

Biogenic amine formation and microbiological profile in Asian seabass and short-bodied mackerel during refrigerated storage

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Abstract

Biogenic amines (BAs) are key chemical indicators of fish spoilage and potential food safety hazards. This study investigated the formation of four common BAs-histamine, cadaverine, putrescine, and tyramine-and monitored microbial populations in Asian seabass (*Lates calcarifer*) and short-bodied mackerel (*Rastrelliger brachysoma*) stored at 0°C and 4°C for 7 days. Total viable bacteria, staphylococci, and Enterobacteriaceae were enumerated using culture-based methods, while BA concentrations were quantified by high-performance liquid chromatography (HPLC). The results revealed that microbial growth was significantly higher ($p<0.05$) in both fish species stored at 4°C when compared with those stored at ice temperature (0°C). The counts of total viable bacteria, staphylococci, and Enterobacteriaceae of short-bodied mackerel stored at 4°C reached 5.32-6.47 Log CFU/g after 7 days. Histamine levels in short-bodied mackerel stored at 4°C reached 292.67 ± 1.53 mg/kg by day 7, exceeding the Codex safety limit of 200mg/kg. Cadaverine, putrescine, and tyramine levels were also increased to 615.00 ± 1.00 , 461.67 ± 1.53 , and 731.67 ± 1.53 mg/kg, respectively. In contrast, histamine remained undetectable in Asian seabass under the same storage conditions. However, cadaverine, putrescine, and tyramine were detected in Asian seabass at 17.67 ± 1.53 , 17.00 ± 2.65 , and 8.33 ± 2.08 mg/kg, respectively. These findings provide essential reference data for assessing spoilage in marine fish species commonly consumed in Thailand and highlight the need for strengthened seafood safety standards on histamine. They further emphasize the critical role of effective cold chain management in traditional markets and local distribution systems.

Keywords: biogenic amines, fish spoilage, refrigerated storage, Asian seabass, short-bodied mackerel

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Introduction

Biogenic amines (BAs) are low molecular weight organic nitrogenous compounds that occur naturally in various foods. They are primarily formed through the microbial decarboxylation of free amino acids, a process facilitated by specific bacterial enzymes. Additionally, BAs can arise via transamination or reductive amination of aldehydes and ketones, especially during food fermentation, spoilage, or under certain physiological conditions (Sivamaruthi et al., 2021). According to their chemical structure, BAs can be classified as aliphatic amines (putrescine, cadaverine, spermine, and spermidine), aromatic amines, (tyramine and 2-phenethylamine), and heterocyclic amines (histamine and tryptamine) (Li et al., 2023). Some of these compounds, such as histamine and tyramine, exhibit vasoactive properties and have been linked to adverse health effects in humans (del Rio et al., 2020). Scombroid poisoning, which presents similar symptoms to those of an allergic reaction arises from the consumption of spoiled fish that induce the production of high histamine levels (Tunç et al., 2025). The most common symptoms include skin rash and itching, dizziness, and headache with the onset typically occurring within a few minutes to one hour after ingestion (Konandana et al., 2021).

Fish freshness is significantly influenced by storage conditions, the duration before processing, and the effectiveness of cold chain management. After harvest, fish undergo biochemical and microbiological changes as a result of endogenous enzymatic activity and microbial proliferation on the skin, gills, and gastrointestinal tract. The rate of spoilage varies with factors such as fish species, storage time and temperature, and the level of microbial contamination (Arulkumar et al., 2023). During spoilage, proteins degrade into peptides, free amino acids, and BAs-thermally stable compounds that serve as reliable indicators of raw material freshness and spoilage. Elevated levels of BAs such as histamine, cadaverine, and putrescine are closely associated with spoilage and potential health risks (Akkaya et al., 2024). This underscores the importance of proper cold chain management to prevent BAs formation in fish and fish products.

Biogenic amines naturally occur in many aquatic food products, reflecting their broad distribution in marine and freshwater species, including tuna, mahi mahi, blue fish, bonito, skip jack, saury, mackerel and others (Food and Drug Administration, 2021). Their formation is closely related to microbial activity as demonstrated in the studies on salted mackerel stored under soft frozen conditions (Tao et al., 2022). In Morocco, investigations of BA formation in commonly consumed species such as sardines, anchovies, mackerel and horse mackerel revealed that the most effective strategies to prevent histamine accumulation are rapid cooling of fish immediately after capture and strict adherence to good hygiene practices along the food chain (El Hariri et al., 2018). Although the global literature has extensively documented BA accumulation in marine fish, much of this research has focused on temperate species. In contrast, studies on tropical, market-sourced fish in Southeast Asia-particularly those sold and consumed in countries like Thailand-remain limited. This lack of data is especially concerning given the region's high reliance on fresh fish and the logistical challenges associated with maintaining a reliable cold chain. Specifically, comparative data on BA accumulation in Thai fish species under local market storage conditions are scarce, with little research examining in Asian seabass (*Lates calcarifer*) and short-bodied mackerel (*Rastrelliger brachysoma*) in Thailand. To address this knowledge gap, the present study investigated the formation of BAs

and associated microbiological changes in Asian seabass (*Lates calcarifer*) and short-bodied mackerel (*Rastrelliger brachysoma*) during refrigerated storage under conditions representative of typical local handling practices. This study focuses on quantifying BAs accumulation in relation to microbial proliferation over time to provide insights into spoilage progression and potential food safety risks in Thai fish market.

Methodology

1. Sample preparation

Asian seabass (*Lates calcarifer*) and short-bodied mackerel (*Rastrelliger brachysoma*) were purchased dead from Thonburi marketplace, Bangkok. Immediately after purchase, the fish were packed in ice in a high-density polyethylene (HDPE) box and transported to the laboratory within approximately 2h. Upon arrival, each ungutted and unfileted fish was weighed and sectioned. For the study, tissue from multiple fish of the same species were pooled to prepare standardized 2kg samples. Samples from Asian seabass and short-bodied mackerel were processed independently and were not combined at any stage. The 2kg samples were then randomly assigned to two storage groups (0°C and 4°C) for 7 days. Triplicate samples were collected on day 0 and day 7 for microbial and biogenic amine analyses. The experimental flow is illustrated in (Figure 1).

2. Microbial analysis by the culture-dependent method

The counts of total viable bacteria, staphylococci and Enterobacteriaceae were conducted using a culture-dependent method as described by Pinto de Rezende et al. (2022) and Rashid et al. (2021). For each analysis, a 25g portion of fish sample was aseptically sampled and transferred to a sterile stomacher bag and 225ml of sterile physiological saline was added. The mixture was homogenized using a stomacher for 2min at room temperature. To enumerate total viable bacteria and staphylococci, 0.1ml of serial dilutions of homogenates was spread on plate count agar (PCA) and mannitol salt agar (MSA), respectively. These plates were incubated at 37°C for 48h. For Enterobacteriaceae enumeration, 1ml of serial dilutions of the homogenates was inoculated into 10ml of molten violet red bile glucose agar (VRBGA). After solidification, an overlay of 10ml of the same molten agar was added and the plates were incubated at 30°C for 24h. The bacterial counts were expressed in the logarithm of colony forming units per gram (Log CFU/g).

3. Determination of biogenic amines

Four biogenic amines, namely, histamine, cadaverine, putrescine, and tyramine, were quantified using high-performance liquid chromatography (HPLC) as described by Abré et al., (2023) with slight modifications. Ten milliliters of perchloric acid (0.2M) and 100µl of 1-3-diaminopropane (0.8mg/ml) were added to 5g of minced fish sample. The resulting homogenate was centrifuged at 7000×g at 4°C for 5min. For the derivation of the extracted BAs, 300µl of saturated sodium carbonate and 400µl of dansyl chloride solution (7.5mg/ml in acetone) were added to 100µl of supernatant. The reaction mixture was vortexed and incubated at 60°C for 5min in the dark, then cooled with water. Subsequently, 100µl of proline (100mg/ml) was added to quench residual dansyl chloride, followed by a 15-min dark incubation period to ensure complete neutralization. To purify the derivatives, 500µl of toluene was added, and the samples were incubated at -20°C for a minimum of 30min. The upper organic phase was then

collected and evaporated under a stream of nitrogen. The dry residue was adjusted to 5ml using acetonitrile and filtered through a 0.45- μ m regenerated cellulose membrane filter. Ten microliters of the solution were injected into HPLC (Model HP 1100, Agilent Technologies, USA). A Zorbax Eclipse-XDB-C18 column (4.6 \times 150mm, 5 μ m, Agilent Technologies, U.S.A.) was used. Chromatographic separation was performed using a gradient elution of (A) acetonitrile (100%), (B) acetonitrile (50%) as follows: 0-8 min, A 70% B 30%; 8-12min, A 80% B 20%; 12-16min, A 5% B 95%; 16-20min, A 70% B 30%; at a column temperature of 28°C. The analytical method for the quantification of biogenic amines in seafood samples was validated by evaluating recovery, the limit of detection (LOD), and the limit of quantification (LOQ) in accordance with conventional protocols from international guidelines (Food and Drug Administration, 2019; AOAC International, 2023).

4. Statistical analysis

Statistical analyses were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine specific group differences. All analyses were performed using SPSS software, version 17.0 (IBM Corporation, USA). Differences between group means were considered statistically significant at $p < 0.05$.

Results and discussion

1. Microbiological profile during refrigerated storage

The microbiological analysis revealed a progressive increase in bacterial load in both Asian seabass (*Lates calcarifer*) and short-bodied mackerel (*Rastrelliger brachysoma*) over the 7-day storage period. The counts of total viable bacteria, staphylococci, and Enterobacteriaceae exhibited higher growth rates in samples stored at 4°C compared to those stored at 0°C (Figure 2). On day 0, the total viable bacterial counts of Asian seabass and short-bodied mackerel stored at 0°C were 1.95 and 1.96 Log CFU/g, respectively, indicating satisfactory freshness. After 7 days of storage, the counts increased more than threefold. In comparison, samples stored at 4°C for 7 days showed substantial microbial proliferation, particularly in short-bodied mackerel stored at 4°C (MF47), which exhibited the highest total bacterial counts (6.47 Log CFU/g) across all tested groups. These results are in accordance with the previous report (Syropoulou et al., 2021) that revealed temperatures facilitated the growth of bacteria in fresh and chill-stored fish. The increased bacterial activity in short-bodied mackerel may be attributable to species-specific differences in surface microbiota or intrinsic biochemical composition. Additionally, the higher counts of Enterobacteriaceae (5.32 Log CFU/g) and staphylococci (5.74 Log CFU/g) in this species after 7 days of storage at 4°C indicate a greater susceptibility to spoilage-associated microbial activity. Similar microbial patterns have been reported in scombroid species, which are known for their rapid spoilage and high potential for amine accumulation (Visciano et al., 2020; Ucar & Ozogul, 2024). These findings reinforce the well-established link between microbial activity and the formation of spoilage indicators such as biogenic amines. In particular, members of the Enterobacteriaceae family are well recognized for their amino acid decarboxylase activity, which facilitates the production of biogenic amines during fish spoilage and fermentation (Li et al., 2025; Zhang et al., 2025). Further metagenomic profiling could help elucidate

the composition and functional roles of these microbial communities (Nam et al., 2023). One limitation of the present study is the lack of sensory evaluation data, which is important for correlating microbial spoilage and biogenic amine formation with consumer-perceived quality. Previous studies have reported that sensory evaluation detects fish spoilage earlier than chemical and microbiological indicators. Notably, the development of initial off-flavors in fish occurred when the total viable counts of bacteria and BAs contents increased (Çakmak & Sancak, 2023; Ntzimani et al., 2024). Another limitation of this study is that all fish were obtained from a single market, which may limit the generalizability of the findings. Future studies should therefore include replication from different geographic sources or supply chains to better reflect variability in real-world conditions.

2. Biogenic amine accumulation in fish samples

Biogenic amine (BA) content was determined and validated using high-performance liquid chromatography (HPLC), showing a pattern consistent with microbial proliferation (Table 1). The recovery of BA was 100.25%. The limit of detection (LOD) and the limit of quantitation (LOQ) were 5.00 and 20.00mg/kg, respectively. At day 0, none of the four targeted amines-histamine, cadaverine, putrescine, and tyramine-were detected in any sample, confirming the initial freshness of the fish. As storage progressed, BA levels remained relatively low in fish kept at 0°C but rose markedly in samples stored at 4°C. Short-bodied mackerel stored at 4°C for 7 days (MF47) exhibited the highest concentrations of all four amines. Histamine levels reached 292.67 ± 1.53 mg/kg, while cadaverine, putrescine, and tyramine measured 615.00 ± 1.00 , 461.67 ± 1.53 , and 731.67 ± 1.53 mg/kg, respectively. These concentrations far exceeded acceptable safety thresholds, particularly for histamine, which is regulated at 50mg/kg by the U.S. Food and Drug Administration (2021) and at 200mg/kg by the Codex Alimentarius Commission (Codex, 2024) for scombroid fish and/or products. These findings closely align with the previous report of histamine levels exceeding 300mg/kg in tuna samples collected during a survey in Northern Italy (Morello et al., 2024). The faster accumulation observed in the present study may be due to higher initial microbial load or specific conditions within the local supply chain. By contrast, Asian seabass stored at 4°C for the same duration (AF47) showed much lower amine levels, with histamine remaining undetectable. However, histamine concentrations have been reported with greater than 198.03mg/kg in Asian seabass or barramundi (*Lates calcarifer* Bloch) fillets kept at 8°C over 20 days (Yassoralipour et al., 2016). Cadaverine and putrescine were present at 17.67 ± 1.53 and 17.00 ± 2.65 mg/kg, respectively, while tyramine was measured at 8.33 ± 2.08 mg/kg. These values suggest that Asian seabass is less prone to amine accumulation under short-term refrigerated storage, possibly due to lower decarboxylase activity in its associated microbiota or lower free amino acid content. Investigating these biochemical and microbiological mechanisms further could yield useful insights for improving post-harvest handling protocols. The differences between the two species point to the role of intrinsic and extrinsic factors in BA formation. Similar conclusions were drawn by Barp et al., (2024), who noted that scombroid fish exhibited rapid spoilage and BA accumulation which can increase toxicity by inhibiting intestinal metabolic enzymes. Cadaverine, putrescine, histamine, and tyramine are among the predominant biogenic amines commonly detected in seafood. Furthermore, the concentrations of these amines tend to rise markedly as storage duration increases (Qu et al., 2022). These findings

have direct implications for fish vendors, retailers, and food safety regulators. Early in the cold chain, improved temperature control and shorter storage durations, particularly for vulnerable species like short-bodied mackerel, are critical to mitigating health risks. Vendors should prioritize ice storage at or near 0°C, and regulators could use this baseline data to establish more species-specific inspection thresholds and guidelines. Implementing real-time monitoring of temperature and quality indicators could enhance compliance and reduce foodborne illness incidents.

In Thailand, there is still a lack of detailed research on the formation of biogenic amines (BAs) in marine species that are commonly consumed locally. This study aims to address that gap by comparing two widely marketed species-Asian seabass (*Lates calcarifer*) and short-bodied mackerel (*Rastrelliger brachysoma*)-under typical refrigerated storage conditions that reflect real-world practices in households and retail settings. Although the accumulation of BAs in marine fish has been well-documented internationally (Soares, 2021; Sánchez-Parra et al., 2024), there is comparatively little data from tropical regions, especially Southeast Asia. This is particularly concerning given the region's challenges in maintaining effective cold chain logistics and Thailand's high consumption of fresh fish. Currently, Thailand has no officially published regulatory limit for histamine in fish, and the Codex Alimentarius Commission limit of 200mg/kg for scombroid and other histidine-rich fish species is generally applied (Codex, 2024). In the present study, short-bodied mackerel (*Rastrelliger brachysoma*) stored at 4°C reached a histamine concentration of 292.67±1.53mg/kg by day 7 of storage, well above the established safety threshold. This finding underscores the public health risk associated with inadequate cold chain practices. This result highlights a significant risk of histamine poisoning when cold chain practices are not properly implemented. Importantly, these findings provide valuable baseline data to support risk evaluations and inform regulatory oversight. They also offer practical insights for developing species-specific monitoring protocols, establishing amine limits tailored to tropical storage scenarios, and improving consumer education on fish handling.

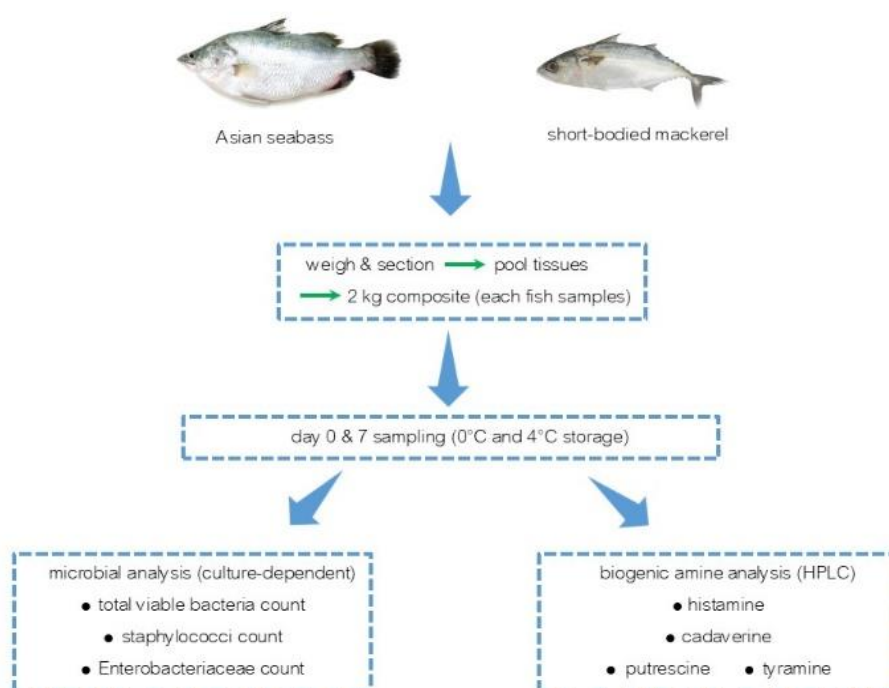


Figure 1 Overview of the experimental workflow in this study.

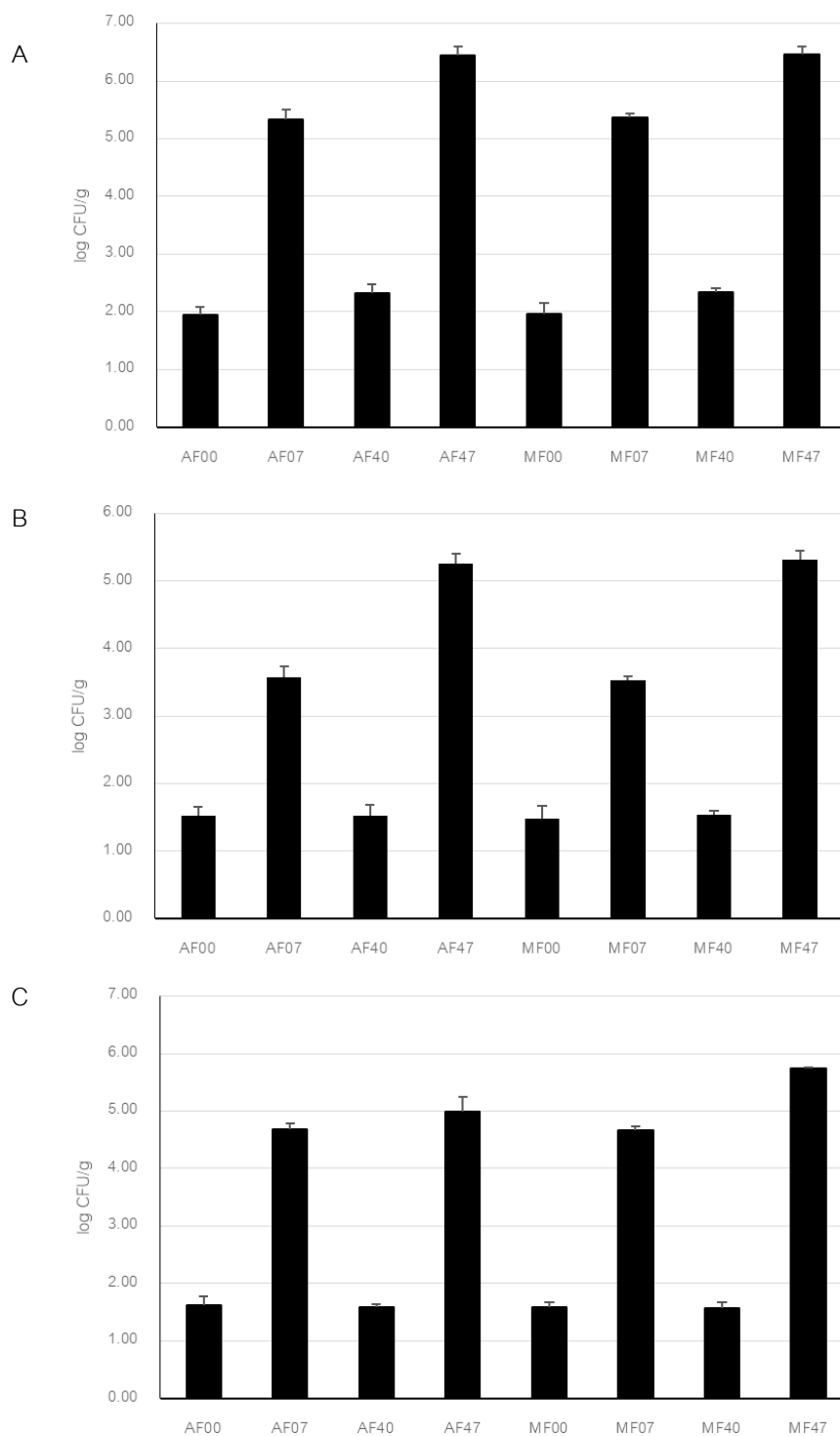


Figure 2 Changes in total viable count (A), staphylococci count (B) and Enterobacteriaceae count (C) in Asian seabass and short-bodied mackerel during storage at 0 and 4°C for 7 days. Error bars represent standard deviations. AF00, Asian seabass at 0°C and day 0; AF07, Asian seabass at 0°C and day 7; AF40, Asian seabass at 4°C and day 0; AF47, Asian seabass at 4°C and day 7; MF00, short-bodied mackerel at 0°C and day 0; MF07, short-bodied mackerel at 0°C and day 7; MF40, short-bodied mackerel at 4°C and day 0; MF47, short-bodied mackerel at 4°C and day 7.

Table 1 Biogenic amine content (mg/kg) of Asian seabass and short-bodied mackerel during refrigerated storage.

sample	biogenic amine content (mg/kg)			
	histamine	cadaverine	putrescine	tyramine
AF00	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
AF07	0.00±0.00 ^b	10.00±2.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
AF40	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
AF47	0.00±0.00 ^b	17.67±1.53 ^b	17.00±2.65 ^b	8.33±2.08 ^c
MF00	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
MF07	0.00±0.00 ^b	19.00±1.00 ^b	0.00±0.00 ^c	76.30±1.13 ^b
MF40	0.00±0.00 ^b	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
MF47	292.67±1.53 ^a	615.00±1.00 ^a	461.67±1.53 ^a	731.67±1.53 ^a

note: each value represents the mean±standard deviation of triplicates followed by different letters in the column are significantly different ($p<0.05$); AF00, Asian seabass at 0°C and day 0; AF07, Asian seabass at 0°C and day 7; AF40, Asian seabass at 4°C and day 0; AF47, Asian seabass at 4°C and day 7; MF00, short-bodied mackerel at 0°C and day 0; MF07, short-bodied mackerel at 0°C and day 7; MF40, short-bodied mackerel at 4°C and day 0; MF47, short-bodied mackerel at 4°C and day 7.

Conclusions

This study highlights the significant impact of storage temperature and fish species on biogenic amine formation and microbial spoilage during refrigeration. Short-bodied mackerel (*Rastrelliger brachysoma*) stored at 4°C showed rapid spoilage, with histamine levels exceeding the Codex safety limit of 200mg/kg by day seven. In contrast, Asian seabass (*Lates calcarifer*) was more resistant under the same conditions, indicating species-specific differences in spoilage rates. To our knowledge, this is the first study to examine biogenic amine accumulation and microbial changes in whole, ungutted specimens of these two species stored at 0°C and 4°C. The results provide important baseline data for assessing spoilage in fish commonly sold in Thai markets and emphasize the need for strict cold storage-especially near 0°C-to minimize microbial growth and histamine formation. Routine monitoring of microbial load and amine content is recommended to ensure seafood safety and quality. Moreover, these findings could provide essential reference data for the development and refinement of food safety regulations in Thailand and address a critical knowledge gap on microbial dynamics and biogenic amine accumulation in tropical fish in Southeast Asia. These results highlight the impact of local storage and handling conditions on food safety. Future research should investigate the effects of gutting, freezing, vacuum packaging, and supply chain variability on microbial dynamics, microbiome composition, and biogenic amine accumulation in these and other tropical fish species in the region.

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