such as color, odor, texture, and general acceptability in red tilapia fillets at –18 °C (Karami *et al.,* 2013), chilled tilapia fillets at 0 °C (Liu *et al.,* 2010), and *Oreochromis niloticus*, *Labeo rohita*, and *Cirrhinus mrigala* (Yasin *et al.,* 2018). The strong correlations between general acceptability and juiciness (r = +0.72, p < 0.001), odor (r = +0.51, p < 0.001), and firmness (r = +0.47, p < 0.001) suggest that moisture retention, aroma and perceived texture were key determinants of panelist preference for raw fillets (Table 3).

Initial scores for steamed fillets were "like moderately" for color (4.14), odor (4.71), flavor (4.57), and mouthfeel (4.43), with juiciness at 3.86 ("like slightly") and general acceptability at 8.00 ("like very much"). Similar to raw samples, most attributes declined gradually over storage (Fig. 4). Changes in color, odor, flavor, and mouthfeel were not significant (p > 0.05), ending at 3.71, 3.86, 3.57, and 3.57, respectively, by Day 90. Juiciness decreased significantly (p < 0.05) to 2.57 ("neither like nor dislike"), and general acceptability dropped significantly (p < 0.05) to 6.00.

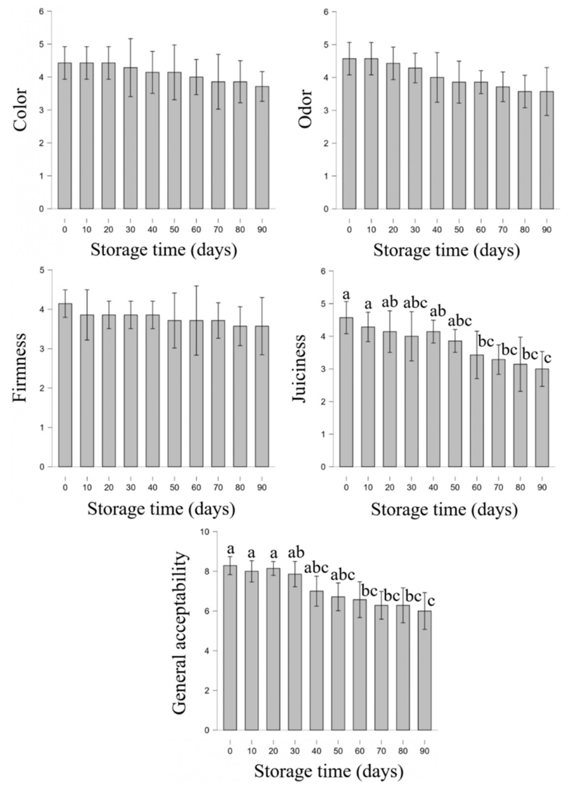

Fig. 3. Changes in sensory attributes of raw (thawed) *Brama orcini* fillets during frozen storage for 90 days. Values with similar superscripts are statistically not significantly different based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test ($p < 0.05$). Vertical bars indicate the standard deviation of the mean (n=7).

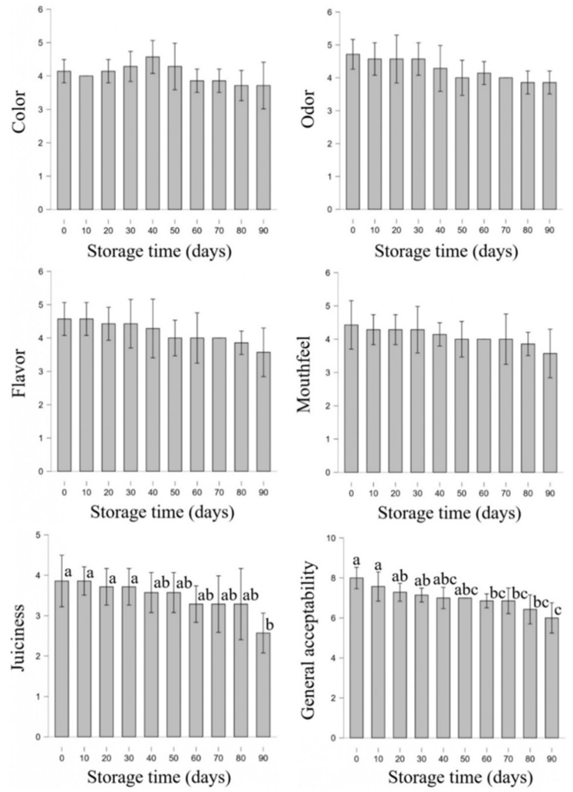

Fig. 4. Changes in sensory attributes of steamed *Brama orcini* fillets during frozen storage for 90 days. Values with similar superscripts are statistically not significantly different based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test ($p<0.05$). Vertical bars indicate the standard deviation of the mean (n=7).

Juiciness loss was more pronounced in steamed fillets, consistent with literature showing that heat treatment causes fish to lose about 20 % of their initial weight due to protein denaturation and fat melting, which release chemically bound water (Leygonie *et al.,* 2012; Nsoga *et al.,* 2021). Similar moisture losses, which also diminish flavor by removing amino acids and nucleotides (Zuanazzi *et al.,* 2020), have been documented in *Cyprinus carpio*, *Arius parkii*, and *Ethmalosa fimbriata* after smoking and boiling (Christophe *et al.,* 2024). For steamed fillets, general acceptability correlated moderately with odor (r = +0.44, p < 0.001) and juiciness (r = +0.39, p < 0.001) (Table 4). These relationships matched panelist feedback, where dryness was the most frequent comment for both raw and steamed samples.

Panelists also noted gradual lightening and slight yellowing of raw fillets from Day 80 onward, consistent with Hematyar *et al.* (2017), who observed similar color shifts in common carp fillets during frozen storage at –20 °C for 24 weeks. Samples were perceived as fresh until Day 70 for raw fillets and Day 80 for steamed fillets, after which slight fishy odors and very mild sourness were detected. These off-odors in fish are often linked to microbial activity producing volatile compounds such as ammonia and sulfur derivatives (Duarte *et al.,* 2020).

However, biochemical analyses indicated that spoilage thresholds were not reached. At Day 60, TVC was only 133.33 CFU/g; by Day 70 it reached 303.33 CFU/g, coinciding with most sensory parameters in the "like slightly" ratings for raw fillets.

At Day 80, steamed fillets had a TVC of 727.78 CFU/g, with most sensory scores also in the "like slightly" range. These values remained far below the typical sensory rejection threshold of 10^7–10^8 CFU/g for fish fillets (Duarte *et al.,* 2020).

While microbial activity remained low, prolonged frozen storage can still lead to oxidative deterioration, which may contribute to flavor and aroma changes. Secondary lipid oxidation products are known to impart rancid or pungent flavors (Zuanazzi *et al.*, 2020; Christophe *et al.,* 2024), and later stages of lipid peroxidation can also alter both color and nutritional quality (Karami *et al.,* 2013).

Overall, the combined physicochemical, microbiological, and sensory results of this study confirm that while *B. orcini* fillets remain safe and acceptable after 90 days at –25 °C.

Table 4. Correlation matrix among sensory characteristics of steamed *Brama orcini* fillets during frozen storage for 90 days

Variable	Color	Odor	Flavor	Mouth feel	Juiciness
Odor	+0.28*				
Flavor	+0.25*	+0.29*			
Mouth feel	+0.21	+0.02	+0.24*		
Juiciness	+0.12	+0.39***	+0.14	+0.08	
General acceptability	+0.24*	+0.44***	+0.35**	+0.21	+0.29*

Note: ***Correlation is significant at the 0.001 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

CONCLUSION

Based on the results of this study, *B. orcini* fillets stored at –25 °C retained acceptable physicochemical, microbiological, and sensory quality for 90 days. Juiciness was the most affected sensory attribute. Future research should explore longer storage periods and profile specific spoilage organisms. Complementary analyses of nutritional quality and consumer preference could support broader utilization of this underexploited species.

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