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Scientific Article

Bacterial community associated to histamine contamination in sardines: a case study in the canned fish industry

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ABSTRACT

This study aimed to identify the predominant bacterial populations in sardine samples with high histamine content (≥ 200 mg·kg^{-1}) and histamine-free (< 1 mg·kg^{-1}). For this study, 1,000 g of muscle samples were collected from 40–45 sardines per batch, all obtained from the same supplier, fishing area and period, for histamine analysis. Samples (1 g) from five different batches with ≥ 200 mg·kg^{-1} of histamine (named histamine group) and five with < 1 mg·kg^{-1} (named no-histamine group) were submitted to microbial analysis by extracting the total DNA, followed by polymerase chain reaction of the V3-V4 region of 16S rRNA gene and Ilumina sequencing (Mi-Seq, paired-end). In total, 18 different operational taxonomic units were identified in all samples. Sardines with high histamine content exhibited lower microbial diversity and a higher abundance of the genera *Photobacterium* and *Shewanella*. These bacteria thrive under mild temperatures and indicate fish spoilage and the production of biogenic amines. In contrast, *Psychrobacter* and *Pseudoalteromonas*, known to withstand harsh conditions, including low temperatures, were more prevalent in histamine-free sardines. Our findings point out to a marked change in the bacterial community of sardines with high and no histamine content, even though they are from the same fishing area, period, and shipment.

Keywords: Histamine; Canned fish; Scombrotoxin; Fish poisoning; Fishing industry.

Comunidade bacteriana associada à contaminação por histamina em sardinhas: um estudo de caso na indústria de conservas de peixe

RESUMO

Este estudo teve como objetivo identificar as populações bacterianas predominantes em amostras de sardinha com alto teor de histamina (≥ 200 mg·kg^{-1}) e sem histamina (< 1 mg·kg^{-1}). Para este estudo, 1.000 g de amostras de músculo foram coletadas de 40–45 sardinhas por lote, todas obtidas do mesmo fornecedor, área de pesca e período, para análise de histamina. Amostras (1 g) de cinco lotes diferentes com ≥ 200 mg·kg^{-1} de histamina (denominado grupo histamina) e cinco com < 1 mg·kg^{-1} (denominado grupo sem histamina) foram submetidas à análise microbiana por meio da amplificação e do sequenciamento Ilumina (Mi-Seq, paired-end) da região V3-V4 do gene 16S rRNA. No total, 18 unidades taxonômicas operacionais diferentes foram identificadas em todas as amostras. As sardinhas com alto teor de histamina exibiram menor diversidade microbiana e maior abundância dos gêneros *Photobacterium* e *Shewanella*. Essas bactérias prosperam em temperaturas amenas e podem ser indicativo de deterioração de peixes e produção de aminas biogênicas. Em contraste, *Psychrobacter* e *Pseudoalteromonas*, conhecidas por suportar condições adversas incluindo baixas temperaturas, foram mais prevalentes nas sardinhas sem histamina. Nossos resultados apontam para uma mudança marcante na comunidade bacteriana das sardinhas com alto e nenhum teor de histamina, embora sejam da mesma área de pesca, período e remessa.

Palavras-chave: Histamina; Peixe enlatado; Escombrotoxina; Envenenamento de peixes; Indústria pesqueira.

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INTRODUCTION

World fish production reached 214 million tons in 2020, of which 90 million came from marine fisheries (FAO, 2022). Fish from the Clupeidae family, such as *Sardina*, *Sardinops* and *Sardinella*, are cosmopolitan and contribute significantly to the world's extractive fisheries. Brazilian fish production is around 1.5 million tons per year, with marine extractive fishing as the primary source (MAPA, 2019). Marine fish are highly susceptible to microbial spoilage due to their high-water content, oxidizable fats, and a pH close to neutral (pH 6.6–6.8). Inadequate handling and storage conditions promote the growth of bacteria that produce the enzyme histidine decarboxylase, which catalyzes the formation of histamine when it interacts with the histidine present in the fish musculature (Rio et al., 2024).

Histamine, a primary and heterocyclic diamine, is the most commonly biogenic amine detected in fish-derived products, especially tuna, mackerel, bonito, and sardines (Rio et al., 2024; Rossano et al., 2006). Despite undergoing commercial sterilization, histamine remains present in these fish without altering their sensory characteristics. Proper handling and storage practices are crucial to prevent histamine formation and ensure consumer safety. Histamine levels in foods must not exceed 200 $mg \cdot kg^{-1}$ to prevent scombroid poisoning, a condition caused by ingesting this substance in high concentrations. Symptoms such as abdominal pain, vomiting, diarrhea, headache, erythema, urticaria, and hypotension may arise, resembling an allergic reaction. In severe cases, it can even lead to anaphylactic shock (FDA, 2020).

Histamine formation is unlikely to occur if the fish is processed under satisfactory hygienic-sanitary conditions. The formation of this biogenic amine in fish-derived products is frequently related to contamination by bacteria and deficiencies in the cold chain. To minimize the risk of excessive histamine formation, it is recommended to freeze fish at -15°C. This temperature helps inactivate most of bacteria that produce the enzyme histidine decarboxylase, responsible for the histamine formation via the decarboxylation of histidine (FDA, 2020; Rio et al., 2024). Histamine is mainly produced by contaminating gram-negative bacteria, including *Morganella morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Vibrio alginolyticus*, *Proteus* spp., and *Photobacterium* spp. (Jay et al., 2005; Rio et al., 2024; Takahashi et al., 2015).

Histamine poses a significant challenge for the sardine canning industry. Peivasteh-Roudsari et al. (2020) assessed the occurrence of histamine in canned fish samples (tuna, sardine, kilka, and mackerel) from Tehran and found it in 46.6% of the samples, with 18.3% of them exceeding the histamine limit established by the Food and Drug Administration of the United States of America. In another study, which analyzed 4,615 samples of fresh and processed fish collected from 2010 to 2015 in southern Italy, histamine levels were detected in 352 (7.6%) of the samples (Cicero et al., 2020). Bita and Sharifian (2024) carried out a careful study on biogenic amines content in long-tail tuna (*Thunnus tonggol*) and yellow-fin tuna (*Thunnus albacares*) samples along distinct stages of the canning process, captured by different methods and transported under different storage conditions. They found that histamine levels were higher in the canned fish samples than the ones collected at the fish port and during storage at the cold room, as the canning process involves higher temperatures and longer storage times, which can promote the growth of histamine-producing bacteria. Additionally, higher levels of histamine were detected in the samples obtained from fish captured by drift gillnet compared to those captured by long line, and in the frozen samples compared to those stored in ice, likely due to differences in handling and storage conditions that affect bacterial activity and histamine formation.

The Brazilian canning industry reports a major concern: in the same shipment, and even within the same batch, high levels of histamine are detected in some fish samples, while very low levels are found in other ones. Regulatory agencies require the entire batch to be discarded under these circumstances (personal communication). Although histamine detection techniques are reliable, the relationship between the bacterial community composition and histamine formation in sardines remains unclear. A better understanding of the microbiota could potentially aid in developing strategies to ensure seafood safety for the canning industry.

Therefore, this study utilized next generation sequencing to identify the predominant bacterial populations in sardine samples with high-histamine content and histamine-free fish samples from the same fishing area and period, transport conditions and batch.

MATERIAL AND METHOD

Sample collection and histamine detection

Biological samples of previously eviscerated *Sardinella pilchardus* were obtained from a supplier in Morocco. The sardines were caught and stored in ice tanks until they arrived at the port, after which they were directly transported to the canning plant. Once in the canning plant, the sardines were subjected to further processing, which involved washing, cutting, and

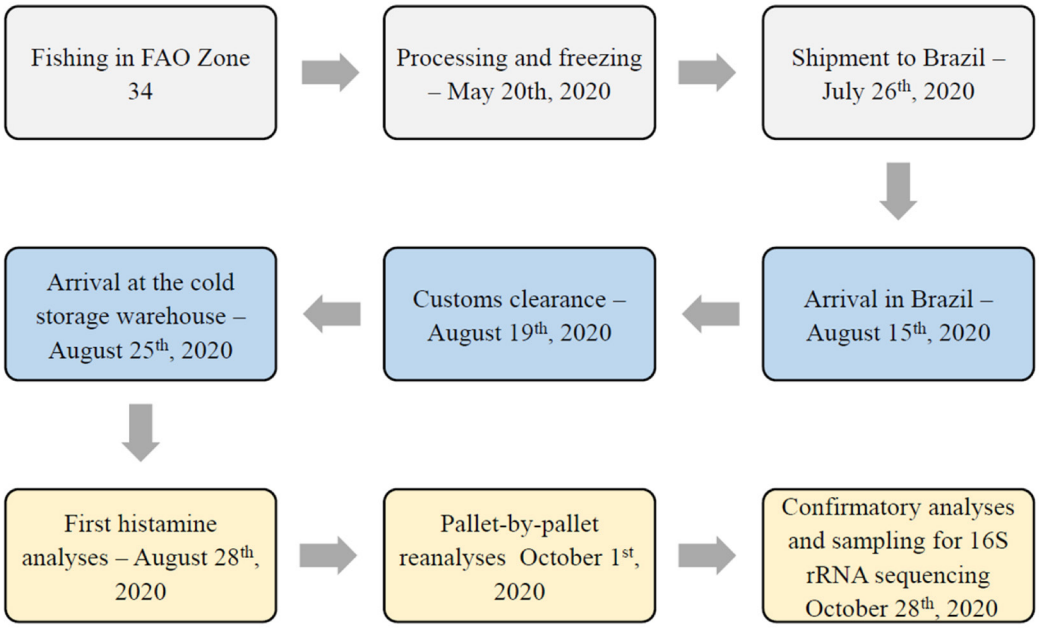
packing in brine. The sardines were then frozen in blocks, each weighing approximately 20 kg, using block freezing technology at -21°C. After freezing, the sardines were placed in a refrigerated container and shipped to Brazil at -21°C, in accordance with the *Regulation of Industrial and Sanitary Inspection of Animal Products* (MAPA, 2020), and collected at the Gomes da Costa (GDC) fish canning plant in Itajaí, Santa Catarina, Brazil. Histamine analysis was performed using the Biofish enzymatic method, confirming the presence of histamine in the collected samples. A reanalysis was conducted on samples from all pallets in the container, revealing varying histamine levels below 1 and 200 $mg \cdot kg^{-1}$ or higher. To ensure reliability, the entire batch was reserved for further experimentation, and the sample collection procedure was repeated. The detailed information about sardine fishing, shipment, storage, and sampling is displayed in Fig. 1.

Sampling for sequencing and confirmatory histamine analysis

Dorsal muscle samples weighing approximately 1,000 g (± 10 g) were obtained from pallets containing sardines with histamine levels of 200 $mg \cdot kg^{-1}$ or higher (named histamine group). Additionally, the same number of samples were collected from histamine-free pallets with histamine levels below 1 $mg \cdot kg^{-1}$ (named no-histamine group). All sample collection was performed in a refrigeration chamber at -18°C. The muscle samples were placed in sterile RNase and DNase-free sample bags (3M sample bag) and transported in a temperature-controlled container to the GDC laboratory, where they were stored at -18°C. Histamine confirmation analysis was conducted on a portion of the samples using the Biofish enzymatic method. Ten 1-g samples were then collected from each group for further analysis. These samples were stored frozen in RNase and DNase-free microtubes, packed with dry ice, and sent to Neoprospecta for total DNA extraction, amplification of the 16S rRNA gene, and sequencing (Fig. 1).

DNA extraction and next generation sequencing

Total DNA extraction was performed using the phenol/chloroform method according to the Neoprospecta laboratory's standard protocol (Christoff et al., 2017). The DNA concentration of each sample was estimated using Picogreen dsDNA (Invitrogen, Carlsbad, California, United States of America). For microbial population identification, the DNA library bank was adjusted to a final concentration of 11 pM, and polymerase chain reaction (PCR) amplification of the V3-V4 region of the 16S rRNA gene was conducted using primers 341F (5' CCTACGGGRSGCAGCAG 3') and 805R (5' GGACTACCAGGGTATCTAAT 3'). Sequencing of the



FAO: Food and Agriculture Organization of the United Nations.

Figure 1. Flowchart displaying the detailed information about fishing area and period, shipment and arrival to Brazil, storage and sampling schedule.

libraries was performed on the Illumina MiSeq platform, employing a 2 × 300 nt paired-end configuration with a volume of 100 K per sample. Operational taxonomic units (OTUs) were generated from the sequences using USEARCH (version 11.0.667) at 97% similarity with the UPARSE algorithm. Taxonomic attribution was determined using the SILVA database (version 138) with 91% identity. Richness and diversity indices were calculated using the vegan R package, and Good's Coverage was determined using the QsRutils R package.

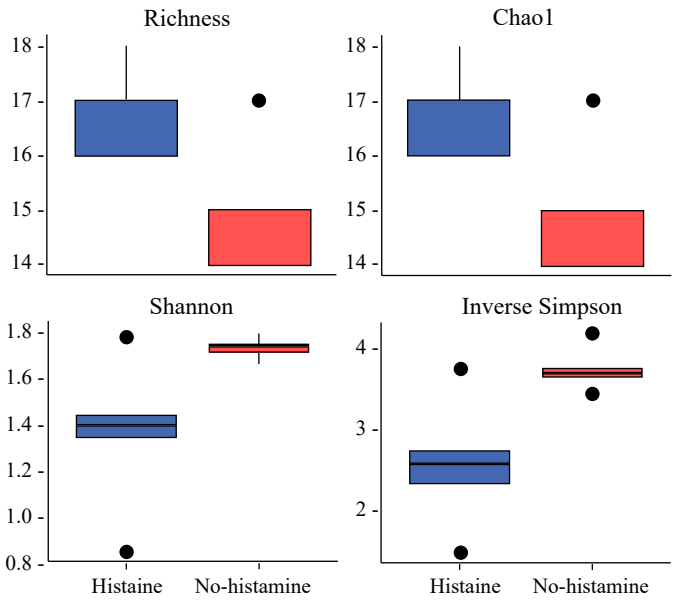
Statistical analysis

Microbiome data were analyzed using R software (version 3.5.1). Due to differences in sequencing depth between samples (up to 9.3x), count data (readings) were normalized using the Bioconductor DESeq2 package. Normalized DESeq2 counts were used to determine beta diversity, non-metric multidimensional scale (NMDS), permutation multivariate analysis of variance, alpha diversity indices and relative abundance (Love et al., 2014). Student's t test was used to identify differences in alpha diversity indices between each treatment in the R environment. Multivariate analysis was performed based on Bray-Curtis' dissimilarity matrices, using the "vegan" package (Oksanen et al., 2019). Generalized univariate linear models (package "mvabund") were used to identify OTUs (Wang et al., 2022). Figures were generated using the "ggplot2" package (Wickham, 2016). The significance level adopted was 5% for all tests.

RESULTS

After processing and filtering the next-generation sequencing data, the sequences were grouped into 18 OTUs. Based on the alpha-diversity analysis (Fig. 2), the richness indices (Richness and Chao1) exhibited opposite behavior compared to the diversity indices (Shannon and Inverse Simpson). Histamine group had higher microbial richness, whereas no-histamine showed higher diversity.

The beta-diversity analysis (NMDS and principal coordinate analysis) based on Bray-Curtis' dissimilarity matrices indicated significant differences ($p < 0.05$) among the samples, suggesting that sardine muscle samples with higher histamine content exhibited different bacterial compositions (Figs. 3a and 3b). The most abundant bacterial families in both treatment groups were Vibrionaceae and Moraxellaceae (Fig. 4). Histamine group exhibited a predominance of the Vibrionaceae family, while Moraxellaceae was the most abundant in no-histamine group. Other minor



● outlier.

Figure 2. Alpha-diversity indices of sardine muscles samples with histamine concentrations ≥ 200 mg·kg^{-1} (histamine) and < 1 m·kg^{-1} (no-histamine).

families identified in both groups were Arcobacteraceae, Planococcaceae, Fusobacteriaceae, Pseudoalteromonadaceae, and Shewanellaceae.

The 18 OTUs identified in the sardine muscle samples belonged to 10 different bacterial genera. Among them, seven comprised more than 95% of the bacterial community, as shown in Fig. 5. In the histamine group, the most abundant genera were *Aliivibrio*, *Cetobacterium*, *Photobacterium*, *Psychrobacter*, *Shewanella*, and *Vibrio*. The no-histamine group showed a predominance of *Photobacterium*, *Pseudoalteromonas*, *Psychrobacter*, and *Vibrio*. In both groups, the most abundant genera were *Photobacterium* and *Psychrobacter*. However, their abundance showed opposite patterns, with *Photobacterium* being more abundant in the histamine group and *Psychrobacter* more abundant in the no-histamine group.

Statistical analysis revealed that samples with histamine showed a higher relative abundance of the genera *Photobacterium* (2 and 17 OTUs) and *Shewanella* (4, 9, and 12 OTUs). Meanwhile, the genera *Psychrobacter* (1, 3, 8, 16, and 18 OTUs), *Pseudoalteromonas* (OTU 5), and *Vibrio* (OTU 11) were more abundant in no-histamine samples. Regarding the genera *Aliivibrio* and *Cetobacterium*, they were more abundant in histamine group, but the difference was not statistically significant (Fig. 4 and Fig. 6).

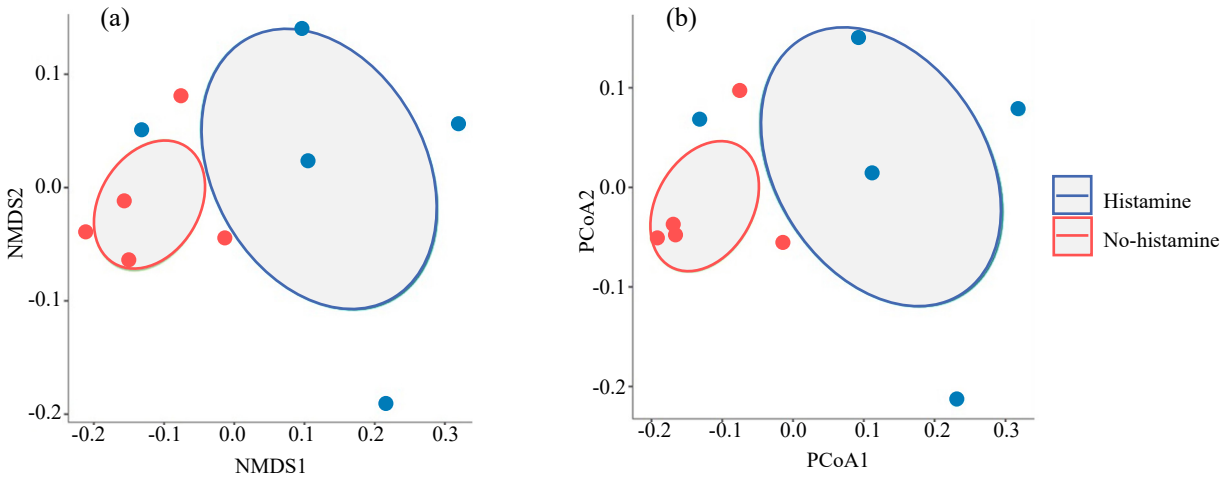


Figure 3. Evaluation of the microbiota composition of sardine muscles samples with histamine concentrations ≥ 200 mg·kg^{-1} (histamine) and < 1 mg·kg^{-1} (no-histamine) by beta diversity indices, (a) non-metric multidimensional scale (NMDS), and (b) principal coordinates analysis (PCoA), based on the Bray-Curtis' dissimilarity matrix.

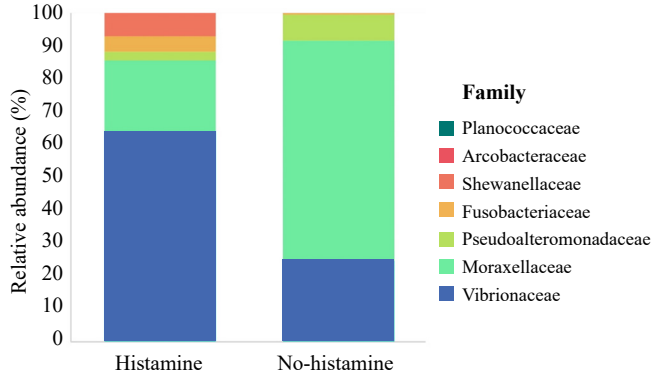


Figure 4. Relative abundance of the main bacterial families identified in samples with histamine concentrations ≥ 200 mg·kg^{-1} (histamine) and < 1 mg·kg^{-1} (no-histamine).

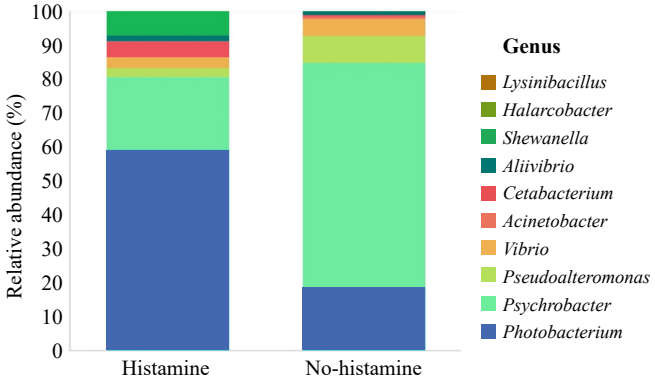


Figure 5. Relative abundance of the main bacterial genera identified in samples with histamine concentrations ≥ 200 mg·kg^{-1} (histamine) and < 1 mg·kg^{-1} (no-histamine).

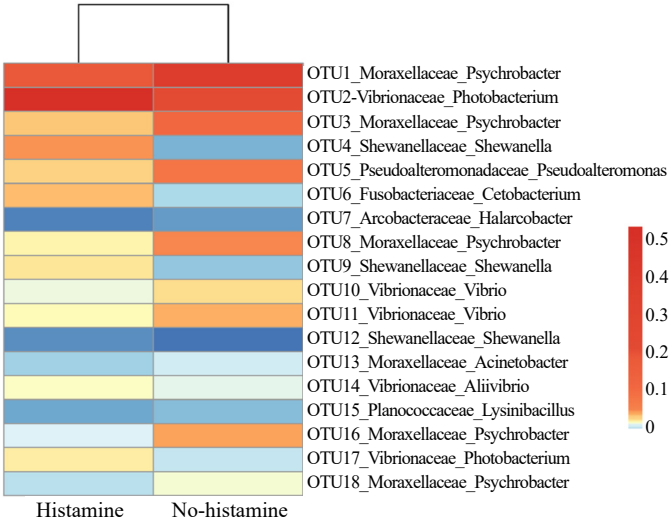


Figure 6. Heatmap of relative percentage of the 18 OTUs identified in samples with histamine concentrations ≥200mg·kg^{-1} (histamine) and <1mg·kg^{-1} (no-histamine).

DISCUSSION

Samples with high-histamine content (≥ 200 mg·kg^{-1}) exhibited higher bacterial richness but lower diversity, indicating a greater number of bacterial species. However, there was a predominance of certain bacteria in terms of abundance, particularly *Photobacterium* spp. and *Shewanella* spp. These two genera are among the main decomposers of saltwater fish (Saelens & Houf, 2022). Therefore, these results suggest that batches with high levels of histamine were exposed to

conditions that favored the proliferation of decomposing bacteria. *Photobacterium* strains produce secondary metabolites that inhibit the growth of other bacteria, indicating that the high dominance of *Photobacterium* spp. in histamine group and the subsequent lower microbial diversity could be attributed to their ability to suppress the growth of other bacterial strains involved in fish decomposition (Oku et al., 2008). On the other hand, samples from the no-histamine group exhibited the opposite pattern, with higher bacterial diversity, but lower bacterial richness. This distinct bacterial composition was further confirmed by beta diversity indices, which indicated significant dissimilarities between the two bacterial communities.

Regarding the bacterial families, Moraxellaceae and Vibrionaceae were more abundant in the non-histamine and histamine group, respectively. Bacteria belonging to the Moraxellaceae family (*Proteobacteria phylum*) are commonly isolated from marine and freshwater fish (González et al., 2000). They are predominant in the spoilage microbiota of aerobically stored animals, but their participation in the spoilage process is still uncertain, since members of this family do not produce harmful by-products from amino acids as do other species, such as *Pseudomonas* or *Shewanella* (Yang, 2014).

Members of the Vibrionaceae family (*Proteobacteria phylum*) are found in a variety of aquatic biotopes and include extremophiles such as the psychrophile *Photobacterium profundum*, which lives at high-ocean depths, as well as highly specialized symbionts such as *Vibrio fischeri*, and pathogens, such as *Vibrio cholerae* (Thompson & Swings, 2006). Bacteria of this family are among the main decomposers of seafood at room temperature, e.g., *Photobacterium phosphoreum* and *Aeromonas* spp. (Saelens & Houf, 2022). Based on the abundance of these families, it may suggest that the samples with histamine had ideal conditions for the proliferation of bacteria closely related to seafood spoilage.

The genus *Photobacterium* was detected in all samples, constituting 52% of the microbiota samples with high content of histamine and 21% in samples with no histamine. This genus is widely distributed in the aquatic environment and is associated with symbiotic interactions with fish and squid, commensalism in the intestines of various marine organisms, pathogenicity, and, primarily, decomposition of fish (Burtseva et al., 2020; Gram, 2009; Martini et al., 2013). It is also involved in the production of biogenic amines, including histamine, putrescine, and cadaverine in fish and shrimp (Bjornsdottir-Butler et al., 2020; Lakshmanan et al., 2002). Additionally, this genus is directly linked to scombroid poisoning and is frequently identified as a significant contributor to histamine production, particularly in fish samples stored at temperatures between 5 and 15°C (Lehane & Lewis, 2000; Kanki et al., 2004).

Photobacterium spp. can thrive in low temperatures, exhibiting optimal growth typically between 5 and 25°C (Moi et al., 2017). However, histamine production by *Photobacterium* spp. significantly decreases at temperatures below 5°C (Takahashi et al., 2015). Additionally, a decrease from 42.8 to 26.6% in the overall abundance of this genus strains was observed in ice-cold (< 0°C) shrimp *Penaeus vannamei* samples (Jia et al., 2019). Therefore, treatment involving temperatures below 5°C appears to be effective in suppressing the growth of this bacterial genus and, consequently, the production of histamine.

The *Shewanella* genus also showed a higher relative abundance in histamine group. This genus is composed of mesophilic bacteria that decompose and exhibit optimal growth at a pH close to neutrality (Møretrø & Langsrud, 2017). They are affected by low pH, which limits their spoilage potential in many types of meat products. *Shewanella* spp. are found almost exclusively in the microbiota of decomposing fish, in addition to being responsible for the characteristic odor of "rotten fish" by reducing trimethylamine oxide to trimethylamine, in addition to producing H_2S (Gram, 2009).

The presence of *Shewanella* spp. and *Photobacterium* spp. in these samples may indicate that the products were deteriorating and that they may not have received adequate cold chain treatment from fishing to processing and freezing. The samples with (≥ 200 mg·kg-1) and without (< 1 mg·kg-1) histamine were taken from the same container. The logistics and unloading process in Brazil did not result in temperature variations that could affect the samples differently. If there were variations in this process, all samples would have been subjected to high temperatures and there would probably not be significant differences in the microbiota composition between the samples.

On the other hand, the genus *Psychrobacter* showed a higher relative abundance in histamine-free samples. The bacteria are psychotropic and halotolerant, capable of growing in a wide temperature range from -10 to 42°C. They are isolated from various regions of the globe, including inhospitable Antarctica (Ayala-del-Río et al., 2010; Bozal et al., 2003; Møretrø et al., 2016). As members of the resident microbiota of fish and other seafood, they are unrelated to the production of H_2S or histamine, and they are unable to compete with spoilage bacteria (González et al., 2000; Møretrø & Langsrud, 2017).

Another genus that showed greater abundance in sardine muscle samples without histamine was *Pseudoalteromonas*, which is abundant in the marine environment and has high

resistance to hostile marine environments, such as the very low temperatures of polar seas and the high pressure of deep-ocean waters (Parrilli et al., 2021). This ability is due to the peculiar metabolic mechanisms of this group, which confer resistance and enable survival in these places with extreme conditions (Bowman et al., 1997).

Species of the *Vibrio* genus commonly colonize fish, marine invertebrates, and algae. They can form biofilms on biotic and abiotic surfaces, which play an essential role in their environmental persistence (Farmer & Janda, 2015). The genus *Vibrio* has been associated with spoilage and histamine formation in seafood (Ding & Li, 2024; Houicher et al., 2021). Deng et al. (2024) even observed a higher correlation of *Vibrio* and *Bacillus* with total biogenic amine content in refrigerated raw hairtails (*Trichiurus lepturus*). Conversely, biogenic amine production is intimately linked to the *quorum sensing* (QS) system, and inactivation or disruption of the QS system prevents biogenic amine production (Ding & Li, 2024). Although the abundance of OTU 11 (*Vibrio* sp.) was greater in the no-histamine group, it had low representation in the whole bacterial community (< 3%). This community was more diverse and had a distinct composition compared to the histamine group. These differences in the bacterial community might influence bacterial physiology and interactions, as no histamine production was detected in the no-histamine samples even though they showed an increased abundance of *Vibrio* and a substantial abundance of *Photobacterium*.

Hence, our results, particularly the contrasting behavior observed in the genera *Psychrobacter*, *Pseudoalteromonas*, *Shewanella*, and *Photobacterium* in the no-histamine and histamine groups, suggest differences in the preservation of sardines from fishing to processing. This occurred despite being caught in the same fishing area and period, by the same fishing fleet, and undergoing the same processing and shipping procedures. One possible reason is the scarcity of fresh water in Morocco. They predominantly use desalinated seawater, which increases the price of ice, often surpassing the cost of the fish. Consequently, vessels use minimal ice, which can eventually be insufficient for proper temperature control. Fish from various vessels are unloaded at the factories and eviscerated before freezing. This variability in fishing practices can lead to histamine formation in some fish prior to freezing.

On the other hand, despite low temperatures typically inhibiting histamine-producing bacteria, there are reports of psychrotolerant amine-forming bacteria in the natural microbiota of fish that can survive freezing conditions. Lakshmanan et al. (2002) reported that some amine-forming bacteria, particularly *Aeromonas*, *Photobacterium*, and *Micrococcus*, can survive to some extent during ice storage of fish. Additionally, *Pseudomonas fluorescens* and *Shewanella putrefaciens* seem capable of forming biogenic amines even during cold storage (Ding & Li, 2024). Thus, the resilience of the natural fish microbiota might contribute to, or even underscore, the role of specific handling conditions in histamine production, even under frozen conditions. However, these are still open issues that need to be addressed in future studies.

CONCLUSION

Sardine samples with ≥ 200 and < 1 $mg \cdot kg^{-1}$ of histamine exhibited marked differences in their associated microbiota. Samples with high-histamine content showed lower bacterial diversity and higher abundance of *Photobacterium* and *Shewanella* genera. Conversely, the bacterial diversity was higher in no-histamine group (< 1 $mg \cdot kg^{-1}$), and *Psychobacter*, *Pseudoalteromas*, and *Vibrio* were more prevalent in these samples. The differential diversity of the bacterial community and abundance of these genera might be important features associated with high and low levels of histamine in sardines. However, these was one of the few studies that have applied next generation sequencing to address the characterization of the bacterial community related to histamine formation. Therefore, future studies should be done to elucidate the conditions behind these changes and how they affect the production of histamine.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data can be made available upon request.

AUTHORS' CONTRIBUTION

Data curation: Frondana, L., Farias, D.R.; **Formal analysis:** Frondana, L., Farias, D.R., Schleder, D.D.; **Investigation:** Frondana, L.; **Methodology:** Frondana, L.; **Writing – original draft:** Frondana, L., Farias, D.R., Schleder, D.D.; **Writing – review & editing:** Farias, D.R., Schleder, D.D.; **Funding acquisition:** Schleder, D.D.; **Conceptualization:** Schleder, D.D.; **Supervision:** Schleder, D.D.; **Visualization:** Schleder, D.D.; **Final approval:** Scheleder, D.D.

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REFERENCES

Ayala-del-Río, H. L., Chain, P. S., Grzymski, J. J., Ponder, M. A., Ivanova, N., Bergholz, P. W., Di Bartoli, G., Hauser, L. L. M., & Bakermans, C. (2010). The genome sequence of *Psychrobacter arcticus* 273-4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. *Applied and Environmental Microbiology*, 76(7), 2304-2312. https://doi.org/10.1128/AEM.02101-09

Bita, S., & Sharifian, S. (2024). Assessment of biogenic amines in commercial tuna fish: Influence of species, capture method, and processing on quality and safety. *Food Chemistry*, 435, 137576. https://doi.org/10.1016/j.foodchem.2023.137576

Bjornsdottir-Butler, K., May, S., Hayes, M., Abraham, A., & Benner Jr., R. A. (2020). Characterization of a novel enzyme from *Photobacterium phosphoreum* with histidine decarboxylase activity. *International Journal of Food Microbiology*, 334, 108815. https://doi.org/10.1016/j.ijfoodmicro.2020.108815

Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S., & McMeekin, T. A. (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Applied and Environmental Microbiology*, 63(8), 3068-3078. https://doi.org/10.1128/aem.63.8.3068-3078.1997

Bozal, N., Montes, M. J., Tudela, E., & Guinea, J. (2003). Characterization of several *Psychrobacter* strains isolated from Antarctic environments and description of *Psychrobacter luti* and *Psychrobacter fozii*. *International Journal of Systematic and Evolutionary Microbiology*, 53(4), 1093-1100. https://doi.org/10.1099/ijs.0.02457-0

Burtseva, O., Kublanovskaya, A., Baulina, O., Fedorenko, T., Lobakova, E., & Chekanov, K. (2020). The strains of bioluminescent bacteria isolated from the White Sea finfishes: Genera *Photobacterium*, *Aliivibrio*, *Vibrio*, *Shewanella*, and first luminous *Kosakonia*. *Journal of Photochemistry and Photobiology B: Biology*, 208, 111895. https://doi.org/10.1016/j.jphotobiol.2020.111895

Cicero, A., Cammilleri, G., Galluzzo, F. G., Calabrese, I., Pulvirenti, A., Giangrosso, G., Cicero, N., Cumbo, V., Vella, A., Macaluso, A., & Ferrantelli, V. (2020). Histamine in fish products randomly collected in Southern Italy: a 6-year study. *Journal of Food Protection*, 83(2), 241-248. https://doi.org/10.4315/0362-028X.JFP-19-305

Christoff et al. (2017). *White paper Neoporspeta: Bacterial identification through accurate library preparation and high-throughput sequencing*. Retrieved from https://www.neoprospecta.com/conteudo/whitepaper-microbioma-e-iras/

Deng, H., Wu, G., Zhou, L., Chen, X., Guo, L., Luo, S., & Yin, Q. (2024). Microbial contribution to 14 biogenic amines accumulation in refrigerated raw and deep-fried hairtails (*Trichiurus lepturus*). *Food Chemistry*, 443, 138509. https://doi.org/10.1016/j.foodchem.2024.138509

Ding, T., & Li, Y. (2024). Biogenic amines are important indices for characterizing the freshness and hygienic quality of aquatic products: A review. *LWT - Food Science and Technology*, 194, 115793. https://doi.org/10.1016/j.lwt.2024.115793

Farmer, J. J., & Janda, M. J. (2015). Vibrionaceae. In: W. B. Whitman (ed.), *Bergey's Manual of Systematic of Archeae and Bacteria* (pp. 491-494). Wiley-Blackwell.

Food and Agriculture Organization of the United Nations (FAO) (2022). *The state of world fisheries and aquaculture: Towards blue transformation*. FAO. https://doi.org/10.4060/cc0461en

Food and Drug Administration (FDA) (2020). Scombrotoxin (histamine) formation. In B. Leonard (Ed.), *Fish and fishery products hazards and controls guidance* (pp. 83-102). FDA.

González, C. J., Santos, J. A., García-López, M. L., & Otero, A. (2000). Psychrobacters and related bacteria in freshwater fish. *Journal of Food Protection*, 63(3), 315-321. https://doi.org/10.4315/0362-028X-63.3.315

Gram, L. (2009). Microbiological spoilage of fish and seafood products. In W. Sperber & M. Doyle (Eds.), *Compendium of the microbiological spoilage of foods and beverages* (pp. 87-119). Springer.

Houicher, A., Bensid, A., Regenstein, J. M., & Ozogul, F. (2021). Control of biogenic amine production and bacterial growth in fish and seafood products using phytochemicals as biopreservatives: A review. *Food Bioscience*, 39, 100807. https://doi.org/10.1016/j.fbio.2020.100807

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Processed meats and seafoods. In J. M. Jay, M. J. Loessner & D. A. Golden (Eds.). *Modern food microbiology* (7. ed., pp. 101-124). Springer. https://doi.org/10.1007/0-387-23413-6_5

Jia, S., Liu, Y., Zhuang, S., Sun, X., Li, Y., Hong, H., Lv, Y., & Luo, Y. (2019). Effect of ε-polylysine and ice storage on microbiota composition and quality of Pacific white shrimp (*Litopenaeus vannamei*) stored at 0°C. *Food Microbiology*, 83, 27-35. https://doi.org/10.1016/j.fm.2019.04.007

Kanki, M., Yoda, T., Ishibashi, M., & Tsukamoto, T. (2004). *Photobacterium phosphoreum* caused a histamine fish poisoning incident. *International Journal of Food Microbiology*, 92(1), 79-87. https://doi.org/10.1016/j.ijfoodmicro.2003.08.019

Lakshmanan, R., Shakila, R., & Jeyasekaran, G. (2002). Survival of amine-forming bacteria during the ice storage of fish and shrimp. *Food Microbiology*, 19(6), 617-625. https://doi.org/10.1006/fmic.2002.0481

Lehane, L., & Lewis, R. J. (2000). Ciguatera: recent advances but the risk remains. *International Journal of Food Microbiology*, 61(2-3),91-125.https://doi.org/10.1016/S0168-1605(00)00382-2

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 550. https://doi.org/10.1186/s13059-014-0550-8

Martini, S., Al Ali, B., Garel, M., Nerini, D., Grossi, V., Pacton, M., Casalot, L., Cuny, P., & Tamburini, C. (2013). Effects of hydrostatic pressure on growth and luminescence of a moderately-piezophilic luminous bacterium *Photobacterium phosphoreum* ANT-2200. *PLoS One*, 8(6), e66580. https://doi.org/10.1371/journal.pone.0066580

Ministério da Agricultura e Pecuária (MAPA) (2019). *Government eliminates import tariffs on sardines for one year*. MAPA. Retrieved from https://www.gov.br/agricultura/pt-br/assuntos/noticias/governo-reduz-tarifa-de-importacao-de-sardinha-a-zero-por-um-ano

Ministério da Agricultura e Pecuária (MAPA) (2020). *Regulation of Industrial and Sanitary Inspection of Animal Products (RIISPOA)*. MAPA.

Moi, I. M., Roslan, N. N., Leow, A. T. C., Ali, M. S. M., Rahman, R. N. Z. R. A., Rahimpour, A., & Sabri, S. (2017). The biology and importance of *Photobacterium* species. *Applied Microbiology and Biotechnology*, 101, 4371-4385. https://doi.org/10.1007/s00253-017-8300-y

Møretrø, T., & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive Reviews in Food Science and Food Safety*, 16(5), 1022-1041. https://doi.org/10.1111/1541-4337.12283

Møretrø, T., Moen, B., Heir, E., Hansen, A. A., & Langsrud, S. (2016). Contamination of salmon fillets and processing plants with spoilage bacteria. *International Journal of Food Microbiology*, 237, 98-108. https://doi.org/10.1016/j.ijfoodmicro.2016.08.016

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., & Wagner, H. (2019). *Vegan: Community Ecology Package. R package version 2.5-5*. Retrieved from https://CRAN.R-project.org/package=vegan

Oku, N., Kawabata, K., Adachi, K., Katsuta, A., & Shizuri, Y. (2008). Unnarmicins A and C, new antibacterial depsipeptides produced by marine bacterium *Photobacterium* sp. MBIC06485. *Journal of Antibiotics*, 61, 11-17. https://doi.org/10.1038/ja.2008.103

Parrilli, E., Tedesco, P., Fondi, M., Tutino, M. L., Giudice, A., Pascale, D., & Fani, R. (2021). The art of adapting to extreme environments: The model system *Pseudoalteromonas*. *Physics of Life Reviews*, 36, 137-161. https://doi.org/10.1016/j.plrev.2019.04.003

Peivasteh-Roudsari, L., Rahmani, A., Shariatifar, N., Tajdar-Oranj, B., Mazaheri, M., Sadighara, P., & Khaneghah, A. M. (2020). Occurrence of histamine in canned fish samples (tuna, sardine, kilka, and mackerel) from markets in Tehran. *Journal of Food Protection*, 83(1), 136-141. https://doi.org/10.4315/0362-028X.JFP-19-288

Rio, B., Fernandez, M., Redruello, B., Ladero, V., & Alvarez, M. A. (2024). New insights into the toxicological effects of dietary biogenic amines. *Food Chemistry*, 435, 137558. https://doi.org/10.1016/j.foodchem.2023.137558

Rossano, R., Mastrangelo, L., Ungaro, N., & Riccio, P. (2006). Influence of storage temperature and freezing time on histamine level in the European anchovy *Engraulis encrasicholus* (L., 1758): a study by capillary electrophoresis. *Journal of Chromatography B*, 830(1), 161-164. https://doi.org/10.1016/j.jchromb.2005.10.026

Saelens, G., & Houf, K. (2022). Systematic review and critical reflection on the isolation and identification methods for spoilage associated bacteria in fresh marine fish. *Journal of Microbiological Methods*, 203, 106599. https://doi.org/10.1016/j.mimet.2022.106599

Takahashi, H., Ogai, M., Miya, S., Kuda, T., & Kimura, B. (2015). Effects of environmental factors on histamine production in the psychrophilic histamine-producing bacterium *Photobacterium iliopiscarium*. *Food Control*, 52, 39-42. https://doi.org/10.1016/j.foodcont.2014.12.023

Thompson, F. L., & Swings, J. (2006). Taxonomy of the Vibrios. In F. L. Thompson, B. Austin & J. Swings (Eds.), *The Biology of Vibrios* (pp. 29-43). ASM Press. https://doi.org/10.1128/9781555815714

Wang, Y., Naumann, U., Eddelbuettel, D., Wilshire, J., Warton, D., Byrnes, J., Silva, V. S., Niku, J., Renner, I., & Wright, S. (2022). *mvabund: Statistical Methods for Analysing Multivariate Abundance Data. R package version 4.0.1*. Retrieved from https://CRAN.R-project.org/package=mvabund

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Yang, X. (2014). Moraxellaceae. In G. W. Smithers (Ed.), *Encyclopedia of Food Microbiology* (2. ed., pp. 826-833). Academic Press. https://doi.org/10.1016/B978-0-12-384730-0.00441-9