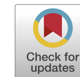
International Journal of Food Microbiology 415 (2024) 110641

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro



Microbiological safety of dry-cured fish from the raw material to the end of processing

Valentina Indio [a], Federica Savini [a,*], Fausto Gardini [b], Federica Barbieri [b], Laura Prandini [a], Yitagele Terefe Mekonnen [a], Federico Tomasello [a], Federica Giacometti [a], Alessandro Seguino [a], Andrea Serraino [a], Alessandra De Cesare [a]

[a] *Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia (BO), Italy*
[b] *Department of Agricultural and Food Science, University of Bologna, Viale Fanin 44, Bologna, Italy*

A R T I C L E I N F O

Keywords:
Ready to eat fish
Process parameters
Microbiological quality
Biological hazards
Shotgun metagenomic

A B S T R A C T

The commercialization of processed fish products is rising in restaurants and small to medium enterprises. However, there is a lack of data related to the microbiological safety of such products. In this study total aerobic colony count and Enterobacteriaceae, as proxy of process hygiene criteria, and detection of *Listeria monocytogenes* and concentration of histamine, as food safety criteria, were investigated in *Salmo salar* (salmon), *Xiphias gladius* (swordfish) and *Thunnus albacares* (yellowfin tuna), before, during, and at the end of a dry-curing process, performed in a dedicated cabinet, at controlled temperature, relative humidity and ventilation, up to 240 h. The microbiological parameters were investigated in the tested fish products by culture methods and shotgun metagenomic, while the presence of histamine, and other biogenic amines, was quantified by High Performance Liquid Chromatography.

In the raw material, and up to the end of the dry curing process, the concentration of Enterobacteriaceae was always lower than 10 CFU/g, while total aerobic colony counts ranged between 3.9 and 5.4 Log CFU/g in salmon; 5.5 and 5.9 Log CFU/g in swordfish; 4.4 and 4.8 Log CFU/g in tuna. The pH values were significantly different between fish species, in the raw materials and during processing except for T4, occurring 70 h after the start of the process for salmon and after 114 h for swordfish and tuna. Water activity was different at specific sampling points and at the end of processing. Overall, 79 % of the sequences identified in the tested fish samples were assigned to y bacteria. The most abundant phyla were Pseudomonadota, Bacillota and Mycoplasmatota. The microbial populations identified by shotgun metagenomic in the tested fish species clustered well separated one from the other. Moreover, the microbial richness was significantly higher in salmon and tuna in comparison to swordfish.

Listeria monocytogenes was not detected in the raw material by using the reference cultural method and very few reads (relative abundance <0.007) were detected in swordfish and tuna by shotgun metagenomic. Histamine producing bacteria, belonging to the genera *Vibrio*, *Morganella*, *Photobacterium* and *Klebsiella*, were identified primarily in swordfish. However, histamine and other biogenic amines were not detected in any sample.

To the best of our knowledge this is the first paper reporting time point determinations of microbiological quality and safety parameters in salmon, swordfish and tuna, before, during and at the end of a dry-curing process. The data collected in this paper can help to predict the risk profile of ready to eat dry-cured fish products during storage before consumption.

1. Introduction

The commercialization of processed fish products is rising in restaurants and small to medium enterprises. However, there is a lack of data related to the microbiological safety of such products. Fish meat provides favourable conditions for the growth of microorganisms, due to low acidity (pH > 6), high water activity, as well as high content in proteins, free amino acids and non-protein nitrogenous compounds

* Corresponding author at: Federica Savini, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy.
E-mail address: federica.savini3@unibo.it (F. Savini).

https://doi.org/10.1016/j.ijfoodmicro.2024.110641
Received 4 December 2023; Received in revised form 15 February 2024; Accepted 22 February 2024
Available online 28 February 2024
0168-1605/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(Gram and Huss, 1996). The growth of spoilage microorganisms results in alterations in appearance (production of slime), development of off-odours and off-flavours and damage of texture, reducing fish shelf life and quality. Spoilage caused by microorganisms can result in the formation of biogenic amines (BAs), volatile amines, sulphides, organic acids, alcohols, ketones and aldehydes with safety risks and unacceptable off-flavours and off-odours (Visciano et al., 2014). During storage, BAs, such as histamine, tyramine, cadaverine and putrescine, are generated by the decarboxylation of specific free amino acids by microorganisms and their accumulation in seafood can be regarded as a good indicator of spoilage. Among BAs, histamine is the most toxic for humans, causing a disease known as scombroid fish poisoning, causing headache, hypotension, vomiting, diarrhoea, flushing of the face and neck, asthma attacks (Barbieri et al., 2019). *Morganella*, *Hafnia*, *Enterobacter*, *Photobacterium*, *Proteus*, *Pseudomonas* spp., former *Lactobacillus* and *Enterococcus* genera can produce BAs (Barbieri et al., 2019; Comas-Basté et al., 2019). Breaking down of nucleotides, especially ATP-related compounds, is also correlated with flavor deterioration of fishery products. The potential of microbial spoilage is primarily assessed by employing quality indices, such as total volatile basic nitrogen (TVB-N) content, BAs, volatile compounds and muscle degradation (K-value).

According to the FAO, 35 % of the fish harvested every year is either lost or wasted (FAO, 2022). Preserving fish meat means to keep its quality, texture and nutritional content (Maulu et al., 2020). A number of preservation strategies can be used to delay spoilage of fresh fish, increase the fish product shelf life, thus reducing waste of this crucial protein source. Those strategies affect the product temperature, pH and water activity to such an extent that the fish is unsuitable for microbial growth and undesired enzymatic reactions. Salting is a well-known technique for preserving fish. There are mainly three salting procedures: brining, where fish fillets are immersed into a solution of salt in water; dry salting, where the fish is in contact with the salt; mixed salting, which is dry salting performed in closed containers (Marchetti et al., 2021). In the dry salting the fish is dehydrated under the high osmotic pressure of salts.

Dry salting has been applied for centuries to store fresh fish, but nowadays is very popular especially among chefs of exclusive restaurants, because the addition of spices to the salt and the control of the process can drive specific fish fermentations enhancing the flavor and nutritional value of the product. The flavor of the cured fish is produced by the microbial metabolism of carbohydrates, the interaction of endogenous proteases and the fat degradation (Liu et al., 2023).

The use of different terms to indicate raw fish which have been cured, dried, fermented, aged using different natural or innovative process parameters selected according to the fish species, local recipe and consumer preference, make difficult to classify the derived products in specific food categories. The fish products tested in this study were cured, dried and aged. Therefore, they have been classified by the authors as dry-cured, according to the review of Liu et al., 2023 (Liu et al., 2023). The latter addressed the influence of microorganisms on the quality aspects of dry-cured fish, while we focused on the microbiological safety parameters because data are lacking while the market of these fish products is quickly rising, especially in restaurants and in small to medium food companies (Niland, 2019). In particular, in our study, we investigated the microbiological safety of dry-cured *Salmo salar* (salmon), *Xiphias gladius* (swordfish) and *Thunnus albacares* (yellowfin tuna), before, during, and at the end of a dry-curing process, performed in a dedicated cabinet named Stagionello Fish Curing Device®., at controlled temperature, relative humidity and ventilation, up to 240 h. At the end of the process, the dry-cured fish is consumed as ready to eat (RTE) product, without cooking.

In the Commission Regulation 2073/2005, process hygiene criteria for the food category dry-cured fish are not defined. Therefore, in the tested fish we investigated total aerobic colony count and Enterobacteriaceae, as proxy of process hygiene criteria. As far as food safety criteria are concerned, *Listeria monocytogenes* was considered relevant in terms of detection because dry-cured fish is a ready to eat product. Moreover, histamine, along with other biogenic amines was included as food safety criteria and tested in all fish species although the food safety criteria for histamine applies only to Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scombresosidae and was therefore relevant for tuna only. Besides the microbiological criteria listed above the microbial population associated with the tested products was assessed through shotgun metagenomic to map in each sample all the microorganisms belonging to different domains (e.g., bacteria, fungi, viruses, parasites, etc), including not cultivable microorganisms. Durazzi et al., 2021 (Durazzi et al., 2021) compared taxonomic results obtained by metataxonomic (i.e., 16S rRNA gene sequencing) and metagenomic (i.e., shotgun metagenomic sequencing) to investigate their reliability for bacteria profiling. The results showed that shotgun sequencing has more power to identify less abundant taxa than 16S sequencing. However, the low abundant taxa are important to detect, because they change between the experimental conditions, as the most abundant ones, detected by both sequencing strategies, and can drive important biological changes. In previous studies changes in the fish microbiome composition have been described according to the fish species (Chiarello et al., 2018; Givens et al., 2015; Larsen et al., 2013), stage of development (Hansen and Olafsen, 1999), sex (Dhanasiri et al., 2011), diet regimen (Parata et al., 2019), geographic location (Xavier et al., 2020), or captive state (Dhanasiri et al., 2011; Parata et al., 2019).

2. Materials and methods

2.1. Dry-curing processes, samples tested and sample collection

The fish species tested in this research include *Salmo salar* (salmon), *Xiphias gladius* (swordfish) and *Thunnus albacares* (yellowfin tuna). Salmon and yellowfin tuna were caught in the FAO zone 34 (i.e., Atlantic Ocean), while swordfish in the FAO zone 87 (i.e., Pacific Ocean). All fish batches were purchased from the same supplier located in Spain. Four eviscerated salmons, four cuts of tuna and four cuts of swordfish were shipped to the laboratory already thawed and stored at 0–4 °C. Fillets and cuts belonging to the same species were obtained from the same batch. The four salmons were filleted in the laboratory, while the tuna and swordfish cuts were used as such (Fig. 1). One fillet of salmon and one cut each of tuna and swordfish were immediately tested at the arrival in the laboratory, while the three remaining salmon fillets and 6 remaining tuna and swordfish cuts were dry-cured as detailed below. The samples immediately tested are described in the text as raw material. For each fish species, the raw material samples were represented by 3 subsamples of 10 g each for the enumeration of total aerobic colony count (TBC) and Enterobacteriaceae (ENT); 3 subsamples of 25 g each for the detection of *L. monocytogenes*; 3 subsamples of 10 g each for the measurement of superficial pH; 3 subsamples of 10 g each for the measurement of superficial water activity (aw). Moreover, two composite samples of 25 and 50 g each, obtained by 5 subsamples of 5 and 10 g each, were collected for shotgun metagenomic sequencing and histamine measurement, respectively.

The remaining three salmon fillets were submitted to a dry-curing process including different steps. Step 1 consisted of curing for 48 h at 2 °C, 85 % relative humidity (RH), and ventilation 3.4 m/s. The salting mixture contained salt, sucrose, dextrose, along with a mix of spices and antioxidants. At the end of this curing step the samples labelled as T0 were collected. Step 2 was dripping for 4 h at 24 °C, 80 % RH and ventilation of 3.4 m/s. At the end of this dripping step the samples labelled as T1 were collected. Step 3 was drying for 6 h at 19 °C, 51 % RH and ventilation of 3.4 m/s. At the end of this first drying step the samples labelled as T2 were collected. Step 4 was drying for 6 h at 14 °C, 54 % RH and ventilation of 3.4 m/s. At the end of this second drying step the samples labelled as T3 were collected. Step 5 was drying for 6 h at 11 °C, 57 % RH and ventilation of 3.4 m/s. At the end of this third drying step

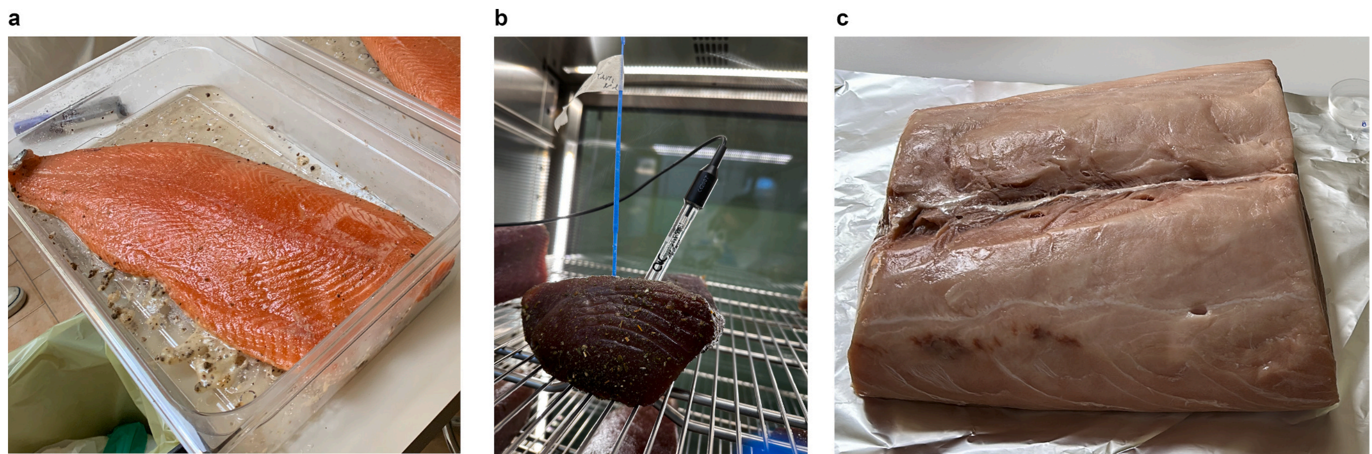


Fig. 1. Fish species tested in this research included *Salmo salar* (salmon) (a), *Thunnus albacares* (yellowfin tuna) (b) and *Xiphias gladius* (swordfish) (c).

the samples labelled as T4 were collected. Step 6 was drying for 12 h at 8 °C, 60 % RH and ventilation of 3.4 m/s. At the end of this fourth drying step the samples labelled as T5 were collected. Step 7 was drying for 12 h at 5 °C, 63 % RH and ventilation of 3.4 m/s. At the end of this fifth drying step the samples labelled as T6 were collected. Step 8 was aging for 40 h at 4 °C, 67 % RH and ventilation of 2.1 m/s. At the end of this first aging step the samples labelled as T7 were collected. Step 9 consisted of aging for 32 h at 4 °C, 60 % RH and ventilation of 2.1 m/s. At the end of this second aging step the samples labelled as T8 were collected. The dry-cured process for salmon lasted 166 h (Table 1).

The three swordfish and three tuna cuts were processed with a slightly different dry-curing process (Table 2). For step 1 the process parameters were set as for salmon, while few changes were made in composition and concentration of spices and antioxidants in the curing mixture. At the end of the first step the samples labelled as T0 were collected. Step 2 was dripping for 12 h at 18 °C, 78 % RH and ventilation of 3.4 m/s. At the end of this dripping step the samples labelled as T1 were collected. Step 3 was drying for 18 h at 15 °C, 55 % RH and ventilation of 3.4 m/s. At the end of this first drying step the samples labelled as T2 were collected. Step 4 was drying for 18 h at 15 °C, 58 % RH and ventilation of 3.4 m/s. At the end of this second drying step the samples labelled as T3 were collected. Step 5 consisted of 18 h at 15 °C, 51 % RH and ventilation of 3.4 m/s. At the end of this third drying step the samples labelled as T4 were collected. Step 5 was drying for 18 h at 10 °C, 54 % RH and ventilation of 3.4 m/s. At the end of this fourth drying step the samples labelled as T5 were collected. Step 6 consisted in drying for 18 h at 8 °C, 57 % RH and ventilation of 3.4 m/s. At the end of this fifth drying step the samples labelled as T6 were collected. Step 7 was the last drying step, lasting 18 h at 6 °C with 60 % RH. At the end of this sixth drying step the samples labelled as T7 were collected. Step 8 consisted of aging for 72 h, at 4 °C, 50 % RH and ventilation of 1.2 m/s. Overall, the dry-curing process for swordfish and tuna lasted 240 h.

The samples collected and analyzed in each fillet and cut at T0 until T8 were the same described above for the raw material. The only difference concerned the detection of *L. monocytogenes*, which was performed in the raw material only, and the composite samples for metagenomic sequencing and histamine determination, collected only at T0, T4 and T8. All the samples for the enumeration of TBC and ENT as well as pH and aw measurements were processed immediately after sampling. All the samples for shotgun metagenomic were flash frozen in liquid nitrogen at each sampling point and stored at −80 °C until DNA extraction. All the samples for histamine analysis were stored at −20 °C at each sampling point until further processing.

2.2. Microbiological culture methods and measurement of physico-chemical parameters

Total aerobic colony count (TBCs) and Enterobacteriaceae (ENT) were enumerated in samples of 10 g each following the international standard protocols 4833-2 (International Organization for Standardization (ISO), 2022) and 21528-2 (International Organization for Standardization (ISO), 2017a), respectively. The detection of *L. monocytogenes* was performed in samples of 25 g each using the ISO method 11290-1 (International Organization for Standardization (ISO), 2017b). The pH was measured in samples of 10 g using the ISO 2917 (International Organization for Standardization (ISO), 1999) and the pH meter Five Go (Mettler Toledo, Columbus, Ohio, USA). Finally, the water activity (a_w) was measured following the ISO 18787 ("International Organization for Standardization (ISO), 2017c - Foodstuffs - Determination of water activity") and the Aqualab CX 4-TE.

Biogenic amine content (including histamine) was determined according to Montanari et al. (2023) (Montanari et al., 2023). In particular, after a dansyl-chloride derivatization (Sigma-Aldrich, St Louis, USA), samples were injected into HPLC Agilent Technologies 1260 Infinity with the automatic injector (G1329B ALS 1260, loop of 20 μl), equipped with a C18 Waters Spherisorb ODS-2 (150 × 4.6 mm, 3 μm)

Table 1
Parameters of the dry curing process for salmon, step description and sampling points.

Step	Time (h)	Temperature (°C)	RH (%)	Ventilation (m/s)	Step description	Sampling point
1	48	2	85	3.4	Curing	T0
2	4	24	80	3.4	Dripping	T1
3	6	19	51	3.4	Drying	T2
4	6	14	54	3.4	Drying	T3
5	6	11	57	3.4	Drying	T4
6	12	8	60	3.4	Drying	T5
7	12	5	63	3.4	Drying	T6
8	40	4	67	2.1	Aging	T7
9	32	4	60	2.1	Aging	T8

Table 2
Parameters of the dry curing process for swordfish and tuna, step description and sampling points.

Step	Time (h)	Temperature (°C)	RH (%)	Ventilation (m/s)	Step description	Sampling point
1	48	2	85	3.4	Curing	T0
2	12	18	78	3.4	Dripping	T1
3	18	15	55	3.4	Drying	T2
4	18	15	58	3.4	Drying	T3
5	18	15	51	3.4	Drying	T4
6	18	10	54	3.4	Drying	T5
7	18	8	57	3.4	Drying	T6
8	18	6	60	3.4	Drying	T7
9	72	4	50	1.2	Aging	T8

column and a UV detector (G1314F VWD 1260) set at 254 nm, to confirm the BA production (histidine, tyramine, putrescine, cadaverine and 2-phenylethylamine). The BA amount was measured with reference to a calibration curve obtained through the injection of dansyl-chloride-derivatized BA standards. Under the adopted conditions, the detection limit for all biogenic amine was 3 mg/l. All the analyses were performed in triplicate.

2.3. DNA extraction and shotgun metagenomic sequencing

The 25 g of fish samples stored at −80 °C were thawed in the refrigerator for 4 h and diluted in 100 ml of sterile water for molecular biology, homogenized in the stomacher (MAYO HG 400 V, Italy) at normal speed for 1 min and centrifuged at 4 °C for 20 min at 9980 ×*g*. The DNA was extracted from the pellet using a bead-beating procedure followed by the PowerFood® Microbial DNA Isolation Kit (MO BIO-Qiagen). The DNA extracted from each sample was quantified using a BioSpectrometer® (Eppendorf, Milan, Italy) and then fragmented and tagged with sequencing indexes and adapters using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Shotgun metagenomic sequencing was performed using the NextSeq500 (Illumina) 2 × 150 bp in paired-end mode. All shotgun metagenomic sequences tested as part of this study were deposited in the SRA-NCBI data portal and are publicly available under the project named PRJNA1040009.

2.4. Bioinformatic and statistical analysis

Sequencing reads were trimmed using AdapterRemoval with the aim of removing sequencing adapters and stretches of low-quality bases. Cleaned reads were then aligned against the corresponding host genome (Ssal_v3.1 for salmon, GCF_016859285 for swordfish, GCF_914725855 for tuna) with bowtie2 algorithm. Unmapped reads were considered to run the kaiju pipeline adopted to determine the taxonomical abundance by comparing metagenomics reads to the db_nr_euk database. The kaiju pipeline is based on protein-level sequence comparison and the reference adopted includes the non-redundant set of proteins contained in the NCBI BLAST database, covering Archaea, bacteria and viruses, as well as fungi and microbial eukaryotes. Taxonomic profiles were reported as relative abundance, while the evaluation of bacteria species of interest was done by quantifying the abundances as fragment per kilobases of bacteria genome per million of reads (FPKM). The indices of Shannon Alpha diversity and Bray-Curtis Beta diversity were calculated adopting the R functions diversity and vegdist (package vegan) respectively; beta diversity was also showed as principal coordinate analysis (PCoA) that was implemented with the R function cmdscale (package stats). To evaluate the significance of differences between the taxa relative abundances between fish species, the One-way-ANOVA, followed by post hoc test (either Dunnet or Tukey HSD test), was performed using the R pakage rstatix.

3. Results

3.1. Microbiological and physico-chemical parameters in salmon, swordfish and tuna before and during the dry-cured processes

In salmon TBC in the raw material was 3.9 Log CFU/g and increased up to 5.4 Log CFU/g at the end of the process (T8) (Table 3). The highest TBC count was quantified at T7 and was statistically significative higher in comparison to the TBC enumerated in the raw material ($p = 0.04$) and at T0 ($p = 0.03$), T1 ($p = 0.02$) and T2 ($p = 0.03$).

In swordfish, the TBC in the raw material was 5.5 Log CFU/g and increased up to 5.9 Log CFU/g at the end of the process (T8) (Table 3). The TBC count at T1 was significantly lower in comparison to that at T0 ($p = 0.01$) as well as T2, T3, T5, T7 and T8 ($p < 0.00$). Moreover, the TBC count at T2 was significantly higher than those at T4 ($p = 0.00$) and T6 ($p = 0.03$). Finally, the TBC count at T8 was significantly higher than those at T4 ($p = 0.01$) and T6 ($p = 0.00$).

In tuna, the TBC count in the raw material was 4.4 Log CFU/g and increased up to 4.8 Log CFU/g at the end of the process (T8) (Table 3). The TBC count at T7 was significantly higher in comparison to that in the raw material ($p = 0.00$) as well as at T0, T1, T2, T3, T4 and T6 ($p < 0.00$). Moreover, the TBC count at T0 was significantly higher than that at T2 ($p = 0.01$); the TBC at T1 was significantly lower than that at T5 ($p = 0.04$); the TBC at T2 was significantly lower than those at T5 ($p < 0.00$), T6 ($p = 0.03$) and T8 ($p < 0.00$). Finally, the TBC count at T3 was significantly lower than that at T5 ($p = 0.03$).

In the raw material, the TBC count in swordfish was significantly higher than in salmon and tuna ($p < 0.00$ and $p = 0.01$, respectively). The same result was observed at T0 ($p < 0.00$ and $p = 0.01$), T2 ($p < 0.00$ for both) and T5 ($p < 0.00$ and $p = 0.01$). At T6 TBC in tuna was significantly lower than both TBC in salmon ($p < 0.00$) and swordfish ($p < 0.00$). The same result was observed at T8 ($p < 0.00$), when we detected also a significative difference between TBC counts in salmon and swordfish ($p < 0.00$) being the latter the most contaminated. All the tested samples showed Enterobacteriaceae counts lower than the quantification threshold of the cultural method, corresponding to 10 CFU/g.

The pH values measured on the surface of the raw material ranged between 5.58 in swordfish at T8 to 6.32 in tuna raw material (Table 4). The pH measured on the different fish species at each sampling point was significantly different except for T4. The a_w values measured on the different fish species tested as raw material, at T0, T5 and T7were not significantly different, while they showed statistically significative differences at T1 to T4, at T6 and T8 (Table 4).

3.2. Taxonomic profile of salmon, swordfish and tuna before and during the dry-curing processes

Shotgun metagenomic sequencing produced an output of 45.98 Giga bases corresponding to an average of 25.54×10^6 sequences per sample (min 15.78×10^6; max 47.01×10^6). The Kaiju analysis showed that in all samples the most represented domain was Bacteria (mean 79 %),

Table 3
Mean values (± standard deviation) of total aerobic colony count (TBC) and Enterobacteriaceae (ENT) in the fillets tested at different sampling points (SP) between raw material and the end of the dry-curing process (T8). *P* values <0.05 were considered statistically significant different in the comparison between fish species.

SP	TBC (log_{10} CFU/g)			p value	ENT (log_{10} CFU/g)		
	Salmon	Swordfish	Tuna		Salmon	Swordfish	Tuna
Raw	3.9 ± 0.3	5.5 ± 0.3	4.4 ± 0.31	<0.00	<1	<1	<1
T0	3.8 ± 0.4	5.6 ± 0.3	4.6 ± 0.35	<0.00	<1	<1	<1
T1	3.8 ± 0.5	5.0 ± 0.2	4.2 ± 0.15	0.01	<1	<1	<1
T2	3.8 ± 0.2	5.9 ± 0.1	3.8 ± 0.34	<0.00	<1	<1	<1
T3	4.1 ± 1.2	5.8 ± 0.1	4.2 ± 0.11	0.01	<1	<1	<1
T4	4.1 ± 1.0	5.3 ± 0.1	4.4 ± 0.35	0.13	<1	<1	<1
T5	4.7 ± 0.5	5.7 ± 0.2	4.9 ± 0.06	<0.00	<1	<1	<1
T6	5.4 ± 0.4	5.2 ± 0.1	4.5 ± 0.17	<0.00	<1	<1	<1
T7	5.6 ± 0.0	5.7 ± 0.1	5.5 ± 0.18	0.20	<1	<1	<1
T8	5.4 ± 0.1	5.9 ± 0.1	4.8 ± 0.10	<0.00	<1	<1	<1

Table 4
Mean values (± standard deviation) of pH and water activity (aw) in the fillets tested at different sampling points (SP) between raw material and the end of the curing process (T8). *P* values <0.05 were considered statistically significant different in the comparison between fish species.

SP	pH on the fish surface				a_w on the fish surface			
	Salmon	Swordfish	Tuna	P value	Salmon	Swordfish	Tuna	P value
Raw	6.19 ±0.11	5.90 ±0.03	6.32 ±0.14	0.00	0.970 ±0.001	0.983 ±0.007	0.979 ±0.012	0.21
T0	5.96 ±0.12	5.94 ±0.05	6.31 ±0.01	0.00	0.897 ±0.003	0.884 ±0.010	0.896 ±0.004	0.10
T1	5.88 ±0.03	5.96 ±0.03	5.91 ±0.00	0.02	0.893 ±0.006	0.896 ±0.004	0.926 ±0.010	0.00
T2	6.15 ±0.01	5.82 ±0.01	5.91 ±0.03	<<0.00	0.879 ±0.016	0.915 ±0.001	0.933 ±0.004	0.00
T3	6.07 ±0.12	5.76 ±0.15	5.96 ±0.02	0.03	0.899 ±0.014	0.927 ±0.007	0.944 ±0.011	0.00
T4	6.01 ±0.02	6.13 ±0.03	6.02 ±0.11	0.10	0.964 ±0.019	0.895 ±0.004	0.926 ±0.007	0.00
T5	6.21 ±0.03	5.85 ±0.05	5.97 ±0.06	0.00	0.920 ±0.006	0.908 ±0.004	0.906 ±0.013	0.19
T6	6.12 ±0.07	5.78 ±0.11	5.96 ±0.03	0.00	0.936 ±0.007	0.925 ±0.008	0.956 ±0.002	0.00
T7	6.10 ±0.09	5.84 ±0.04	6.04 ±0.03	0.00	0.939 ±0.027	0.944 ±0.017	0.942 ±0.008	0.94
T8	6.19 ±0.05	5.58 ±0.28	6.03 ±0.06	0.01	0.939 ±0.022	0.898 ±0.023	0.957 ±0.015	0.02

followed by Eukaryota (mean 5.4 %), Viruses (mean 0.5 %) and Archaea (mean 0.0 %). Overall, 15 % of sequences were unclassified.

Fig. 1 shows the top 20 phyla quantified in the raw material and at T0, T4 and T8 for each fish species in terms of relative abundance. The phylum Pseudomonadota was the most abundant in all tested samples (min 41.4 %; max 80.2 %; mean 55.2 %) followed by Bacillota (min 4.5 %; max 25.4 %; mean 17.9 %) and Mycoplasmatota (min 0.0 %; max 5.1 %; mean 2.4 %) (Fig. 2). The relative abundance of Bacillota was homogeneous among the three fish species ($p = 0.15$), while the phylum Pseudomonadota was significantly more abundant in swordfish ($p < 0.00$) and Mycoplasmatota in tuna ($p < 0.00$) (Fig. S1).

Fig. 3 shows the top 20 genera quantified in the raw material and at T0, T4 and T8 for each fish species in terms of relative abundance. The most abundant genera within the phylum Pseudomonadota were *Bradyrhizobium*, *Pantoea* and *Massilia* in salmon (18.4, 6.2 and 3.8 %) and tuna (26.2, 5.6 and 4.9 %), while they were represented by *Photobacterium* (53.5 %) and *Vibrio* (5.4 %) in swordfish. The most abundant genera within Bacillota were *Bacillus* and *Neobacillus* in salmon (8.8 and 4.9 %), *Bacillus* and *Enterococcus* in tuna (8.3 and 2.6 %), *Dellaglioa* and *Carnobacterium* in swordfish (4.3 and 4.4 %) (Fig. 3). Moreover, in tuna there was a significant increase of *Staphylococcus* at T8 (5%) mostly represented by the specie *S. equorum* and to a small percentage by *S. agnetis* (< 0.7 %) and *S. aureus* (< 0.3 %). *Mycoplasmopsis* was the only genus found within the phylum Mycoplasmatota which was primarily detected in salmon (2.6 %) and tuna (4.5 %).

The genera showing a relative abundance >0.1 % in at least one of the fish species were tested with the ANOVA analysis, followed by post hoc, in order to identify statistically significative differences between fish species (Table S1). As far as the top 20 genera are concerned *Enterococcus*, *Klebsiella*, *Phanerochaete*, *Tetrahymena*, *Pantoea*, *Lingulodinium* and *Methylocystis* were significantly more abundant in salmon in comparison to swordfish. *Neobacillus* was significantly higher in salmon in comparison to tuna. *Carnobacterium* was significantly higher in swordfish compared to salmon. *Mycoplasmopsis*, *Raoultella*, *Bradyrhizobium* and *Massilia* were significantly higher in tuna compared to swordfish. *Photobacterium* and *Vibrio* were significantly higher in swordfish in comparison to tuna. Finally, the relative abundance of *Bacillus* was significantly higher in salmon and tuna in comparison to swordfish (Table S1).

Our data showed clear differences among the three fish species-. This observation is formally highlighted by the beta-diversity analysis, in which three well separated clusters, corresponding to the different fish species, were identified (Fig. 4). The Shannon's diversity index, showing the microbial community richness, was significantly different among the three tested fish species (ANOVA $p < 0.00$) with some fluctuation during the time series (Fig. 5). The difference in terms of alpha diversity was particularly significant in the comparison salmon vs swordfish ($p < 0.00$) and swordfish vs tuna ($p = 0.03$).

3.3. Biological hazards in salmon, swordfish and tuna before and during the dry-cured processes

The dry-aged fish is mostly consumed as ready to eat (RTE) product, without cooking. Therefore, according to Commission Regulation 2073/2005, the food safety criteria for dry-aged fish are detection of *L. monocytogenes* and concentration of histamine. *L. monocytogenes* was not detected in three samples of raw material of 25 g each tested for each fish species by using the culture method. Sequences of *L. monocytogenes* were not detected in salmon using shotgun metagenomics, while very few (relative abundance <0.007) were quantified in swordfish and tuna. Any read of *L. monocytogenes* was counted in swordfish the raw material, while few of them were detected at T0 but decreased upon T8. In tuna *L. monocytogenes* was detected in the raw material, increased upon T4 to then decrease until T8 (Fig. S2).

Histamine and other biogenic amines were not quantified (<3 mg/kg) in the samples analyzed. Nonetheless, in the metagenomic dataset we looked for histamine producing bacteria (Fig. 6) such as *Morganella psychrotolerans* and *Photobacterium* spp. (Emborg and Dalgaard, 2008;

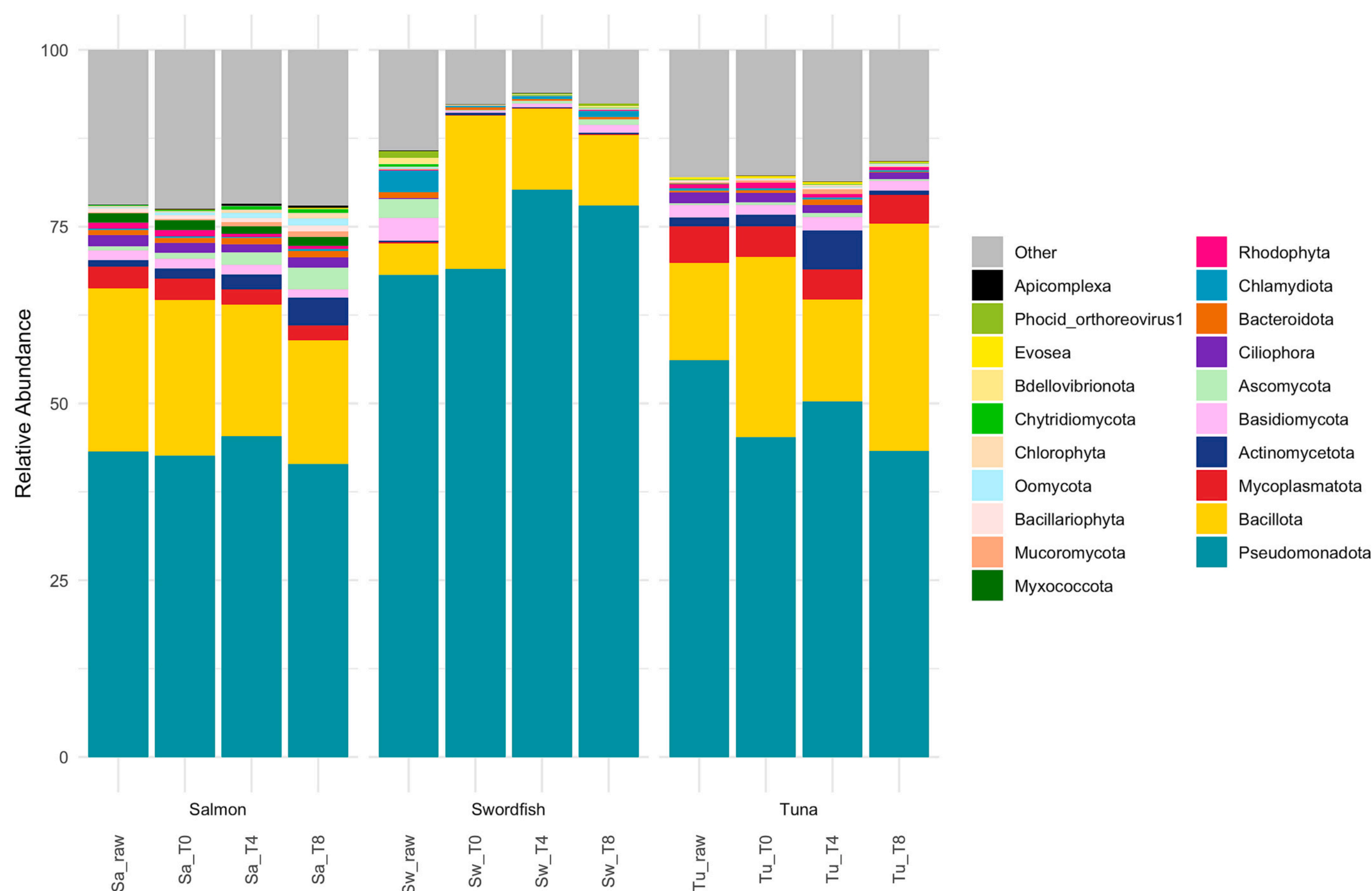


Fig. 2. Barplots showing the relative abundance of the top 20 most abundant phyla identified in salmon, swordfish and tuna testing raw fish and samples collected at the sampling points T0, T4 and T8.

Kanki et al., 2007). Reads of *Klebsiella pneumoniae* were observed in all tested samples at all sampling points, while *P. phosphoreum* was detected only in salmon and swordfish, with the highest abundance, as calculated by the FPKM parameter, in swordfish at T0. Swordfish cuts appeared the richest in terms of histamine producing bacteria. For some of them (e.g., *Vibrio palustris*, *Vibrio vulnificus*) the abundance seemed to decrease between T0 and T8, while for others (e.g., *Photobacterium damselae*, *Vibrio nigripulchritudo*) was very stable over time. The highest abundance of histamine producing bacteria was detected for *V. fluvialis* at T0, and *P. phosporeum* and *P. kishithanii* at the other sampling points.

Besides the selected food safety criteria, additional genera, including human foodborne hazards, detected using shotgun metagenomic sequencing, with relative abundance values >1 % in the tested samples included *Enterococcus* and *Pseudomonas* in salmon; *Klebsiella* and *Bacillus* in salmon and tuna; *Vibrio*, *Shewanella* and *Acinetobacter* in swordfish; *Staphylococcus* in tuna.

4. Discussion

In this study we investigated the microbiological safety of different types of dry-cured fishes, from the raw material to the end of processing, lasting 166 h for salmon and 240 h for swordfish and tuna. In the Commission Regulation 2073/2005, process hygiene criteria for the food category dry-cured fish are missing; therefore, we enumerated total aerobic colony count and Enterobacteriaceae as proxy of these criteria. On the contrary, the food safety criteria for ready to eat foods apply also to dry-cured fish and are detection of *L. monocytogenes* and concentration of histamine. These food safety criteria were investigated by targeted approaches, as microbiological culture methods and HPLC (for histamine), and by an untargeted strategy, represented by shotgun metagenomic.

All the tested samples showed Enterobacteriaceae counts lower than the quantification threshold of the cultural method, corresponding to 10 CFU/g. On the contrary, aerobic colony counts were always higher than 3 Log CFU/g in all the tested samples. In the raw material TBC were significantly higher in swordfish than in salmon and tuna. This result can be due to differences in the microbiome of the different species and to the impact of the operations before the fish purchase and transport to the laboratory. Indeed, the salmon was purchased eviscerated and fileted in our laboratory, while for swordfish and tuna were purchased the cuts already manipulated elsewhere. Aerobic colony counts increased in all fish species during the process. The highest TBC counts were enumerated at T7 with any statistical significative difference between species. This result can be explained by the time/temperature conditions applied in the processing steps before this specific sampling point (Table 1).

Besides cultural methods to investigate specific microbiological hazards we applied shotgun metagenomics to map additional microorganisms in the tested samples. In the studies reported in the literature, investigating the diversity and activity of fish spoilage microorganisms in seafood products using culture-based approaches (Boziaris and Parlapani, 2017; Gram and Dalgaard, 2002; Gram and Huss, 1996), the spoilage microorganisms identified included *Pseudomonas*, *Psychrobacter*, *Lactobacillus*, and other lactic acid bacteria (LAB), *Shewanella*, *Photobacterium*, and *Aeromonas*. In our study, performed by shotgun metagenomic, Pseudomonadota and Bacillota, formerly classified as Proteobacteria and Firmicutes, were the prevalent phyla. The same result was reported by other authors in salted and dried fish products (Wang et al., 2021). *Bradyrhizobium* was the prevalent genus identified in salmon and tuna, both originating from the Atlantic Ocean, and persisted during processing. In the scientific literature *Bradyrhizobium* is

Fig. 3. Barplots showing the relative abundance of the top 20 most abundant genera identified in salmon, swordfish and tuna testing raw fish and samples collected at the sampling points T0, T4 and T8.

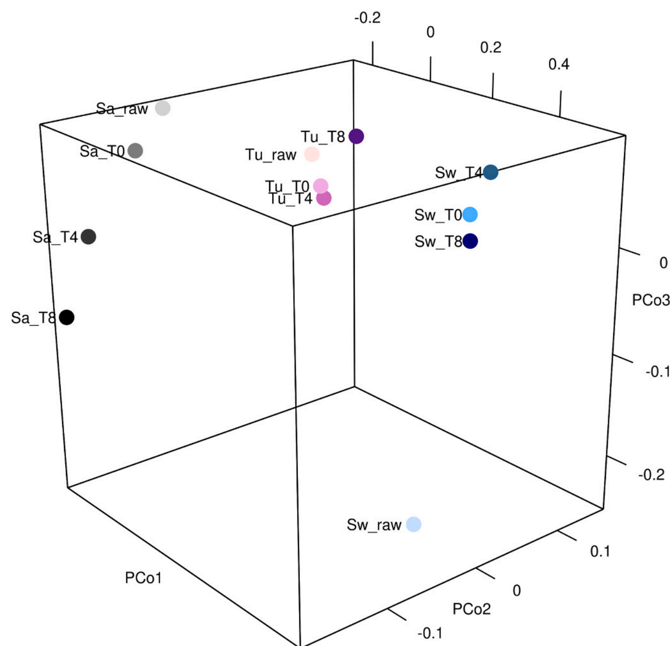


Fig. 4. Principal coordinate 3D projection of distances between genera identified in salmon, swordfish and tuna testing raw fish (Sa_raw, Sw_raw, Tu_raw) and samples collected at the sampling points T0 (Sa_T0, Sw_T0, Tu_T0), T4 (Sa_T4, Sw_T4, Tu_T4) and T8 (Sa_T8, Sw_T8, Tu_T8) computed by Bray-Curtis beta diversity analysis.

not described as fish spoilage bacteria but it was isolated in the gut of some fish species. Back in 2015, McDonald et al. (McDonald et al., 2015) showed that fish gastrointestinal tract can provide a suitable environment for nitrogen fixation that may facilitate production of reduced nitrogen by the resident microbial population, including *Bradyrhizobium.* Whether this community provides reduced nitrogen to the host in an active or passive manner, and whether it is present in a permanent or transient relationship, remains to be determined (McDonald et al., 2015).

Photobacterium was the prevalent genus in samples of raw swordfish and increased during the curing process. A specific association between swordfish and *Photobacterium* is not reported in the literature, while *Photobacterium* has been identified as prevalent genus in liver and gut of tuna (Gadoin et al., 2021). *Photobacterium* is ubiquitous in marine environments and includes different species (Thyssen et al., 2005). It is a psychrotrophic microorganism and therefore can survive in cold stored foods, including fish and fish products (Betts, 2006). *Photobacterium* is facultative anaerobic (Bjørkevoll et al., 2003). Moreover, it is halotolerant and has been used as starter for fish sauce fermentation (Zheng et al., 2017). *Photobacterium* can be a fish commensal microorganism (Bjornsdottir-Butler et al., 2015; Egerton et al., 2018), but includes also species, as *P. damselae* and *P. piscicida*, which are pathogenic for both fish and humans (Rivas et al., 2013).

Besides the presence of common genera in the tested fish samples, before and during processing, the beta diversity analysis showed a clear separation between the microbial composition of the different samples, which is likely associated to the fact that the fish species were different as well as the catching and slaughtering areas. The lower Shannon index associated to the swordfish samples means that the number of different microbial species in those samples was lower in comparison to that of

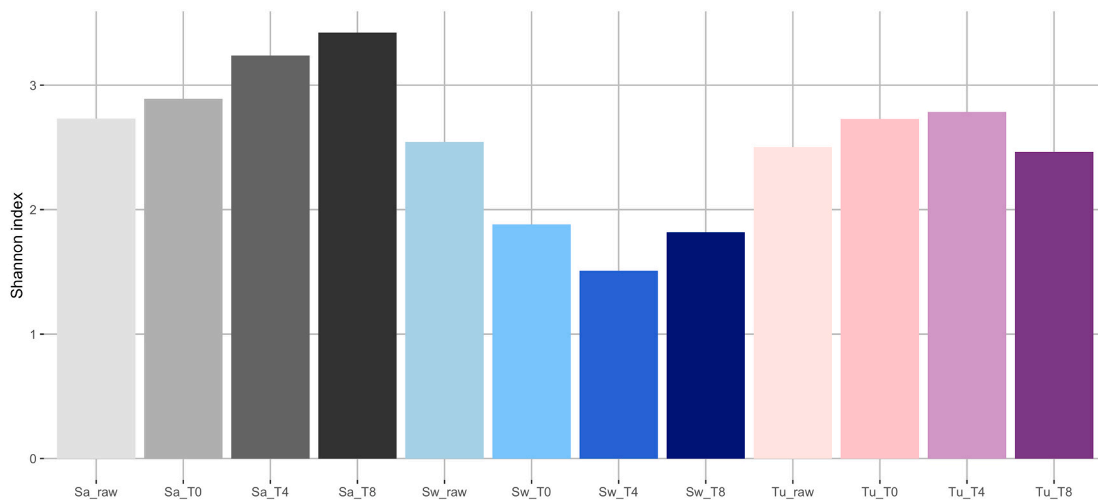


Fig. 5. Alpha-diversity indexes calculated for the genera with the Shannon's diversity index in salmon, swordfish and tuna testing raw fish (Sa_raw, Sw_raw, Tu_raw) and samples collected at the sampling points T0 (Sa_T0, Sw_T0, Tu_T0), T4 (Sa_T4, Sw_T4, Tu_T4) and T8 (Sa_T8, Sw_T8, Tu_T8).

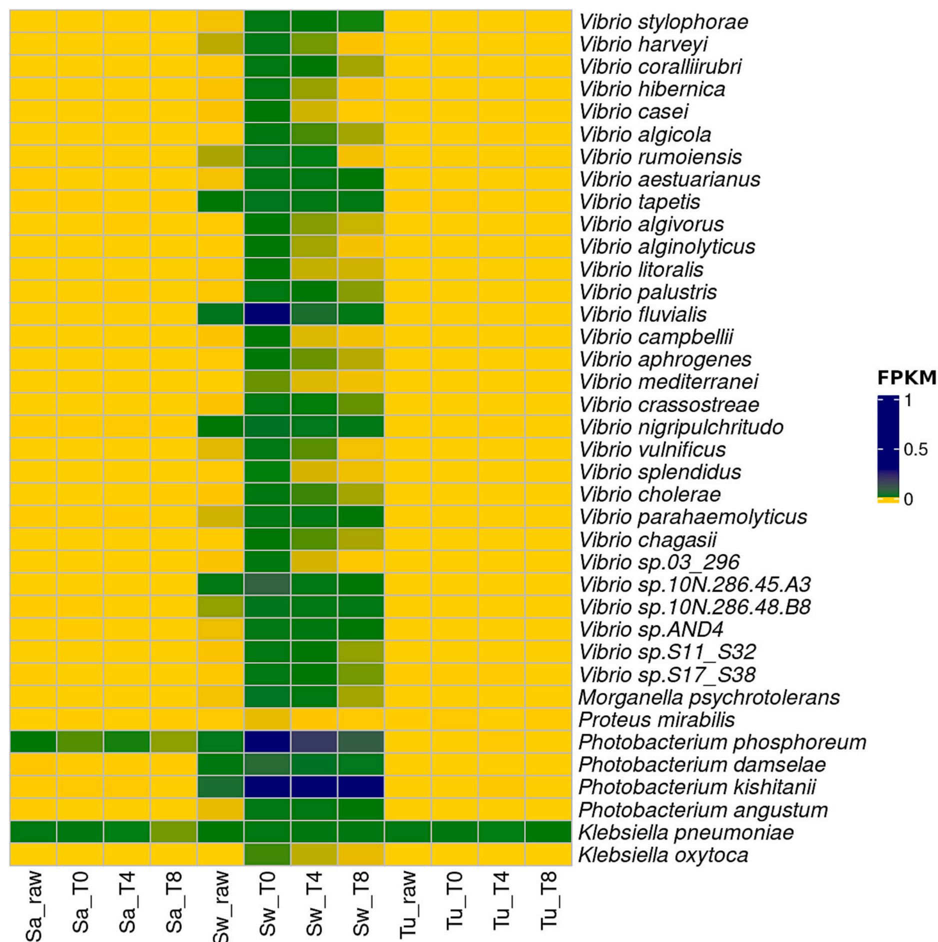


Fig. 6. Abundance of histamine producing bacteria (FPKM) in salmon raw material (Sa_raw) and at different sampling points (Sa_T0, Sa_T4, Sa_T8); in swordfish raw material (Sw_raw) and at different sampling points (Sw_T0, Sw_T4, Sw_T8); in tuna raw material (Tu_raw) and at different sampling points (Tu_T0, Tu_T4, Tu_T8).

salmon and tuna. This result is explained by the high relative abundance of *Photobacterium* in swordfish (Fig. 2).

Staphylococcus was not detected in salmon or swordfish, while in tuna started to increase at T4 and significantly raised at T8. In cured fish *Staphylococcus* can be added as starter culture given its ability to promote the formation of flavor and delay of lipid oxidation (Zhao et al., 2022). The most abundant *Staphylococcus* species identified in our study was *S. equorum* isolated also in Korean high-salt-fermented seafood products (Lee et al., 2018) as well as in high-salt fermented meat products and cheeses in Europe (Blaiotta et al., 2004; Bockelmann et al., 2005; Kastman et al., 2016). The genomic characterization of *S. equorum* strains highlighted that these microorganisms are able to produce

acetoin, butanediol and branched chain fatty acid. Moreover, they seem to be able to produce bacteriocin in vitro. Nonetheless, none of the genes needed for histamine and tyramine production were identified in the characterized isolates (Lee et al., 2018).

L. monocytogenes was investigated in the raw material using the qualitative ISO method, including two enrichment steps, but was not detected in three samples of 25 g each. In 2021, fish and fish products were the fourth most frequently reported vehicle in strong-evidence foodborne outbreaks in the EU, leading to 190 human cases, 41 hospitalizations and four deaths. All the deaths occurred in the Netherlands and were due to listeriosis linked to the consumption of smoked fishes (i.e., smoked salmon, eel and mackerel) (European Food Safety Authority and European Centre for Disease Prevention and Control, 2022). Using shotgun metagenomic very few reads of *L. monocytogens* were detected in swordfish and tuna, while they were completely lacking in salmon. This result can be explained considering that, in contrast to salmon, swordfish and tuna were cut and manipulated before freezing and shipping to our laboratory. Although *L. monocytogenes* was not an issue in our study because was not identified in the raw material, it is a ubiquitous microorganism able to grow at low temperatures and to persist in the food production environments (Fagerlund et al., 2022). Persistence of L. *monocytogenes* in food processing environments is considered to be the major source of RTE food contamination (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2018), due to both improper hygiene conditions but also to the ability of this foodborne pathogen to adapt to environmental stressors, also forming biofilms. This problem can be limited by producing RTE fish in close systems as the cabinets applied in this study, where the cleaning and disinfection procedures are more effective in comparison to those applied at larger scales in both industrial and artisanal settings.

Besides *L. monocytogenes*, in 2021 the highest number (i.e., 14) of foodborne outbreaks associated to the food category fish and fishery products was due to histamine and scombrotoxin. In our samples histamine was not detected by HPLC. However, reads of different histamine producing species, belonging to the genera *Vibrio*, *Morganella*, *Proteus*, *Photobacterium* and *Klebsiella* were detected by shotgun metagenomic sequencing (Fig. 6, Table 1S). The relative abundance of the *Vibrio* genus in swordfish was 5.4 % and included >30 species. *Vibrio* spp. are Gram-negative, rod-shaped and mostly halophilic bacteria found in aquatic ecosystems, such as oceans, estuaries and aquaculture (Thompson et al., 2004). The *Vibrio* species causing foodborne human diseases due to the consumption of raw or partially cooked fish, primarily shellfish, are *V. vulnificus*, *V. cholerae* and *V. parahaemolyticus* (Tsironi et al., 2017). Human outbreaks caused by *V. vulnificus* and *V. parahaemolyticus* have been reported in different countries of the Mediterranean area (Lozano-LeÃn et al., 2003; Ottaviani et al., 2013). Although the occurrence of *Vibrio* is mostly relevant in shellfish, the presence of *Vibrio* in cured fish should be further investigated, because a positive correlation between water temperature and *Vibrio* concentrations has been demonstrated in several regions around the world (https://mobil.bfr.bund.de/cm/349/bacterial-foodborne-vibrio-infections-health-risk-assessment-of-the-occurrence-of-vibrio-spp-in-food.pdf).

A classification of histamine producing bacteria has been suggested based on the amount of histamine they produce at 15 °C for 48 h in tuna fish infusion broth or in trypticase soy broth containing 1 to 2 % histidine (Oktariani et al., 2022). Following this classification, the high histamine producing bacteria are those producing >1000 mg/l, while the low histamine producing bacteria are those producing <500 mg/l (Oktariani et al., 2022). Among the bacteria identified in our study, *M. psychrotolerans*, *K. pneumoniae*, *K. oxytoca*, *P. damselae*, *P. kishitanii*, and *P. angustum* were all classified as high histamine producing bacteria, while *Vibrio alginolyticus* as low histamine producing bacteria (Oktariani et al., 2022). Within the genus *Photobacterium*, the species known as histamine producers are *P. aquimaris*, *P. angustum*, *P. kishitanii*, *P. damselae*, and *P. phosphoreum* and they were all identified in our samples, except for *P. aquimaris* (Fig. 6). *P. phosphoreum* was identified in salmon and swordfish at all sampling points while the other species primarily in swordfish. According to the literature *Photobacterium* was identified as histamine producer in Atlantic cod (Kuuliala et al., 2018), haddock (Reynisson et al., 2010) and Atlantic salmon (Jääskeläinen et al., 2019).

One important limitation of this study is the limited number of sample replicates investigated, especially for the metagenomic analysis. Testing multiple samples would have provided a better estimation of the variability in the microbiome. All in all, the results of this study showed that that the dry-curing process under controlled time, temperature, relative humidity and ventilation parameters in a close cabinet does not negatively affect the microbiological safety of different types of fish species. To the best of our knowledge this is the first paper detailing time point determinations of microbiological safety parameters in salmon, swordfish and tuna before, during and at the end of a dry-curing process. The data collected in this paper can help to predict the risk profile of dry-cured fish during storage before consumption. However, challenge studies are needed to assess the growth potential of foodborne pathogens in dry-cured fish during processing and storage at refrigeration temperature.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Valentina Indio: Writing – original draft, Formal analysis, Data curation. **Fausto Gardini:** Writing – review & editing, Data curation. **Laura Prandini:** Investigation, Data curation. **Yitagele Terefe Mekonnen:** Investigation. **Federico Tomasello:** Investigation, Data curation. **Federica Giacometti:** Investigation, Data curation. **Alessandro Seguino:** Writing – review & editing. **Andrea Serraino:** Writing – review & editing, Methodology, Conceptualization. **Alessandra De Cesare:** Writing – original draft, Investigation, Data curation.

Declaration of competing interest

None of the authors declare to have financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110641.

References

Barbieri, F., Montanari, C., Gardini, F., Tabanelli, G., 2019. Biogenic amine production by lactic acid bacteria: a review. Foods 8, 17. https://doi.org/10.3390/foods8010017.

Betts, G., 2006. Other spoilage bacteria. In: Food Spoilage Microorganisms. Elsevier, pp. 668–693. https://doi.org/10.1533/9781845691417.5.668.

Bjørkevoll, I., Olsen, R.L., Skjerdal, O.T., 2003. Origin and spoilage potential of the microbiota dominating genus Psychrobacter in sterile rehydrated salt-cured and dried salt-cured cod (Gadus morhua). Int. J. Food Microbiol. 84, 175–187. https://doi.org/10.1016/S0168-1605(02)00418-X.

Bjornsdottir-Butler, K., Bowers, J.C., Benner, R.A., 2015. Prevalence and characterization of high histamine-producing bacteria in Gulf of Mexico fish species. J. Food Prot. 78, 1335–1342. https://doi.org/10.4315/0362-028X.JFP-15-012.

Blaiotta, G., Pennacchia, C., Villani, F., Ricciardi, A., Tofalo, R., Parente, E., 2004. Diversity and dynamics of communities of coagulase-negative staphylococci in

traditional fermented sausages. J. Appl. Microbiol. 97, 271–284. https://doi.org/10.1111/j.1365-2672.2004.02298.x.
Bockelmann, W., Willems, K.P., Neve, H., Heller, K.H., 2005. Cultures for the ripening of smear cheeses. International Dairy Journal, The Fourth IDF Symposium on Cheese: Ripening, Characterization and Technology 15, 719–732. https://doi.org/10.1016/j.idairyj.2004.08.022.
Boziaris, I.S., Parlapani, F.F., 2017. Chapter 3 - specific spoilage organisms (SSOs) in fish. In: Bevilacqua, A., Corbo, M.R., Sinigaglia, M. (Eds.), The Microbiological Quality of Food, Woodhead Publishing Series in Food Science. Technology and Nutrition. Woodhead Publishing, pp. 61–98. https://doi.org/10.1016/B978-0-08-100502-6.00006-6.
Chiarello, M., Auguet, J.-C., Bettarel, Y., Bouvier, C., Claverie, T., Graham, N.A.J., Rieuvilleneuve, F., Sucré, E., Bouvier, T., Villéger, S., 2018. Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. Microbiome 6, 147. https://doi.org/10.1186/s40168-018-0530-4.
Comas-Basté, O., Luz Latorre-Moratalla, M., Sánchez-Pérez, S., Teresa Veciana-Nogués, M., Del Carmen Vidal-Carou, M., 2019. Histamine and other biogenic amines in food. From scombroid poisoning to histamine intolerance. In: Proestos, C. (Ed.), Biogenic Amines. IntechOpen. https://doi.org/10.5772/intechopen.84333.
Dhanasiri, A.K.S., Brunvold, L., Brinchmann, M.F., Korsnes, K., Bergh, Ø., Kiron, V., 2011. Changes in the intestinal microbiota of wild Atlantic cod Gadus morhua L. upon captive rearing. Microb. Ecol. 61, 20–30. https://doi.org/10.1007/s00248-010-9673-y.
Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., De Cesare, A., 2021. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. Sci. Rep. 11, 3030. https://doi.org/10.1038/s41598-021-82726-y.
EFSA Panel on Biological Hazards (BIOHAZ), Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P.S., Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Takkinen, J., Wagner, M., Arcella, D., Da Silva Felicio, M.T., Georgiadis, M., Messens, W., Lindqvist, R., 2018. Listeria monocytogenes contamination of ready-to-eat foods and the risk for human health in the EU. EFS2 16. https://doi.org/10.2903/j.efsa.2018.5134.
Egerton, S., Culloty, S., Whooley, J., Stanton, C., Ross, R.P., 2018. The gut microbiota of marine fish. Front. Microbiol. 9, 873. https://doi.org/10.3389/fmicb.2018.00873.
Emborg, J., Dalgaard, P., 2008. Modelling the effect of temperature, carbon dioxide, water activity and pH on growth and histamine formation by Morganella psychrotolerans. Int. J. Food Microbiol. 128, 226–233. https://doi.org/10.1016/j.ijfoodmicro.2008.08.016.
European Food Safety Authority, European Centre for Disease Prevention and Control, 2022. The European Union One Health 2021 Zoonoses Report, 20. EFS2. https://doi.org/10.2903/j.efsa.2022.7666.
Fagerlund, A., Wagner, E., Møretrø, T., Heir, E., Moen, B., Rychli, K., Langsrud, S., 2022. Pervasive *Listeria monocytogenes* is common in the Norwegian food system and is associated with increased prevalence of stress survival and resistance determinants. Appl. Environ. Microbiol. 88, e00861-22 https://doi.org/10.1128/aem.00861-22.
FAO, 2022. The State of World Fisheries and Aquaculture 2022. FAO. https://doi.org/10.4060/cc0461en.
Gadoin, E., Desnues, C., Bouvier, T., Roque d'Orbcastel, E., Auguet, J.-C., Crochemore, S., Adingra, A., Bettarel, Y., 2021. Tracking spoilage Bacteria in the tuna Necrobiome. SSRN Journal. doi:https://doi.org/10.2139/ssrn.3985243.
Givens, C., Ransom, B., Bano, N., Hollibaugh, J., 2015. Comparison of the gut microbiomes of 12 bony fish and 3 shark species. Mar. Ecol. Prog. Ser. 518, 209–223. https://doi.org/10.3354/meps11034.
Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria – problems and solutions. Curr. Opin. Biotechnol. 13, 262–266. https://doi.org/10.1016/S0958-1669(02)00309-9.
Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology, Specific Spoilage Organisms 33, 121–137. https://doi.org/10.1016/0168-1605(96)01134-8.
Hansen, G.H., Olafsen, J.A., 1999. Bacterial interactions in early life stages of marine cold water fish. Microb. Ecol. 38, 1–26. https://doi.org/10.1007/s002489900158.
International Organization for Standardization (ISO), 1999. Meat and Meat Products — Measurement of pH — Reference Method. ISO 2917:1999.
International Organization for Standardization (ISO), 2017a. Microbiology of the Food Chain - Horizontal Method for the Detection and Enumeration of Enterobacteriaceae - Part 2: Colony Count Technique. ISO 21528-2:2017a.
International Organization for Standardization (ISO), 2017b. Microbiology of the Food Chain – Horizontal Method for the Detection and Enumeration of Listeria monocytogenes and of Listeria spp. – Part 1: Detection Method. ISO 11290-1:2017b.
International Organization for Standardization (ISO), 2017c. Foodstuffs - Determination of Water Activity. ISO.pdf n.d.
International Organization for Standardization (ISO), 2022. Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. In: Part 2, Colony Count at 30 °C by the Surface Plating Technique. ISO 4833-2:2013+A1:2022.
Jääskeläinen, E., Jakobsen, L.M.A., Hultman, J., Eggers, N., Bertram, H.C., Björkroth, J., 2019. Metabolomics and bacterial diversity of packaged yellowfin tuna (Thunnus albacares) and salmon (Salmo salar) show fish species-specific spoilage development during chilled storage. Int. J. Food Microbiol. 293, 44–52. https://doi.org/10.1016/j.ijfoodmicro.2018.12.021.
Kanki, M., Yoda, T., Tsukamoto, T., Baba, E., 2007. Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. Appl. Environ. Microbiol. 73, 1467–1473. https://doi.org/10.1128/AEM.01907-06.
Kastman, E., Kamelamela, N., Norville, J., Cosetta, C., Dutton, R., Wolfe, B., 2016. Biotic interactions shape the ecological distributions of Staphylococcus species. MBio 7. https://doi.org/10.1128/mBio.01157-16.
Kuuliala, L., Al Hage, Y., Ioannidis, A.-G., Sader, M., Kerckhof, F.-M., Vanderroost, M., Boon, N., De Baets, B., De Meulenaer, B., Ragaert, P., Devlieghere, F., 2018. Microbiological, chemical and sensory spoilage analysis of raw Atlantic cod (Gadus morhua) stored under modified atmospheres. Food Microbiol. 70, 232–244. https://doi.org/10.1016/j.fm.2017.10.011.
Larsen, A., Tao, Z., Bullard, S.A., Arias, C.R., 2013. Diversity of the skin microbiota of fishes: evidence for host species specificity. FEMS Microbiol. Ecol. 85, 483–494. https://doi.org/10.1111/1574-6941.12136.
Lee, J.-H., Heo, S., Jeong, D.-W., 2018. Genomic insights into Staphylococcus equorum KS1039 as a potential starter culture for the fermentation of high-salt foods. BMC Genomics 19, 136. https://doi.org/10.1186/s12864-018-4532-1.
Liu, J., Mai, R., Liu, P., Guo, S., Yang, J., Bai, W., 2023. Flavor formation in dry-cured fish: regulation by microbial communities and endogenous enzymes. Foods 12, 3020. https://doi.org/10.3390/foods12163020.
Lozano-LeÃn, A., Torres, J., Osorio, C.R., Martãìnez-Urtaza, J., 2003. Identification of *tdh* -positive *Vibrio parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. FEMS Microbiol. Lett. 226, 281–284. https://doi.org/10.1016/S0378-1097(03)00604-9.
Marchetti, M.D., Gomez, P.L., Yeannes, M.I., Garcia Loredo, A.B., 2021. Effect of salting procedures on quality of hake (Merluccius hubbsi) fillets. Heliyon 7, e07703. https://doi.org/10.1016/j.heliyon.2021.e07703.
Maulu, S., Hasimuna, O., Concilia, M., Mweemba, M., 2020. An assessment of post-harvest fish losses and preservation practices in Siavonga district, Southern Zambia. Fish. Aquat. Sci. 23 https://doi.org/10.1186/s41240-020-00170-x.
McDonald, R., Zhang, F., Watts, J.E.M., Schreier, H.J., 2015. Nitrogenase diversity and activity in the gastrointestinal tract of the wood-eating catfish Panaque nigrolineatus. ISME J. 9, 2712–2724. https://doi.org/10.1038/ismej.2015.65.
Montanari, C., Barbieri, F., Lorenzini, S., Gottardi, D., Šimat, V., Özogul, F., Gardini, F., Tabanelli, G., 2023. Survival, growth, and biogenic amine production of Enterococcus faecium FC12 in response to extracts and essential oils of Rubus fruticosus and Juniperus oxycedrus. Front. Nutr. 9, 1092172. https://doi.org/10.3389/fnut.2022.1092172.
Niland, J., 2019. The Whole Fish Cookbook. Hardie Grant Books.
Oktariani, A.F., Ramona, Y., Sudaryatma, P.E., Dewi, I.A.M.M., Shetty, K., 2022. Role of marine bacterial contaminants in histamine formation in seafood products: a review. Microorganisms 10, 1197. https://doi.org/10.3390/microorganisms10061197.
Ottaviani, D., Leoni, F., Rocchegiani, E., Mioni, R., Costa, A., Virgilio, S., Serracca, L., Bove, D., Canonico, C., Di Cesare, A., Masini, L., Potenziani, S., Caburlotto, G., Ghidini, V., Lleo, M.M., 2013. An extensive investigation into the prevalence and the genetic and serological diversity of toxigenic *Vibrio parahaemolyticus* in Italian marine coastal waters. Environ. Microbiol. 15, 1377–1386. https://doi.org/10.1111/j.1462-2920.2012.02839.x.
Parata, L., Nielsen, S., Xing, X., Thomas, T., Egan, S., Vergés, A., 2019. Age, gut location and diet impact the gut microbiome of a tropical herbivorous surgeonfish. FEMS Microbiol. Ecol. fiz179 https://doi.org/10.1093/femsec/fiz179.
Reynisson, E., Lauzon, H.L., Thorvaldsson, L., Margeirsson, B., Rúnarsson, Á.R., Þór Marteinsson, V., Martinsdóttir, E., 2010. Effects of different cooling techniques on bacterial succession and other spoilage indicators during storage of whole, gutted haddock (Melanogrammus aeglefinus). Eur. Food Res. Technol. 231, 237–246. https://doi.org/10.1007/s00217-010-1273-z.
Rivas, A.J., Lemos, M.L., Osorio, C.R., 2013. Photobacterium damselae subsp. damselae, a bacterium pathogenic for marine animals and humans. Front. Microbiol. 4 https://doi.org/10.3389/fmicb.2013.00283.
Thompson, J.R., Randa, M.A., Marcelino, L.A., Tomita-Mitchell, A., Lim, E., Polz, M.F., 2004. Diversity and dynamics of a North Atlantic coastal *Vibrio* community. Appl. Environ. Microbiol. 70, 4103–4110. https://doi.org/10.1128/AEM.70.7.4103-4110.2004.
Thyssen, A., Ollevier, F., Brenner, D., Krieg, N., Staley, J., Garrity, G., 2005. Genus II. In: Photobacterium Beijerinck 1889, 401AL. Springer.
Tsironi, T., Ronnow, P., Giannoglou, M., Taoukis, P., 2017. Developing suitable smart TTI labels to match specific monitoring requirements: the case of Vibrio spp. growth during transportation of oysters. In: Food Control, Special issues from the '29th EFFoST International Conference', 73, pp. 51–56. https://doi.org/10.1016/j.foodcont.2016.06.041.
Visciano, P., Schirone, M., Tofalo, R., Suzzi, G., 2014. Histamine poisoning and control measures in fish and fishery products. Front. Microbiol. 5, 500. https://doi.org/10.3389/fmicb.2014.00500.
Wang, Y., Shen, Y., Wu, Y., Li, C., Li, L., Zhao, Y., Hu, X., Wei, Y., Huang, H., 2021. Comparison of the microbial community and flavor compounds in fermented mandarin fish (Siniperca chuatsi): three typical types of Chinese fermented mandarin fish products. Food Res. Int. 144, 110365 https://doi.org/10.1016/j.foodres.2021.110365.
Xavier, R., Pereira, A., Pagan, A., Hendrick, G., Nicholson, M., Rosado, D., Soares, M., Perez-Losada, M., 2020. The effects of environment and ontogeny on the skin microbiome of two Stegastes damselfishes (Pomacentridae) from the eastern Caribbean Sea. Mar. Biol. 167 https://doi.org/10.1007/s00227-020-03717-7.
Zhao, D., Hu, J., Chen, W., 2022. Analysis of the relationship between microorganisms and flavour development in dry-cured grass carp by high-throughput sequencing, volatile flavour analysis and metabolomics. Food Chem. 368, 130889 https://doi.org/10.1016/j.foodchem.2021.130889.
Zheng, B., Liu, Y., He, X., Hu, S., Li, S., Chen, M., Jiang, W., 2017. Quality improvement on half-fin anchovy (*Setipinna taty*) fish sauce by *Psychrobacter* sp. SP -1 fermentation. J. Sci. Food Agric. 97, 4484–4493. https://doi.org/10.1002/jsfa.8313.