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Impact of fish dry-curing on the behaviour of *Listeria monocytogenes* during the production of ready to eat fishery products

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ABSTRACT

Fish dry-curing technologies performed within dedicated cabinets are gaining popularity in restaurants and food companies. These technologies are based on a constant control and adjustment of temperature, relative humidity and ventilation parameters and are used to transform raw fillets or whole fish into ready to eat products. To date no scientific data are available regarding the impact of these technologies on the survival of foodborne pathogens such as *Listeria monocytogenes*. Thus, we performed challenge tests to evaluate the behaviour of *L. monocytogenes* throughout the curing process conducted within a patented cabinet on salmon, yellowfin tuna and swordfish. A significant decrease of *L. monocytogenes* count was recorded during salting for salmon, yellowfin tuna and swordfish (0.72, 0.51 and 0.84 Log_{10} CFU/g; $p < 0.05$ and < 0.001) and during drying and aging for tuna and swordfish (0.77 and 0.49 Log_{10} CFU/g; $p < 0.01$ and < 0.05). However, an increase of the pathogen was expected applying two predictive microbiology models using the parameters of the challenge tests. Further studies are therefore necessary to include into predictive models relevant parameters which may affect the behavior of *L. monocytogenes*, such as ventilation, relative humidity and effect of competitive microflora.

1. Introduction

Curing is a common method for preserving seafood, and specifically fish curing comprises drying, salting, smoking, pickling and marinating, or various combinations of these methods, as well as fermentation and ripening, with the exception of refrigeration and canning (Jarvis, 1988). As the global demand for seafood increases, the consumers' demand for innovative ready to eat (RTE) fishery products with longer shelf-life, unique, intense and desired flavors and odors, and new approaches to fish preservation are needed. Fish and fishery products are indeed food commodities with high commercial value, high-quality protein content, vitamins, minerals and unsaturated fatty acids, which are beneficial to health, but at the same time, are highly perishable. Therefore, the preservation of fish by innovative curing processes is of great interest given that consumers are demanding fresh or fresh-like, minimally processed fishery products that do not alter their natural quality attributes and maintain safety.

Recently, similarly to meat dry-agers, new patented cabinets for fish dry-curing, based on continuous temperature, relative humidity (RH) and ventilation monitoring and management, are entering the market. These dry-curing systems transform raw fillets or whole fish in RTE products through maturation, smoking, fermentation, and drying. The popularity of such products has risen in the last years to such an extent that are currently consumed by Ho.Re.Ca (hotel/restaurant/catering) customers or, less frequently, at home (personal communications from: Eater Los Angeles, 2022; San Francisco Chronicle, 2023; https://www.meatico.it/dryagingfish.html) probably owing to the shorter preparation time needed, ease of use, and freshness (Rebezov et al., 2021).

In the literature, the available studies report mostly the role of microorganisms in relation to flavor development (Liu et al., 2023; Zhao et al., 2022) or the characterization of the microbiota during traditional fish curing (Dias et al., 2021; Kobayashi et al., 2016; Zhang et al., 2021).

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However, to date only few papers address the microbiological safety of the cured fish investigating the presence and/or concentration of spoilage, indigenous or pathogenic bacteria such as *Listeria monocytogenes* and other foodborne pathogens (Ganapathiraju et al., 2019; Lalitha & Surendran, 2002; Pasonen et al., 2019; Sánchez-Parra et al., 2023). Besides, during fish dry-curing in dedicated cabinets no scientific data is available on survival ability and growth potential of *L. monocytogenes* while few data describe the degree of variability of this curing process, which is reflected in the physico-chemical characteristics of this product (Indio et al., 2024). Nevertheless, since the dry-cured fish is commercialized as RTE product and *L. monocytogenes* is listed as food safety criteria based on Regulation CE 2073/2005, the collection of data is needed about the safety of the product.

Within the RTE food category, the highest occurrence of *L. monocytogenes* (from 2.3% to 2.6%) is reported in fish and fishery products. Indeed, *L. monocytogenes* has been observed on the fish surface and in the stomach lining, gills, and intestines, while the flesh is usually not contaminated by the microorganism unless it spreads from the intestinal content or by cross-contamination due to manipulation of fish using contaminated equipment and to inappropriate transport (Jami et al., 2014; Zakrzewski et al., 2024 reported a considerable pooled prevalence of *Listeria* sp. in raw fish (12.2%). Salmonidae was reported to be the most contaminated family, with pooled prevalence of 28.5% (with an occurrence of 30.3% in *Salmo salar* species), whereas very low isolation of *L. monocytogenes* was found among *Xiphiidae* (3.2%, all in *Xiphias gladius* species) and *Scombridae* (0.7 %) (Zakrzewski et al., 2024). In the United States L. *monocytogenes* seafood contamination accounted for 30% of the overall recalls and recall classification from 2002 to 2020, with salmon and tuna accounting respectively for 53% and 10% (Rene Blickem et al., 2023). *L. monocytogenes* resulted also the primary cause of recalls in food products containing tuna as an ingredient, rather than fresh, frozen or uncooked tuna, accounting for 148 and 6 out of the overall 154 tuna recalls respectively (Blickem et al., 2022). In Europe, *L. monocytogenes* was ranked as the second hazard (6.4%) among the overall Rapid Alert System for Food and Feed (RASFF) notifications for seafood over the period 1996–2020 (Pigłowski, 2023). Studies reporting quantification of *L. monocytogenes* in fishery products, report that in salmon the level of contamination is usually below 10 CFU/g but sporadic higher levels of contamination (i.e., 5–6 Log_{10} CFU/g) were reported in cold smoked fish products (Acciari et al., 2017; Eicher et al., 2020). Beyond that, in recent years, in the EU several outbreaks involving RTE seafood products were reported (European Food Safety Authority & European Centre for Disease Prevention and Control, 2023).

These data demonstrate the importance associated with the control of *L. monocytogenes* during traditional and well-validated fish processing, thus making it even more difficult in case that innovative processing technologies are applied for the preservation and shelf-life extension of seafood products.

Thus, considering the absence of data related to challenge tests in dry-cured fish and the need of assessing the impact of the process parameters used in the different recipes, the aim of this study was to evaluate the behavior of *L. monocytogenes* during dry curing process and to compare the results obtained through challenge tests, with the ones obtained using two predictive microbiological models.

2. Materials and methods

2.1. Fish curing process

For this study 4 fish fillets of *Salmo salar* (salmon), *Xiphias gladius* (swordfish) and *Thunnus albacares* (yellowfin tuna) each were shipped to the laboratory still frozen at −20 °C and thawed at 4 °C overnight. 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.

One fillet of each fish species was used as a control, while the remaining three were contaminated with *L. monocytogenes*. All the fillets belonged to different batches. Briefly, once thawed the fillets were laid in false-bottomed boxes and covered with a salting mixture (20% w/w) containing salt (37%), sucrose, dextrose along with a mix of spices and antioxidants. The salting step allows to increase the stability of the final product by removing water through the osmotic dehydration and to promote flavor and taste development. After 48 h at 2 °C salt was mechanically removed and fillets underwent a dripping step of 4 h for salmon and 12 h for swordfish and yellowfin tuna, followed by the curing process on horizontal inox grids. Every fish species has been cured following a so called “climatic recipe”, consisting of steps, with a different duration in terms of hours, that are characterized by combinations of temperature, RH and ventilation, set considering the starting characteristics of the raw material and of the expected final product in terms of organoleptic characteristics. Hence, the whole process should enhance the product value with a balance avoiding drying of the product. Tuna and swordfish followed the same process, that was different from the one of the salmon (Table 1).

All the fillets were dry-cured in the patented cabinet Stagionello® Fish Curing Device 150 kg, (Industrial invention patent N°EP2769276B1, N° CA 2852650 “Device and method for the control and management of the conservation and/or processing of food in a closed, mobile or fixed environment”). Within the cabinets, the process parameters of RH, ventilation and temperature can be monitored and controlled.

2.2. Challenge tests

Two separate challenge tests were performed considering different contamination scenarios: the first occurring in the raw material, before salting and dripping steps (i.e., timepoint T0); the second occurring during the mechanical removing of salt, before drying and aging steps (timepoints T0-T8). For each of the two challenge tests, three independent trials were performed on three different fish batches. A mix of three *L. monocytogenes* strains was used in both challenge tests to inoculate the fish fillets, namely ATCC 15313, and the ANSES wild strains 12MOB099LM and 12MOB102LM isolated from fishery products. For each stock culture, the inoculum was prepared following the procedures reported in ISO 20976-1(ISO 20976-1:2019, 2019) and ISO 20976-2 (ISO 20976-2:2022, 2022). For each challenge test, all the fillets were tested for the presence of *L. monocytogenes* before inoculation according to ISO 11290-1 (International Organization for Standardization, 2017).

A concentration of ~3 log CFU/g was obtained on the fish fillets spiked before the salting step in the first challenge test, and after the mechanical removal of the salting mixture in the second challenge test. A volume of 50 μL was used to inoculate each sample, represented by a 10 cm^2 area; control samples were inoculated with 50 μL of sterile saline on the surface and used to perform the physico-chemical analysis. In addition, only for salmon, given its different thickness throughout the fillet, samples were collected from three different areas, namely the upper, central and lower parts, and analyzed in order to assess potential variability within a single fillet. After inoculation the fillets were air-dried for 15 min and then placed in aerobic condition into the Stagionello® Fish Curing Device for the curing process.

Samples were collected at the transition point from one step to the following one of the climatic recipe process. The count of *L. monocytogenes* was performed by serial dilution and direct surface plating onto Chromogenic Listeria Agar (CLA Oxoid, U.K) plates according to ISO 11290-2 (International Organization for Standardization (ISO), 2017). At each sampling point, three samples were analyzed for pH (Mettler-Toledo, USA) and a_w (Aqualab CX 4-TE), in accordance respectively with ISO 2917 (ISO, 1999) and ISO 18787 (ISO, 2017). Temperature of the product was determined at each sampling point by inserting the thermometer into the fillets.

The sodium chloride content (NaCl) was determined through the determination of the chloride anion by ion chromatography with an

Table 1
Parameters set for the dry curing process of salmon, swordfish and yellowfin tuna and identification of sampling points; each sample is collected at the end of the corresponding step.

Step description	Duration (h)		T (°C)		RH (%)		Ventilation (m/s)		Sampling point
	Salmon	Swordfish, Yellowfin Tuna	Salmon	Swordfish, Yellowfin Tuna	Salmon	Swordfish, Yellowfin Tuna	Salmon	Swordfish, Yellowfin Tuna	
Curing	48	48	2	2	85	85	3.4	3.4	T0
Dripping	4	12	24	18	80	78	3.4	3.4	T1
Drying	6	18	19	15	51	55	3.4	3.4	T2
Drying	6	18	14	15	54	58	3.4	3.4	T3
Drying	6	18	11	15	57	51	3.4	3.4	T4
Drying	12	18	8	10	60	54	3.4	3.4	T5
Drying	12	18	5	8	63	57	3.4	3.4	T6
Aging	40	18	4	6	67	60	2.1	3.4	T7
Aging	32	72	4	4	60	50	2.1	2.1	T8

electrochemical conductivity detector.

2.3. Predictive models

Parallel to conducting the challenge tests, two different predictive microbiology models for the growth of *L. monocytogenes*, *i.e.* the ComBase (www.combase.cc) broth growth model and the model implemented in the Food Spoilage and Safety Predictor (FSSP) software (http://fssp.food.dtu.dk/Help/Listeria/Lm-LAB/lm-lab.htm) for *L. monocytogenes* in fishery products, were used to predict the behavior of *L. monocytogenes* in the three fish product during the dry-curing process. Since physico-chemical data of the product were available for each step of the dry-curing process a stepwise approach was applied assuming a gradual transition from one pH value to the following one and from one a_w value to the following one, while the temperatures used were the ones recorded during the process. The models were used to compute the maximum growth rate (μmax) during the dry-curing process and from that to estimate the log increase of *L. monocytogenes* in the products. Furthermore, when the a_w values were below the validated range of the model, a "no growth" was assumed, avoiding the use of the models outside the validated range. It is noteworthy that the models used do not encompass one of the key variables of the dry-curing process (*i.e.*, air speed). Moreover, since we did not enumerate LAB which were not expected to grow, the competition between *L. monocytogenens* and LAB was not considered in the model.

All the simulations were run using Excel version 16.79.2.

2.4. Statistical analysis

The differences in a_w, pH and *L. monocytogenes* counts between the upper, central, and lower part of the salmon fillets at each sampling point were tested using the paired *t*-test considering significative a p-value <0.05. The log reduction (Δlog) was calculated for each batch and a mean Δlog was calculated across the three batches for both challenge tests.

A paired *t*-test was used to compare the load of *L. monocytogenes* at the beginning and at the end of the challenge test across the 3 batches of each fish species, and a $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Challenge tests

Fillets of salmon, swordfish and yellowfin tuna are very perishable products and the dry-curing process is a suitable technology to extend their shelf-life. At the end of the dry-curing process the fish is RTE, and therefore *L. monocytogenes* must be considered a relevant biological hazard. The behaviour as well as the estimation of growth inhibition or growth potential of *L. monocytogenes* based upon hurdle technology has to be judged case by case. The inherent variability linked to the product, the microorganism in question and the processing and storage conditions have to be taken into account, including as many intrinsic characteristics of the matrix and the extrinsic environmental conditions as necessary to fully describe the process and the product.

The thawed fillets of salmon, swordfish and yellowfin tuna showed an initial pH respectively of 6.12 ± 0.06, 5.61 ± 0.07 and 6.49 ± 0.06, and a_w values respectively of 0.991 ± 0.015, 0.985 ± 0.014 and 0.992 ± 0.014. *L. monocytogenes* was not detected in the samples prior to challenge test.

The behaviour of *L. monocytogenes* count, as well as temperature, pH and a_w parameters are summarized for the salting and dripping steps (raw samples and T0) in Tables 2 and 3, and for the drying and aging steps (T0 to T8) in Tables 4 and 5.

In relation to the different thickness for which all the salmon fillets were investigated, no statistical differences in relation to *L. monocytogenes* counts as well as pH and a_w parameters were observed between the samples collected from upper, central and lower parts of the same fillet (data not shown).

The pH generally showed a moderate decrease in the first step after salting that is performed at higher temperatures, indicating a weak fermentation, while in the following steps variable values were recorded without a definite trend. The decrease of pH during initial fermentation was too low to inhibit *L. monocytogenes* as observed in other fish fermented products (Axelsson et al., 2020; Guyer & Jemmi, 1991). The NaCl content observed at T0 (after curing), T4 (after the third step of drying) and T8 (at the end of the dry-curing process) resulted respectively of 4.55, 3.60 and 4.43 g/100 g in salmon, of 3.05, 1.96 and 2.95 g/100 g in swordfish, and of 6.01, 3.13 and 4.62 g/100 g in yellowfin tuna fillets. It is reported that *L. monocytogenes* can easily survive in a salt marinaded containing 6% NaCl and multiplicate in fermented fish products with 6.3% NaCl at 4 and 7 °C of temperature (Axelsson et al., 2020; Guyer & Jemmi, 1991). Similarly, the a_w values varied during the test roughly in relation with the different percentages of RH. At the beginning of the process, after salting, a_w values able to control the *L. monocytogenes* multiplications were measured, while during the process, lowering the temperature and increasing the environmental RH resulted in an increase of the a_w values and in the last three sampling point (T6, T7 and T8, Table 5) conditions permissive for the pathogen multiplication were detected, with the exception of swordfish samples.

Differences among the desiccation obtained for the three species can be attributed both to the different process parameters set for preparation of salmon, tuna and swordfish but also to the different fish composition. Considering the temperature, the process parameters set, the pH, a_w and the NaCl content of the product, the growth of *L. monocytogenes* should be supported. However, after both the salting and dripping steps and the drying and aging steps, a slight decrease of the mean count of *L. monocytogenes* was observed from raw samples to T8 with an overall Δlog ranging from 0.62 to 1.33 Log_{10} CFU/g. A significant decrease of *L. monocytogenes* count was recorded during salting for the three species tested, and further decreased during drying and aging with values that resulted significant for tuna and swordfish but not significant for

Table 2
Mean log CFU/g values (standard deviation) of *Listeria monocytogenes* counts during the curing and dripping steps (raw samples and T0 samples) with the observed average Δlog reduction.

	Salmon				Yellowfin Tuna				Swordfish			
	Batch 1	Batch 2	Batch 3	Mean	Batch 1	Batch 2	Batch 3	Mean	Batch 1	Batch 2	Batch 3	Mean
Raw	3.39	3.45	3.45	3.43	3.29	3.59	2.89	3.26	3.60	3.49	3.27	3.46
	(0.41)	(0.31)	(0.39)	(0.04)	(0.03)	(0.16)	(0.42)	(0.35)	(0.10)	(0.06)	(0.17)	(0.17)
T0	2.65	2.90	2.89	2.81	2.68	2.85	2.72	2.74	2.64	2.77	2.43	2.61
	(0.31)	(0.22)	(0.08)	(0.14)	(0.19)	(0.09)	(0.31)	(0.09)	(0.25)	(0.21)	(0.26)	(0.17)
Δlog	**−0.74**	**−0.55**	**−0.56**	**−0.72****	**−0.61**	**−0.74**	**−0.17**	**−0.51***	**−0.96**	**−0.72**	**−0.84**	**−0.84****

Statistical significance *p < 0.05 **p < 0.01.

Table 3
Mean values (standard deviation) of pH, water activity (a_w) and temperature values observed in the fillets of different fish species during the curing and dripping steps.

		Salmon	Yellowfin Tuna	Swordfish
Raw	**pH**	5.98 (0.03)	6.0 (0.02)	5.89 (0.03)
	a_w	0.991 (0.01)	0.992 (0.014)	0.985 (0.014)
	T(°C)	2.4 (0.10)	2.7 (0.05)	2.8 (0.15)
T0	**Ph**	5.78 (0.04)	5.84 (0.01)	5.86 (0.03)
	a_w	0.898 (0.04)	0.893 (0.06)	0.894 (0.04)
	T(°C)	23.5(0.10)	15.8 (0.10)	15.4 (0.07)

salmon: this might be attributed to the fact that for salmon on two batches there was a slight reduction while in the third a slight increase.

In detail, after the salting and dripping steps, the overall decrease varied from a minimum of 0.51 Log_{10} CFU/g in yellowfin tuna and a maximum of 0.84 Log_{10} CFU/g in swordfish (Table 2). In this respect, when considering the reduction of the Log_{10} CFU count of *L. monocytogenes* during the salting step, the mechanical removal of salt that is expected to detach the microorganism from the surface of the product has to be considered. Also after the drying and aging steps, the mean overall decrease varied from a minimum of 0.11 Log_{10} CFU/g in salmon and a maximum of 0.77 Log_{10} CFU/g in yellowfin tuna.

Considering all the sampling points throughout the process, the data show that the counts of *L. monocytogenes* did not follow a progressive decreasing trend, but rather a fluctuating behaviour, consistent with steps in which intrinsic and extrinsic factors supported or not the growth of the pathogen.

The different behaviour of *L. monocytogenes* in salmon (Table 4) must be taken into account given that *Salmo salar* is the most economically important fish species, usually undergoes many different processing procedures and is therefore the species more frequently contaminated by *L. monocytogenes* (Zakrzewski et al., 2024). According to the literature, smoked salmon and sushi salmon were responsible for several outbreaks of listeriosis (Eicher et al., 2020). In addition, different composition of the three fish species may have influenced the result. As an example, the high fat content of salmon may have had a protective action in inoculated *L. monocytogenes.* The presence of fat droplets in the food matrix is generally assumed to suppress microbial growth if the fat content is sufficiently high (Baka et al., 2017a), nevertheless recent studies have also shown that the presence of fat in the food matrix can also enhance microbial growth under certain conditions (Baka et al., 2017b; Verheyen et al., 2018). Besides the influence of the fish product composition it has to be underlined that we did not count LAB along the

Table 4
Mean values (standard deviation) of *Listeria monocytogenes* counts during the drying and aging steps (samples T0-T8) with the observed average Δlog reduction.

	Salmon				Yellowfin Tuna				Swordfish			
	Batch 1	Batch 2	Batch 3	Mean	Batch 1	Batch 2	Batch 3	Mean	Batch 1	Batch 2	Batch 3	Mean
T0	3.02	3.09	3.66	3.26 (0.35)	3.74	3.67	3.52	3.64 (0.11)	3.61	3.59	3.65	3.62 (0.03)
T1	2.98	3.46	3.28	3.24 (0.24)	3.19	3.52	3.08	3.26 (0.23)	3.03	3.04	3.75	3.27 (0.41)
T2	3.12	3.08	3.19	3.13 (0.06)	3.86	3.81	4.00	3.89 (0.10)	3.17	3.32	3.30	3.26 (0.08)
T3	3.06	3.32	2.71	3.03 (0.30)	3.11	3.84	3.93	3.63 (0.45)	3.26	3.36	3.41	3.35 (0.08)
T4	3.15	3.13	3.21	3.16 (0.04)	2.93	2.90	2.76	2.86 (0.09)	3.45	2.70	3.57	3.24 (0.47)
T5	3.66	3.06	2.98	2.90 (0.21)	2.75	2.26	2.38	2.46 (0.25)	3.05	3.32	3.95	3.44 (0.46)
T6	3.03	3.17	3.04	3.08 (0.08)	2.97	2.89	2.46	2.77 (0.27)	2.91	3.41	3.80	3.38 (0.45)
T7	3.18	3.04	3.08	3.10 (0.07)	3.00	3.08	2.83	2.97 (0.13)	2.73	3.45	3.78	3.32 (0.55)
T8	3.04	3.40	3.00	3.15 (0.22)	3.08	2.90	2.64	2.87 (0.22)	2.85	3.18	3.36	3.13 (0.26)
Δ log	**+0.02**	**+0.30**	**−0.66**	**−0.11**	**−0.66**	**−0.77**	**−0.88**	**−0.77****	**−0.76**	**−0.41**	**−0.29**	**−0.49***

Statistical significance *p < 0.05 **p < 0.01.

Table 5
Mean values (standard deviation) of pH, water activity (a_w) and temperature values observed in the fillets of different fish species during the drying and aging steps (samples T0-T8).

	Salmon			Yellowfin Tuna			Swordfish		
	pH	a_w	T°	pH	a_w	T°	pH	a_w	T°
T0	5.93 (0.12)	0.902 (0.03)	2.5 (0.10)	6.28 (0.01)	0.893 (0.009)	2.7 (0.10)	5.91 (0.04)	0.870 (0.023)	2.8 (0.15)
T1	5.85 (0.04)	0.858 (0.03)	21.5 (0.05)	5.88 (0.01)	0.923 (0.09)	16.3 (0.05)	5.92 (0.03)	0.894 (0.004)	16.2 (0.10)
T2	6.11 (0.01)	0.877 (0.017)	16.9 (0.07)	5.88 (0.03)	0.931 (0.001)	11.7 (0.10)	5.73 (0.06)	0.910 (0.001)	13.2 (0.10)
T3	6.00 (0.08)	0.897 (0.015)	14 (0.10)	5.93 (0.01)	0.942 (0.01)	12.3 (0.07)	5.67 (0.15)	0.930 (0.01)	13.6 (0.05)
T4	5.98 (0.02)	0.942 (0.017)	11.7 (0.10)	5.99 (0.1)	0.924 (0.01)	12.1 (0.10)	6.10 (0.04)	0.900 (0.01)	10.2 (0.07)
T5	6.18 (0.03)	0.918 (0.006)	10.3 (0.15)	5.91 (0.05)	0.920 (0.01)	7.9 (0.10)	5.82 (0.05)	0.910 (0.01)	10 (0.15)
T6	6.09 (0.06)	0.934 (0.008)	9.6 (0.07)	5.93 (0.04)	0.930 (0.01)	8.3 (0.15)	5.75 (0.1)	0.920 (0.01)	9.4 (0.07)
T7	6.07 (0.09)	0.937 (0.021)	4.9 (0.05)	6.00 (0.03)	0.940 (0.01)	5.1 (0.05)	5.81 (0.04)	0.942 (0.02)	6.3 (0.10)
T8	6.16 (0.06)	0.925 (0.028)	5 (0.15)	5.97 (0.03)	0.955 (0.02)	4.2 (0.07)	5.55 (0.27)	0.900 (0.02)	3.9 (0.05)

process.

Moreover, one limitation of this study is the lack of enumeration data for LAB that might be one of the reasons why the pathogen did not grow, as expected looking at the predictive model results. Nevertheless, not only a slight fermentation is expected based on previous findings on this kind of product (Indio et al., 2024) but also in naturally contaminated product where no experimental inoculation of selected LAB is performed, the effective role of such bacteria would be very difficult to be evaluated and quantified.

3.2. Predictive models

When compared with the results obtained from both selected predictive models, the behavior of *L. monocytogenes* during the challenge test differs among the three fish species. Despite substantial differences between the two models, both predicted similar results, with an overall increase in *L. monocytogenes* from T0 to T8 in all fish species. The increase varied from a minimum of 0.2 Log_{10} CFU/g in swordfish to a maximum of 1.8 Log_{10} CFU/g in yellowfin tuna (Figs. 1–3).

The Combase model is a broth model, suitable for a wider application but less specific than the FSSP model. The FSSP model was developed and validated on fishery products (lightly preserved fishery products) as well as on ready-to-eat eat products, making it more specific to the type of product considered in this study.

Both models include physio-chemical parameters such as pH and a_w, but the FSSP model also allows for the inclusion of other parameters, such as organic acid concentration and interactions with lactic acid bacteria. However, the fact that these additional parameters were not included in this study likely led to the similar results, despite the intrinsic differences between the two models.

The models available are not designed to incorporate some extrinsic and characteristic factors of curing process, such as the RH or the ventilation parameters that may influence the behavior of *L. monocytogenes*. Another drawback is that during the challenge test, at different timepoints, the recorded physico-chemical values were outside the validation range of the models, more specifically in some points the a_w values were below the minimum allowed by the model itself. In these cases, we opted for a conservative approach assuming a no growth of the pathogen, rather than using a Non Thermal Survival Model, even though a slight log reduction could occur due to unrefrigerated storage (18–22 °C) and unfavourably low a_w values (food microbiology principle) (food microbiology principle) in the growth range of this microorganism which combined may cause metabolic exhaustion.

Based on the findings of this study and in relation to the EFSA opinion on the microbiological safety of aged meat, two main similarities have to be highlighted: fish dry-curing in cabinets is comparable to dry-aging of meat and predictive microbiology models seem to

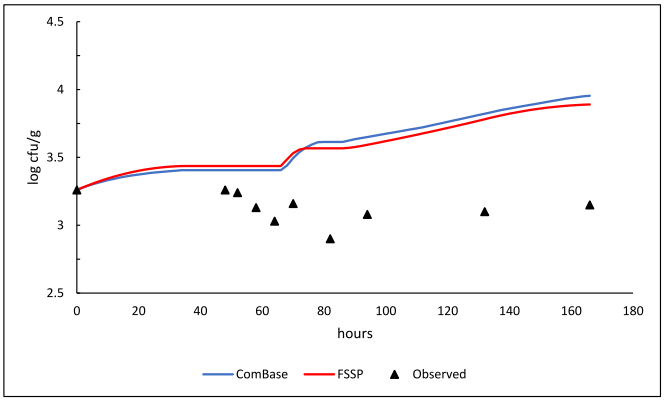

Fig. 1. *Listeria monocytogenes* (Log_{10} CFU/g) values predicted in salmon using the two predictive growth models (Combase and FSSP) and mean (Log_{10} CFU/g) values observed during the challenge test.

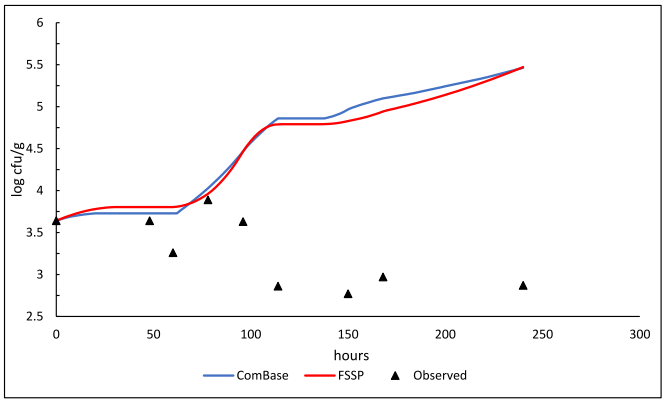

Fig. 2. *Listeria monocytogenes* (Log_{10} CFU/g) values predicted in Yellowfin Tuna using the two predictive growth models (Combase and FSSP) and mean (Log_{10} CFU/g) values observed during the challenge test.

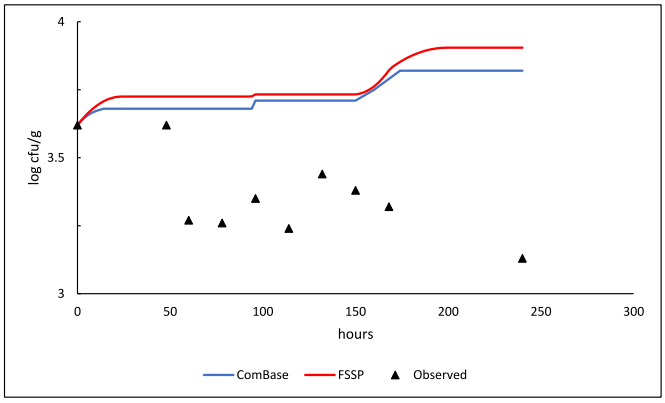

Fig. 3. *Listeria monocytogenes* (Log_{10} CFU/g) values predicted in swordfish using the two predictive growth models (Combase and FSSP) and mean (Log_{10} CFU/g) values observed during the challenge test.

overestimate the growth of *L. monocytogenes* in these kinds of RTE products. The predictive models indeed incorporate information related to the process, but it is not possible to incorporate all parameters affecting the behaviour of the pathogen. Predictive microbiology is used to ensure microbial food safety by facilitating the selection of appropriate processing conditions, but, as observed by Bonilauri and Colleagues (2021), the utility of predictive microbiology models is often somewhat limited from an industry perspective given that the models are usually developed under laboratory conditions or use combinations of parameters not appropriate for that particular food production. On the other hand, challenge tests are time and money-demanding. For these reasons, a standardized collection of high-quality data is essential to build efficient and accurate predictive models or to validate existing ones.

The overestimation of the growth of *L. monocytogenes* in seafood by predictive models was assessed and evaluated by Dalgaard and Jorgensen (1998) who concluded that new expanded models, including more variables, may provide a more accurate prediction in seafood.

If on one side the overestimation of growth of *L. monocytogenes* by predictive models represents a precautional approach, on the other it may represent an additional cost for food business operator (FBO) and society to reach the goal of zero tolerance, for addition of unnecessary preservatives and for reducing the shelf-life of products (Dalgaard and Jorgensen, 1998) with an increase of food waste. Further studies are necessary to gather and include into predictive models some of other relevant factors which may affect the behavior of *L. monocytogenes*, e.g. ventilation, RH during this particular curing process, as well as to

perform the validation of models capable to include additional parameters.

4. Conclusion

These data could give relevant information to the FBOs, helping them to improve the safety of their products. Nonetheless, since in fresh and in lightly preserved seafoods no step determines complete elimination of *L. monocytogenes* FBO have the responsibility to adhere strictly to good manufacturing practices and good hygienic practices, in order to prevent also contamination events, ensure good quality of the raw materials. These measures, taken either by the producers or manufacturers to limit *L. monocytogenes* prevalence become also important to control the growth during the product shelf-life. Lastly the Competent Authority would benefit from information and tools helping to verify the fulfillment of the industry and Ho.Re.Ca to regulation in force and ensure food safety.

As already reported for dry-aged meat, the predictive models tend to overestimate the bacterial growth since not all parameters can be included into the model itself e.g. ventilation and RH. Thus, the data obtained are precautionary and cannot be intended as a unique reference. Further research involving other fish species and different curing conditions is necessary to better understand the safety of such products and for feeding the predictive models as well as for validation of the adopted processes.

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CRediT authorship contribution statement

Federica Savini: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Federica Giacometti:** Writing – review & editing, Methodology, Investigation. **Federico Tomasello:** Writing – review & editing, Writing – original draft, Formal analysis. **Valentina Indio:** Writing – review & editing, Writing – original draft, Formal analysis. **Fausto Gardini:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Federica Barbieri:** Formal analysis, Data curation. **Lia Bardasi:** Writing – review & editing, Writing – original draft, Investigation. **Mattia Ramini:** Investigation. **Laura Prandini:** Investigation, Data curation. **Yitagele Terrefe Mekkonnen:** Investigation. **Sean Alberto Cuomo:** Investigation. **Alessandra De Cesare:** Writing – review & editing, Writing – original draft, Methodology. **Andrea Serraino:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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