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Effect of refrigerated dry-aging on the microbial quality and shelf life of dolphinfish (*Coryphaena hippurus*) marketed by seafood restaurants

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Abstract

Refrigerated dried fish products are a growing trend in restaurants in the northern and southern regions of Brazil, despite data on the microbiological safety and shelf life of these products remaining scarce, and a national regulatory standard not being available. This study evaluated the microbial quality and shelf life of eight refrigerated dry-aged dolphinfish specimens to determine safety thresholds for bacterial growth. Fish loins were stored in controlled conditions (0.7 ± 0.6°C, 60 ± 11% relative humidity, 1.5 m/s airflow) and analyzed in duplicate on days 0, 1, 2, 3, 5, 8, 12, 15, and 19. Microbiological analyses included counts of total aerobic mesophilic and psychrotrophic bacteria, Enterobacteriaceae, coagulase-positive *Staphylococcus*, *Vibrio* spp., *Salmonella* spp., yeasts, and molds. Water activity decreased in fresh loins from 0.925 ± 0.007 to 0.885 ± 0.007 after 5 days of aging. *Salmonella* spp. and *Vibrio* spp. were absent in all 25 g samples. Enterobacteriaceae and coagulase-positive *Staphylococcus* counts remained below 7 log CFU/g, while mesophilic counts reached 4.6 log CFU/g. Psychrotrophic counts surpassed 7 log CFU/g by day 12. These results indicate that refrigerated dry-aging extended the shelf life of dolphinfish loins up to 17 days—significantly longer than that of conventionally refrigerated fresh fish—by maintaining microbiological safety within acceptable limits.

Keywords: dry fish; food safety; seafood restaurant; microbial counts; commercial shelf life.

Practical Application: Nowadays, special attention has been given to sustainable fish production, mainly due to the high perishability of this food matrix and its increasing production and consumption, raising concerns about food safety risks. Refrigerated dry-aging has attracted consumers due to the desirable flavor, aroma, and texture of premium fish products. Our findings achieved a shelf-life extension of refrigerated dry-aged dolphinfish, and its potential application to seafood restaurants and the retail fish production sector.

1 INTRODUCTION

Globally, Brazil is one of the largest consumers of fish products, with an estimated fish consumption of 9.4 kg per capita by the year 2020, according to the Food and Agriculture Organization (FAO, 2022). The country's extensive coastline and numerous rivers provide a rich supply of fish, which is also available through aquaculture. The development of the fish production chain is closely linked to the growing consumer preference for healthier products, as fish offers numerous nutritional benefits (Camargo & Pouey, 2005). Fish meat is particularly rich in vitamins A and D, calcium, omega-3, and phosphorus, while providing high-quality lipids and proteins with significant biological value (Domingo, 2007; Hunter & Roberts, 2000). To meet the rising demand, an increasing number of commercial establishments, such as supermarkets and restaurants, are investing in new recipes for fish preparation, as well as in advanced storage techniques and the optimization of products generated through fish processing (Seafood Brasil, 2023).

In addition to their nutritional benefits, fresh fish are highly perishable, presenting significant challenges for enhancing consumption and public health (Viana et al., 2023). Fresh fish products are subject to both intrinsic and extrinsic factors that contribute to the main mechanisms of spoilage, shortening their shelf life by enzymatic autolysis, oxidation, and microbial growth (Nie et al., 2022). Fish tissues contain autolytic enzymes that break down proteins, fats, and other substances, leading to undesirable changes such as off-flavors, odors, texture degradation, and rancidity. Fish with high fat content, high moisture, and slightly acidic pH levels promote microbial growth and spoilage. Microorganisms naturally inhabit the fish's skin, gills, and digestive tract, and the unsanitary practices during harvesting, transportation, and processing introduce contaminants, accelerating

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spoilage (Panebianco et al., 2024). To reduce spoilage, microbial hazards, and preserve fish freshness, it is essential to strictly control cold storage chain, minimize the time between capture and consumption, and implement proper hygienic practices.

Fish meat can become a potential vehicle for foodborne pathogens, especially those of bacterial origin. Several bacterial pathogens, including *Vibrio* spp., *Salmonella* spp., *Listeria monocytogenes, Staphylococcus aureus, Clostridium botulinum, Shigella* spp., and *Aeromonas* spp., are considered major microbial hazards for seafoods (Novoslavskij et al., 2015). According to current legislation, raw, chilled, or frozen fish and chilled or frozen bivalve mollusks, which are not consumed raw, must be tested for the presence of thermotolerant coliforms/*Escherichia coli*, coagulase-positive staphylococci, and *Salmonella* spp., as well as for the total count for mesophilic and psychrotrophic aerobic microorganisms. The national legislation sets the following standards for frozen raw fish not consumed as raw food: coagulase-positive staphylococci/g, with a tolerance of 10^3 CFU/g (colony forming units/gram); and the absence of *Salmonella* spp. in 25 g of the sample. For pre-cooked frozen fish, the standards include coliforms at 45°C, with a tolerance of 5 x 10 CFU/g; coagulase-positive staphylococci/g, with a tolerance of 10^3 CFU/g; and the absence of *Salmonella* spp. in 25 g of sample (Brasil 1997, 2020, 2022).

These bacteria can contaminate fish through water, domestic waste, post-harvest handling, improper hygiene, and failures in the cold chain from capture to consumption (Ali et al., 2011). Maintaining strict control over the cold chain is one of the key objectives in fish technology and food safety. Research on fish preservation technologies has been bolstered by studies on food safety, aiming to enhance the effectiveness of fish quality control. For this reason, microbiological analysis is essential for establishing the hygienic and sanitary profile of final fish products and identifying critical points for microbial contamination within the production chain.

With this concern, refrigerated dry-aging (DA) has been applied to the reduction of food water activity (Aw) at cooler temperatures, which inhibits microorganisms that are sensitive to Aw below 0.85, resulting in a significant increase of shelf life (Dashdorj et al., 2016; Khazzar et al., 2023). DA has been used to preserve animal-based foods for over 100 years in regions in Northern Europe, Australia, the United States, Africa, Asia, Japan, and Argentina. The process of aging meat involves various methods, including sun drying, hot air drying, dry salting, microwave vacuum drying, and freeze drying (Belton et al., 2022). The refrigerated DA method requires carefully controlled parameters of temperature, humidity, and ventilation to extend the meat's shelf life, depending on the characteristics of each food matrix and the conditions within the chambers. This maturing technique involves applying cold temperatures around 4°C with controlled humidity of 61–85% for periods ranging from 14 to 40 days (Dashdorj et al., 2016). Refrigerated DA has also been applied to fish maturation in a process known as *dry fish* where the fish are stored in refrigerated chambers to dehydrate them, enhancing tenderness and flavor. Several microorganisms develop on the surface of fish, affecting quality through their proteolytic and lipolytic activities, as well as their metabolic products (Kim et al., 2018). *Dry fish* has gained popularity as a gastronomic trend in restaurants worldwide and, more recently, in the culinary markets of Rio de Janeiro, Brazil. Contemporary gastronomy is embracing *dry fish* as a new concept of freshness and flavor, highlighting the appreciation of aged fish meat consumption. The widespread adoption of this aging technique in the restaurant sector can be attributed to an Australian chef, Josh Niland, and his innovative methods, which include DA whole fish carcasses and utilizing all edible parts of the fish (Niland 2019, 2021, 2023). The duration of DA and other process parameters vary based on the characteristics of the fish species. Throughout the maturation process, the skin dries out, the excess fluids are released, and there is a significant change in the texture of the flesh (Niland, 2019).

Due to the widespread popularity of dry-aged meat, the European Food Safety Authority (EFSA) has issued a statement confirming that when meat is aged under strictly controlled conditions, there are no greater risks compared to fresh meat (EFSA, 2023). However, national regulations impose a shelf-life limit on fish, with a maximum refrigeration storage period of 7 days (Brasil, 2004), which prevents DA from being recognized as a long-term fish conservation method by the health surveillance authorities. Since DA fish is a prolonged maturation process, certain microbial agents may adapt to cold storage conditions, potentially leading to spoilage and rancidity (Luqman et al., 2024; Sampels, 2015; Savini et al., 2024). Furthermore, there is limited research supporting the safety and quality of DA as a viable conservation strategy, particularly for large-scale fish production and consumption.

The present study focuses on the dolphinfish (*Coryphaena hippurus*), an important fishery resource worldwide and a prized species, typically weighing between 8.0 and 12 kg. Its meat is increasingly explored in gastronomy, particularly as a raw menu option in Japanese and Mediterranean cuisines. This fish is also known as "sea goldfish" or "mahi-mahi," a member of the Coryphaenidae family. The species is primarily oceanic, captured off the littoral coast of Rio de Janeiro (Brazil), typically restricted to the 20°C isotherm, with the main fishing season occurring from January to June (Alejo-Plata et al., 2011). As a migratory species, dolphinfish requires a higher proportion of dark muscle, lipids, glycogen, and myoglobin for its long journeys. As a result, dolphinfish meat has an excellent flavor, making it ideal for loins and fillet cakes, and it is widely marketed in restaurants throughout northern and southern Brazil (Lira et al., 2020). Our experimental study examined the DA conditions of dolphinfish stored in a restaurant refrigerated chamber at 0.7 ± 0.6°C, 60 ± 11% RH, and 1.5 m/s ventilation, under aerobic conditions from day 0 up to day 19. The goal was to assess shelf-life parameters and microbial quality. The primary objective was to generate new data to promote local fisheries and gastronomy, while advancing the understanding of *dry fish* in the global context of fish technology and food safety.

1.1 Relevance of the work

This research provides evidence that dolphinfish processed through refrigerated dry-aging can extend its shelf life by maintaining the quality and safety, making it a useful method for restaurants and seafood suppliers.

2 MATERIAL AND METHODS

2.1 Sample extraction and preparation

The present study was carried out in April and May 2024 at a local restaurant in Rio de Janeiro, Brazil (Ocyá, Ilha da Gigóia), to assess the safety of DA of fish products in real restaurant conditions. The fish was treated exactly as the procedures applied by the restaurant staff for routinely served products. Fish specimens were obtained from Tijucas Island, located off the Rio de Janeiro coast, and were slaughtered using the Japanese method known as *Ikejime*, which complies with the guidelines set by the EFSA (2004) and follows procedures outlined by Poli et al. (2005) and Sperss (2019). A total of eight dolphinfish specimens (*C. hippurus*) were caught and placed into an icebox maintained at 0 ± 1°C, and shipped within 12 h to the restaurant, whereas *postmortem* procedures were cautiously conducted. First, scales and viscera were removed, and the fish was suspended upside down in a freeze chamber set at 0°C ± 3°C, 45–75% RH, and 1.5 m/s ventilation (COPELAND LP®, CR20K6M-PFV-141, Florissant Avenue, St. Louis-US) for 5 days. The restaurant's refrigerated chamber included software for continuous control of temperature and RH parameters. The system was continuously checked for RH to never exceed 80% at any point of the process, for temperatures to remain within a limited range of fluctuations, and for constant ventilation throughout DA storage. The Aw of one fresh dolphinfish sample was measured by a Pawkit water activity meter (Decagon Devices, Pullman, WA, USA) and resulted in 0.925 ± 0.007. After 5 days in DA conditions, Aw reduced to 0.885 ± 0.007, meaning a water loss of 0.04 units, or 4.32%, from the fresh initial weight. From this point on, the loins of 949 g ± 157 g were extracted from eight dolphinfish specimens and packed in aerobiosis bags and maintained in a refrigerated DA chamber set at 0°C ± 3°C, 45–75% RH, and 1.5 m/s ventilation (COPELAND LP®, CR20K6M-PFV-141) and analyzed in duplicate on days 0, 1, 2, 3, 5, 8, 12, 15, and 19 to establish microbial counts and limit of shelf life.

2.2 Bacteriological analysis

Conventional method protocols were established initially using a sterile stomacher bag containing 10 g of sample aliquot and 90 mL of sterile peptone saline (1% peptone solution (m/v), Difco®, Detroit, MI), which was homogenized for 1 min in a stomacher (model ESH, Merck KGaA, Darmstadt, Germany). After that, serial decimal dilutions were conducted and sequenced plated into specific culture medium in accordance with American Public Health Association (Salfinger & Tortorello, 2015) protocols. The analyses were done in duplicate, and after incubation, the results of colony counting were expressed as log CFU/g. Microbial analysis was performed at the Food Microbiology Laboratory of the Federal University of Rio de Janeiro—Macaé *Campus*. Additionally, culture plating techniques and biochemical tests were performed on DA dolphinfish loin samples to characterize the microbial species present.

Total counts of aerobic mesophilic and psychrotrophic bacteria was conducted by the pour-plate technique on Petri dishes containing plate-count agar (PCA; Difco®, Detroit, MI), which were incubated for 24–48 h at 37°C, and 7 days at 10°C (International Organization for Standardization [ISO], 2003b; Salfinger & Tortorello, 2015), respectively.

To establish the coagulase-positive *Staphylococcus* count, 0.1 mL of each dilution was inoculated on mannitol salt agar (Difco®, Detroit, MI) using spread-plate technique and incubated at 36 ± 1°C from 30 to 48 h (ISO 2003a). After, coagulase, catalase, Gram coloring, thermonuclease, and biochemical tests were applied to determine *Staphylococcus* species (Koneman et al., 2019).

The count of Enterobacteriaceae was established using pour-plate technique on VRB agar (violet red bile, Difco®, Detroit, MI) and incubated at 36 ± 1°C for 18–24 h (ISO, 2006). *Escherichia coli* was confirmed by EMB agar (eosin methylene blue, Difco®, Detroit, MI), typical growth and IMViC (indole, methyl red, Voges–Proskauer, Citrate), TSI test (triple sugar iron), urease, gelatin hydrolysis, and carbohydrate fermentation (Difco®, Detroit, MI) (ISO, 2001; Koneman et al., 2019).

Detection of *Salmonella* spp. protocol was established by buffered peptone water (non-selective enrichment), Rappaport–Vassiliadis, Muller–Kauffmann tetrathionate broth (selective enrichment), Hektoen enteric agar, and xylose lysine deoxycholate agar (Difco®, Detroit, MI) (ISO, 2002).

To detect *Vibrio* spp., dilutions were inoculated by the spread-plate technique onto TCBS agar (Difco®, Detroit, MI), and to confirm Vibrionaceae species, biochemical tests including cytochrome oxidase, fermentation of glucose, sucrose, arabinose, and mannose, growth in different saline concentrations (0, 3, 6, 8, and 10%), amino acid utilization (lysine–ornithine decarboxylase and arginine dehydrolase), acetoin production in the Voges–Proskauer medium, and resistance to vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate) (ISO, 2017) were applied.

The identification of *Pseudomonas* species was performed using biochemical test panels including oxidase test, SIM (sulfide, indole, motility), catalase, carbon and nitrogen assimilation, and penicillin susceptibility (Koneman et al., 2019). The biochemical identification of *Bacillus* spp. was carried out through tests such as Gram staining, catalase activity, urease production, nitrate reduction, motility, and indole production (ISO, 2004).

To establish yeast and mold counts, dilutions were inoculated into potato dextrose agar (Difco®, Detroit, MI), with 3.5 ± 0.1 pH, and incubated at 22–30°C for 7–10 days (ISO, 2008). Colony characteristics, lactophenol cotton blue (LPCB, Merck KGaA®, Darmstadt, Germany) staining, and microscopy were used to assess the morphological identification of molds (Samson et al., 2004).

2.3 Statistical analyses

Microbial counts were expressed as log CFU/g, and the mean and standard deviation were calculated using the Excel® program, and nonlinear modeling to graphical analysis by the statistical environment R using the readxl package (Wickham & Bryan, 2023), and in long format with tidyr (Wickham et al.,

2019). TAPC (total aerobic psychrotrophic count), measurements were used to obtain the fish shelf life, which was defined as the time to achieve 7 log CFU/g (International Commission of Microbial Specifications for Foods [ICMSF], 2011), considering the fitted values from the DMFit primary predictive model (Baranyi & Roberts, 1994).

3 RESULTS AND DISCUSSION

3.1 Microbial quality parameters of dolphinfish samples

Table 1 presents the results of microbial counts for eight dolphinfish loin samples of fresh fish and after 19 days of refrigerated DA in a restaurant chamber under controlled conditions of 0.7 ± 0.6 °C, 60 ± 11% RH, and 1.5 m/s ventilation. The only microbial group that reached the limit of 7 log CFU/g (ICMSF, 2001) was the count of aerobic psychrotrophic bacteria after 12 days of DA storage. Aerobic mesophilic bacteria reached a maximum of 4.6 log CFU/g after 8 days of DA storage, followed by yeasts and molds, which reached 5.8 log CFU/g at 19 days of DA. The counts for coagulase-positive *Staphylococcus* and Enterobacteriaceae reached a maximum of 2.9 log CFU/g at 19 days and 3.7 log CFU/g at 5 days of DA storage, respectively. No typical growth was detected in the *Salmonella* spp. and *Vibrio* spp. by the isolation protocols.

Counts of psychrotrophic aerobic bacteria had an increase of 1.4–4.2 log CFU/g from fresh to DA dolphinfish samples, and surpassed the limit of 7 log CFU/g (ICMSF, 2001) at 12 days of DA storage. Psychrotrophic bacteria have a slow growth rate in cold storage conditions, but may have an adaptable genetic ability to survive and multiply either in long-term refrigeration or freezing periods. Psychrotrophic bacteria are specialized to thrive in colder environments, typically between 0°C and 15°C, making them particularly relevant for the DA fish, due to adaptive genetic traits that enable them to survive and multiply during long-term refrigeration or freezing periods (González et al., 2000; Hébraud & Potier, 1999). These bacteria can directly affect the fish's quality by producing volatile compounds like amines and fatty acids, which contribute to complex aromas and flavors that may be desirable, depending on the maturation process. However, some species, including *Pseudomonas, Shewanella,* and *Carnobacterium*, can produce unpleasant compounds, such as off-odors or bitter tastes. If these bacteria proliferate excessively, they can accelerate the spoilage of the fish. Additionally, species like *Shewanella putrefaciens* and *Pseudomonas* spp. can break down nitrogenous compounds and produce potentially harmful substances, like biogenic amines, with histamine being the most significant, which can pose a toxicity risk to humans (Lin et al., 2014).

Enterobacteriaceae counts reached a maximum value of 3.7 log CFU/g on the 5th day of DA dolphinfish storage; however, all values remained within the limits established by sanitary regulations (Brasil, 2004, 2022). Confirmatory biochemical tests were *E. coli* positive in only one (12.5%) of the samples. The TSI, IMViC, and other biochemical tests were used to characterize other Enterobacteriaceae species, including *Morganella morganii* (12.5%), *Citrobacter* spp. (12.5%), *Proteus vulgaris* (25%), and *Enterobacter* spp. (37.5%). Non-fermentative isolates obtained by the TSI test were further analyzed using the O/F test, which helped to identify 25% of *Pseudomonas* spp. and 12.5% of *Shewanella* spp. Furthermore, pigment production on trypticase soy agar showed negative results for all *Pseudomonas* spp. isolates tested. DA seafood is mostly consumed as ready-to-eat (RTE) product, without cooking, amplifying the risk of histamine and other biogenic amine poisoning. The main histamine-producing bacteria are the Enterobacteriaceae species, *Pseudomonas* spp., *Shewanella* spp., and *Photobacterium* spp. (Emborg & Dalgaard, 2008; Kanki et al., 2007; Lin et al., 2014). Despite Enterobacteriaceae and Pseudomonadales presently being isolated, further biochemical evaluation is needed.

The mesophilic bacteria count of fresh dolphinfish samples was 2.7 log CFU/g and increased to 3.3 log CFU/g within 5 days under DA conditions. A maximum limit of 4.6 log CFU/g was reached at 8 days of DA, but no count exceeded the limit of 10^5–10^6 log CFU/g described by sanitary regulations (Brasil, 2004, 2022). Mesophilic bacteria generally grow at temperatures between 20°C and 45°C. While DA typically occurs at lower temperatures, between 0°C and 5°C for the DA treatment; nevertheless, some mesophilic bacteria can still be present and influence the maturation process. These bacteria may produce acids or other compounds that enhance the flavor and aroma of the fish meat. This is one of the key beneficial effects of dry aging when properly managed. Mesophilic bacteria help break down proteins and lipids, leading to more intense flavors and

Table 1. Microbial counts of fresh and dried-aged dolphinfish samples.

	Fresh dolphinfish	Refrigerated-dried aged dolphinfish loins								
	Aw: 0.925 ± 0.007	Aw: 0.885 ± 0.007								
Microbial Counts (log. CFU/G)		Day 0	Day 1	Day 2	Day 3	Day 5	Day 8	Day 12	Day 15	Day 19
Aerobic Mesophilic Bacteria	2.775	3.389	3.732	3.986	4.371	4.431	4.623	4.462	4.462	4.469
Aerobic Psychrotrophic Bacteria	1.477	4.201	4.201	5.939	6.313	6.267	5.975	8.058	8.489	7.352
Staphylococcus coagulase-positive	0.698	0.698	1.544	1.397	1.653	1.301	1.301	2.267	2.707	2.845
Enterobacteriaceae	0	0	1.176	3.278	3.579	3.716	3.361	2.778	2.954	2.498
Yeast and Mold	2.176	3.176	3.230	3.703	3.716	3.863	4.108	4.149	4.292	5.863
Salmonella spp.	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence	Absence
Vibrio spp.	0	0	0	0	0	0	0	0	0	0

*Refrigerated dry fish conditions: at 0.7 ± 0.6°C, 60 ± 11% RH, and 1.5 m/s ventilation, (COPELAND LP®, CR20K6M-PFV-141, Florissant Avenue, St. Louis-US); Day 0: 5 days of dry fish; day 1: 6 days of dry fish; day 2: 7 days of dry fish; day 3: 8 days of dry fish; day 5: 10 days of dry fish; day 8: 13 days of dry fish; day 12: 17 days of dry fish; day 15: 20 days of dry fish; day 19: 24 days of dry fish; Absence: absence of *Salmonella* spp. in 25 g of the sample; 0: Count with value 10 ≤ CFU/g; Aw: water activity.

making the fish meat more tender, which is desirable during maturation. However, if the growth of mesophilic bacteria becomes excessive, they can cause unwanted decomposition, resulting in off-odors and flavors (Du et al., 2001). Additionally, certain mesophilic bacteria can be pathogenic or produce toxins, potentially compromising the safety of the final product (Antoine et al., 2002).

Mesophilic and psychrotrophic bacteria are the main hygienic markers associated with poor fish sanitary conditions during handling. The methods of fishing and butchering before the drying process can represent a significant source of microbial contamination to fish meat (Poli et al., 2015). Generally, the steps include beheading, eviscerating the gut, and deboning, before filleting or cutting loins. Good hygiene practices of butchering are vital, especially for medium- to large-scale fishing industry, to avoid microbial and fungi involvement in spoilage enzymatic reactions that lead to off-flavors and odors (Luqman et al., 2024). In this context, dolphinfish is typically caught in oceanic tropical waters, often far from its final market destination, and is rarely consumed within a few days after harvest. It may usually take 2–3 weeks for some species to reach the final consumers, during which their quality and safety can be significantly compromised. The present dolphinfish sample collection was obtained from a fast on-ice transportation chain within 12 hours from fishing to maturation, which may have contributed to lower fish contamination. To improve quality and reduce safety risks, good hygienic *postmortem* procedures and lowering water activity by refrigerating the fish shortly after harvest help to slow microbial growth, preventing it from exceeding the safety threshold of 7 log CFU/g for mesophilic aerobic bacteria.

The counts of *Staphylococcus* spp. increased from 0.7 to 2.8 log CFU/g from fresh dolphinfish to 19 days of DA samples, but did not exceed the limit of 10^3 log CFU/g set by national sanitary legislation for non-raw fish consumption (Brasil, 2022). Biochemical panels identified *Staph. aureus* in only one of the DA fish samples (12.5%), while other coagulase-negative species like *S. xylosus* (25%) and *S. sciuri* (12.5%), as well as unidentified coagulase-negative *Staphylococcus* (25%) and *Micrococcus* (25%) species, were also present. A recent study by Indio et al. (2024) detected 5% *Staphylococcus* isolation in dry-cured tuna samples, with *S. equorum* being the most significant species, followed by a small percentage of *S. agnetis* (< 0.7%) and *S. aureus* (< 0.3%). *Staphylococcus aureus* is a global risk associated with poor hygienic practices among food handlers. Both coagulase-positive and -negative *Staphylococcus* species naturally inhabit the skin, nasal passages, and digestive system of humans. Asymptomatic food handlers may inadvertently transfer the bacteria to fish and equipment through contact or respiratory droplets (Bencardino et al., 2021). The growth of these bacteria is closely linked to enterotoxin production, which typically occurs at temperatures between 15°C and 45°C, with an optimal growth temperature of 37°C, conditions that were avoided in our study. However, the bacteria can also grow under refrigeration, even below 7°C, but at a significantly slower rate.

The yeast and mold counts increased from 2.2 to 3.2 log CFU/g in fresh dolphinfish to DA samples and reached the limit of 5.8 log CFU/g after 19 days of DA storage. The morphology and microscopic characteristics identified included species of *Aspergillus* and *Fusarium*. Yeast and molds should also be considered when addressing the risk of contamination in DA fish. Fungal contamination can lead to mycotoxin production, which, when consumed in excess, can lead to serious chronic diseases, including immune system dysfunction, respiratory issues, and liver cancer. Mycotoxins such as aflatoxin B1 (AFB1), T-2 toxin (T-2), ochratoxin A (OTA), and deoxynivalenol (DON) have been identified in open-air and sun-DA fish products, primarily released by fungi from the genera *Fusarium, Penicillium*, and *Aspergillus* (Deng et al., 2021). However, recent studies have focused on developing cost-effective drying methods to assist small-scale sellers (Fitri et al., 2022). Authors recommend that marketplaces and storage facilities for dried fish implement better fungal control measures in order to prevent mycotoxins from contaminating the products (Islam & Kabir, 2019; Syamdidi, 2012).

3.2 Shelf life of refrigerated dry-aging dolphinfish

The present study established TAPC to determine the acceptable parameter of DA dolphinfish shelf life using the predictive microbiology model by Baranyi and Roberts (1994) and the microbiological threshold of 7 log CFU/g for fish samples according to ICMSF (2001) (Figure 1).

According to Figure 1, a TAPC threshold of 7 log CFU/g was reached at the 12th day under DA conditions (equal to 17 days DA). Literature review did not access available data on the shelf life of refrigerated DA dolphinfish loins in restaurant handling conditions, but other DA fish species were taken for comparison. A recent study on the microbiological safety conducted by Panebianco et al. (2024) evaluated DArainbow trout at 3°C and 78% RH storage and reported a 10-day limit based on total mesophilic and psychrophilic counts (5.3 ± 0.7 log CFU/g and 5.8 ± 0.3 log CFU/g, respectively). Indio et al. (2024) reported a limit of 7 days, when the total aerobic colony count (TBC) reached 5.4 log CFU/g for salt-DA salmon (*Salmo salar*) at a similar restaurant storage chamber condition (4°C, 67% RH, and a ventilation rate of 2.1 m/s). Roseiro et al. (2017) studied vacuum-packaged and cured-DA tuna (*Thunnus obesus*) loins and reported a limit of 8 days of storage based on psychrotrophic bacteria counts of 6.4 Log CFU/g at 2–4°C refrigerated chamber.

Refrigerated fish products stored at temperatures between 0°C and 4°C typically have a shelf life ranging from 7 to 12

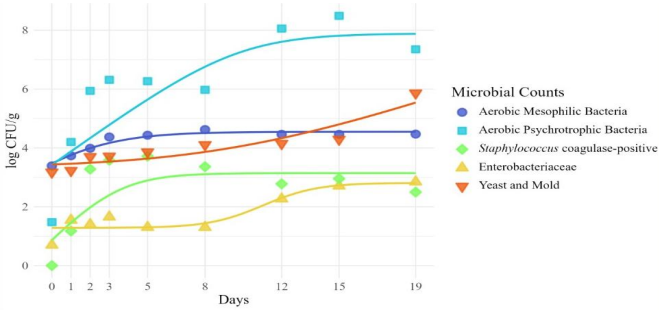

Figure 1. Microbial counts of dolphinfish loin samples at 19 days of dry-aging storage.

days. The Food and Drug Administration (FDA, 2022) has published guidelines for controlling fish hazards, noting that *C. hippurus* (dolphinfish) is a primary species associated with histamine poisoning, as well as risks from environmental chemicals and aquaculture drugs. To mitigate histamine contamination, the FDA recommends that fish be stored at 4°C for no longer than 24 hours after slaughter (FDA, 2022). Du et al. (2001) reported a 5-day shelf life for *C. hippurus* stored at 1.7°C, with total mesophilic aerobic bacterial counts (TMAC) reaching 7 log CFU/g. Antoine et al. (2002) found that *C. hippurus* stored at 7°C had a shelf life of 3 days before reaching 10^6 CFU/g of TMAC. Bai et al. (2019) identified amino acids, aldehydes, and biogenic amines associated with spoilage in *C. hippurus* stored at 4°C, and suggested an 8-day shelf-life limit. When comparing these studies to the present findings, which show a shelf-life extension of up to 17 days for dolphinfish loin samples based on TAPC, it can be concluded that the reduction of water activity played a crucial role in minimizing microbial activity in the final product.

Overall, these results emphasize the high quality of dolphinfish supplied by the local fishery, as the initial microbial counts in fresh samples were below the quantification limit established by national regulations (Brasil, 2020). Upon capture, the muscle tissue of the fish is sterile; however, after death, bacteria present on the skin, gills, and intestines can colonize the flesh (Huss, 1995). The time required for bacteria to reach the muscle varies, but it is limited when stored and transported at 0°C. Since the dolphinfish specimens in this study were transported to the restaurant within 12 hours of capture and maintained at temperatures between 0°C and 1°C, microbial translocation was likely minimized. The short food chain, controlled storage conditions (time/temperature), and adherence to good hygienic practices may have positively contributed to minimizing biological risk hazards. Furthermore, our results highlight the potential of DA as an effective method for maintaining acceptable microbial levels in fish during the maturation process for at least 17 days.

4 CONCLUSIONS

Refrigerated *dry fish* is widely used in restaurants around the world to reduce the Aw of fish to levels below 0.85, under controlled refrigeration conditions. The DA parameters currently under evaluation (0.7 ± 0.6°C, 60 ± 11% RH, and 1.5 m/s ventilation) proved effective in extending the shelf life of dolphinfish up to 17 days, surpassing the duration reported for conventionally refrigerated fresh fish. Microbiological analyses revealed results in accordance with national food safety standards, indicating the method's reliability. This preservation technique shows promise for broader applications, supporting extended shelf life through the use of restaurant refrigerated aging chambers and potentially scaling to commercial operations.

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