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Cured or fresh? Between fish maturation trends in restaurants and food safety: The case of dry-aged rainbow trout

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ABSTRACT

The application of dry aging on fish is increasing in restaurants worldwide. This process determines fluid loss, changes in muscle texture, and the development of a more concentrated flavour appreciated by consumers. Despite the growing popularity in the foodservice industry, there is a lack of data on potential risks and quality conditions of dry-aged fish. This study was aimed at assessing the safety for consumption of whole rainbow trout subjected to dry aging in a local restaurant (Turin, Italy). Quantitative microbiological analyses and detection of foodborne pathogens were performed on dorsal, ventral muscles, and skin samples at day 0, 3, 6, 10, and 14 of dry aging. Changes in water activity (a_w), pH, colour, and biogenic amines in muscle were checked at each sampling point. Almost all the microbiological indicators were below the quantification limits in muscle until day 6 of dry aging, while higher but still acceptable loads were observed at day 10. At day 14, yeasts and moulds exceeded the acceptable level in one case (3.3 ± 0.4 $\log_{10}$ CFU/g) in ventral muscle and counts of *Pseudomonas* spp. (from 6.1 ± 0.8 to 6.4 ± 0.5 $\log_{10}$ CFU/g), total mesophilic (from 4.4 ± 0.3 to 5.3 ± 0.7 $\log_{10}$ CFU/g), and psychrophilic (from 5.4 ± 0.1 to 5.8 ± 0.3 $\log_{10}$ CFU/g) bacteria indicated the approaching of spoilage. Detection of *Listeria monocytogenes* and *Staphylococcus aureus* was always negative during the trials. Minimal changes of pH and colour were observed, while a_w values followed a decreasing trend during dry aging. Putrescine was the only detectable amine, with a maximum concentration of 2.05 ± 0.02 mg/kg at day 14. In this study, dry-aged rainbow trout was safe for consumption and 10 days was the most appropriate curing time with 3 °C and 78% of relative humidity. Further studies at different environmental conditions and on other fish species are needed for a comprehensive risk assessment of this fast-growing practice in the restaurant sector.

1. Introduction

For their intrinsic and extrinsic features, fresh fish products are considered highly perishable and short shelf-life foods. Enzymatic autolysis, oxidation, and microbial growth are the main mechanisms involved in fish spoilage (Ghaly, 2010; Nie et al., 2022). To compensate the perishability and minimize waste, several approaches have been developed to extend the shelf-life of fishery products. These strategies include the employment of a range of techniques or substances, such as MAP technology, plant or algae extracts, essential oils, microbial-derived compounds, temperature/pressure treatments, fermentation, smoking, refrigeration, freezing, traditional and innovative drying approaches (Fitri et al., 2022; Hassoun et al., 2022; Nie et al., 2022; Panebianco et al., 2019). Despite consumers' appreciation for products preserved by these methods, it was often assumed that fish should be eaten 'fresh', minimizing the time between capture and consumption to thoroughly appreciate its flavour characteristics. This popular belief, once shared by many top chefs, is significantly mutating in recent years suggesting a shift in the culinary landscape that could led to a substantial reconsideration of fish products in the contemporary gastronomic era. Recently, in fact, the trend of maturing fish applying the dry aging process has been spreading all over the world in the restaurant sector (Food and Wine, 2019; Food and Wine Italia, 2021; Identità Golose, 2020; Identità Golose, 2021; LA Eater, 2022; San Francisco Chronicle, 2023). Dry aging is a ripening process employed since many years to improve the sensory properties of meat, especially

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beef. Dry-aged meat is extremely appreciated by consumers for its palatability attributes, such as the typical flavour and the remarkable tenderness (Terjung et al., 2021). This maturing technique is based on the application of cold temperatures (0–4 °C) with controlled and monitored humidity (61–85%) for a period that may significantly vary in relation to the products' features (14–40 days) (Dashdorj et al., 2016). Since this practice has become widely popular, even at restaurant level, the European Food Safety Authority (EFSA) has expressed an opinion about the safety of aged meat, concluding that there are no increased risks compared to its fresh counterpart if meat is aged under strictly controlled conditions (EFSA, 2023). The application of dry aging on fish initially appeared as a novelty fad limited to a few high-level restaurants, but it is now a tried-and-true practice employed by many chefs in Europe and beyond (Food and Wine, 2019; Food and Wine Italia, 2021; Identità Golose, 2020; Identità Golose, 2021; LA Eater, 2022; San Francisco Chronicle, 2023). The widespread application of this technique for fish curing in the restaurant sector is mainly thanks to Australian chef Josh Niland and his innovative concept of approaching fishery products, based on dry aging of whole carcasses and using all edible parts of fish. In his books, Niland describes in detail how to perform dry aging on different fish species and the various steps to achieve an optimal result (Niland, 2019, 2021, 2023). In a first phase, fish usually undergoe preliminary operations, such as scaling and gutting, before being hung head-down in dry aging cabinets with controlled temperature (preferably between −2 and 2 °C) and low relative humidity. To prevent the formation of wetness from contact, larger fish should not come into contact with one another or with surfaces, while smaller specimens and fillets can be placed on perforated stainless-steel trays. Dry aging time and the other process parameters are variable depending on the characteristics of the fish species to be treated (Niland, 2019). During the curing, the skin becomes drier, there is a general release of superfluous fluids and a substantial modification in the texture of flesh. Fish subjected to this ripening method tend to develop different aromatic characteristics compared to fresh products, resulting in a more concentrated and extremely appreciated flavour. The same process can be applied to salami, sausages, and other derived products prepared from fish muscle. Given the emphasis on reducing food waste within the framework of a circular economy (Boronat et al., 2023; Greggio et al., 2021), the chance of employing dry aging for improving the palatability of fish parts or species normally not highly appreciated by consumers appears promising.

As previously stated, fish are characterized by high perishability but may also result in human illness since various pathogenic microorganisms could be present in this matrix (Novoslavskij et al., 2016; Parlapani, 2021). In addition to bacteria existing on raw material, contamination at the restaurant level has gained increasing attention, since microorganisms can be transferred to products during handling and by catering equipment (Sheng & Wang, 2021). Fishery products also carry potential chemical hazards, such as the formation of harmful biogenic amines during storage (Visciano et al., 2020). As dry aging of fish is being implemented by an increasing percentage of restaurants and operators, there is an evident need for a thorough microbiological and chemical risk assessment to ensure the safety of these increasingly popular foods. To the best of our knowledge, in fact, there is only one study about the safety of dry-cured seawater fish (salmon, swordfish, yellowfin tuna) (Indio et al., 2024) while no surveys have been conducted on whole freshwater fish subjected to dry aging in restaurants.

The aim of this work was to evaluate the microbiological and chemical quality of rainbow trout subjected to dry aging at foodservice level, intending to prove the safety of these products for consumers and provide new data for the risk assessment of dry-aged freshwater fish.

2. Materials and methods

2.1. Experimental plan and dry aging conditions

The present study was carried out at a local restaurant (Turin, Italy) conducting dry aging of fish, to assess the safety of these products and this curing process in real conditions. The fish were treated exactly with the procedure applied by the restaurant staff for routinely served products. To confirm the trend of monitored microbiological and chemical parameters, the experiment was entirely replicated twice within two distinct time frames in February and March 2022. Henceforth, we will denote the trials as Experiment 1 (E1) and Experiment 2 (E2). Gutted rainbow trout (*Oncorhynchus mykiss*; 1.5–2 kg each) from a local fish farm (Agritrutta s.s., Monodovì, Cuneo, Italy) reached the restaurant in vacuum packaging (0–1 °C) within 24–48 h from slaughter. After the manual removal of gills and scales, the fish were washed in a saline solution (15% NaCl) for 40–60 s to eliminate any residual blood. Afterwards, fish were suspended upside down in a dry aging cabinet (Arris Ager model AA 85135; refrigeration type: ventilated; max absorbed power: 780 W; cooling power: 246 W; voltage: 230V/50 Hz; produced by Gemm s.r.l. (Codognè, Treviso, Italy) for Arris Catering Equipment s.r.l. (Cadoneghe, Padua, Italy)) setting as desired parameters a temperature of 2 °C and a relative humidity (RH) of 70%. The cabinet operated in ventilated cooling mode and included a software for continuous control of parameters. This system ensured that the RH never exceeded 80% at any point of the process, that temperatures remained within a limited range of fluctuations, and that ventilation was constant throughout the entirety of dry aging. Salt blocks were positioned at the bottom of the cabinet and replaced every 24–48 h to absorb liquids dripped from the fish during dry aging. A data logger (Testo 174 H, Testo AG, Lenzkirchen, Germany) was used to monitor temperature and RH variations in the cabinet during fish maturation. In routine restaurant procedures, the chef and his staff determined 9–10 days as the optimal dry aging time to obtain the best organoleptic features of the products. After this period, the fish were portioned into slices with the skin still attached. Subsequently, the slices underwent a pan-cooking process on the skin side for a time required to reach a core temperature of approximately 75–85 °C. For our trials, in order to characterise more accurately the evolution of potential hazards in products, we requested the restaurant to extend the dry aging time until day 14. At day 0, 3, 6, 10, and 14, a whole fish (5 fish analysed for each experiment; 10 fish in total) was transported in cold environment (0–1 °C) to the laboratory of Food Microbiology (Department of Veterinary Sciences, University of Turin). Each fish was then portioned under sterile conditions to obtain samples for microbiological and chemical analyses.

2.2. Microbiological analyses

For quantitative microbiological analyses, distinct determinations for dorsal (epaxial) muscle (30 samples analysed during E1 and E2), ventral (hypaxial) muscle (30 samples analysed during E1 and E2), and skin (30 samples analysed during E1 and E2) were performed. The sampling sites considered in this study are displayed in Fig. 1. For microbiological quantifications, three samples of 10 g collected from each sampling site (dorsal muscle, ventral muscle, skin) were diluted (1:10) in sterile physiological saline peptone (PS; 0.85% NaCl + 0.1% Bacto-Peptone) and homogenised for 30 s (Stomacher 400 Circulator; Seward, London, UK). Serial dilutions were prepared in tubes with 9 mL of sterile PS. The following microbiological enumerations were performed: i) total mesophilic bacteria on Plate Count Agar (PCA; Oxoid, Basingstoke, UK), incubated at 30° for 72 h; ii) total psychrophilic bacteria on PCA (Oxoid), incubated at 4 °C for 10 days; iii) *Pseudomonas* spp. on *Pseudomonas* Agar (Oxoid) with CFC supplement (Oxoid), incubated at 25 °C for 48 h; iv) *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBGA; Oxoid), incubated at 37 °C for 24 h; v) lactic acid bacteria (LAB) on De Man Rogosa and Sharp (MRS) Agar (Oxoid),

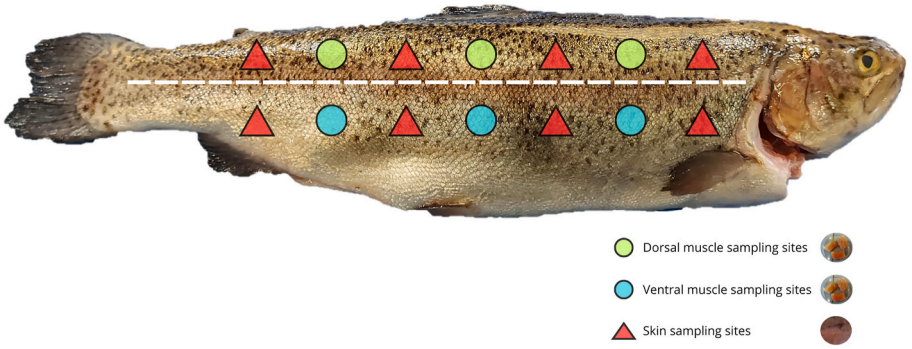

Fig. 1. Sampling sites considered in this study for microbiological analyses of rainbow trout specimens during the dry aging process.

incubated anaerobically at 30 °C for 48 h; vi) coagulase-positive staphylococci on Baird Parker Agar (Oxoid) supplemented with Egg Yolk Tellurite emulsion (Oxoid), incubated at 37 °C for 48 h; vii) yeasts and moulds on Potato Dextrose Chloramphenicol Agar (Condalab, Madrid, Spain), incubated at 30 °C for 48–72 h. To assess the acceptability of products during dry aging, the data obtained from quantitative microbiological analyses were compared to the limits proposed by the Interdepartmental Centre for Research and Documentation on Food Safety of Piedmont region (CeIRSA) for the category 'fish and fish preparation' (CeIRSA, https://www.ceirsa.org/matrice_alim.php#inizio). For microbiological indicators not considered by the CeIRSA, other benchmark limits reported in the literature were considered (Table 1) (Civera et al., 2011; Lyhs, 2002; Mol et al., 2007; Nguyen et al., 2018).

Alongside quantitative microbiological determinations, the detection (presence/absence) of *Listeria monocytogenes* and *Staphylococcus aureus* was carried out on muscle and skin samples at each sampling point. *L. monocytogenes* detection was performed according to the ISO 11290–1:2017 (Anonymous, 2017). Briefly, pools of 25 g of muscle and skin samples, randomly collected from different areas of fish, were suspended (1:10) in half-Fraser broth (Sifin, Berlin, Germany) and incubated at 30 °C for 24 h. An aliquot (0.1 mL) from the half-Fraser was plated on Oxoid Chromogenic *Listeria* Agar (OCLA, Oxoid) and Palcam Agar (Oxoid), incubated at 37 °C for 48 h. Meanwhile, 0.1 mL from the half-Fraser (Sifin) broth were transferred in 10 mL of Fraser broth (Sifin). After 24 h at 37 °C, 0.1 mL from the Fraser broth were plated on OCLA (Oxoid) and Palcam (Oxoid) agar plates, incubated at 37 °C for 48 h and then checked for typical *L. monocytogenes* colonies. For *S. aureus* detection, 25 g of skin and muscle collected from several spots were diluted in 225 mL of PS and serial dilutions were performed. Aliquots (0.1 mL) of each dilution were inoculated by surface plating on Baird Parker Agar (Oxoid) supplemented with Egg Yolk Tellurite emulsion (Oxoid), incubated at 37 °C. Plates were checked after 24–48 h for typical *S. aureus* colonies. Verification of suspected *L. monocytogenes* and *S. aureus* colonies was carried out using MALDI-TOF MS (Microflex LT; Bruker Daltonics, Bremen, Germany).

2.3. Product features and chemical analyses

2.3.1. Water activity (a_w) and pH

Water activity (a_w) and pH were measured on randomly collected muscle samples. In particular, a_w was measured with a water activity meter (Aqualab Series 3 TE; Decagon Devices, Pullman, Washington, DC, USA), while pH was determined (pH 8+ DHS Stirrer; XS Instruments, Carpi, Italy) in 10 g of sample stirred in 10 mL of deionized water for 60 s.

2.3.2. Colour

Colour was measured on muscle samples of ~5 × 5 cm obtained from the same area of epaxial zone. Measurements of L* (lightness), a* (redness), and b* (yellowness) were performed with Minolta CM-600D spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) using 8 mm diameter measuring area, a D65 illuminant, and a 10° standard observer.

2.3.3. Biogenic amines

2.3.3.1. Standards and reagents. Spermidine trihydrochloride, spermine tetrahydrochloride, 1,7-diaminoheptane were employed as internal standard (IS) (Sigma, St Louis, MO, USA). Histamine dihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, and the dansyl chloride were obtained from Acros Organics (Geel, Belgium). Merck (Darmstadt, Germany) supplied all reagents and solvents of HPLC grade.

2.3.3.2. Sample extraction, derivatization, and analysis. The analysis was performed as described by Sagratini et al. (2012) and Nobile et al. (2024), with some modifications. In short, 10 g of homogenised sample (muscle randomly collected from different areas of fish) combined with

Table 1
Benchmark values, valid for fish and fish preparations, considered in this study for the different microbial populations quantified.

Microbiological indicators	Limits (log_{10} CFU/g)				Reference
	Satisfactory	Acceptable	Not satisfactory	Dangerous	
Total mesophilic bacteria	<4	4 ≤ x < 6	≥6	- [a]	CeIRSA, https://www.ceirsa.org/matrice_alim.php#inizio
Total psychrophilic bacteria	<6	–	≥6	–	Mol et al. (2007)
Pseudomonas spp.	<5	5 ≤ x < 7	≥7	–	Civera et al. (2011)
Enterobacteriaceae	<2	2 ≤ x < 4	≥4	–	CeIRSA, https://www.ceirsa.org/matrice_alim.php#inizio
Lactic acid bacteria	<4	4 ≤ x < 6	≥6	–	Lyhs, U. (2002) [b]
Coagulase-positive staphylococci	<2	2 ≤ x < 4	≥4	≥5 and SET (+) [c]	CeIRSA, https://www.ceirsa.org/matrice_alim.php#inizio [d]
Yeasts and moulds	<3	–	≥3	–	Nguyen et al. (2018)

[a] Not specified.
[b] Levels detected in spoiled vacuum packaged rainbow trout.
[c] SET (+) = presence of staphylococcal enterotoxins in food.
[d] Fish and fish preparations to be consumed after cooking.

100 μL of IS (400 μg mL^{-1}) underwent an extraction with 15 mL of 5% Trichloroacetic acid (TCA), following by vortexing for 5 min and centrifugation at 2500*g* for 10 min at 4 °C. Afterwards, the supernatant was removed and further 10 mL of 5% TCA were added to the solid sample to repeat the extraction. Then, both extracts were mixed and 1 mL of the supernatant was subjected to derivatization by the addition of 1 mL of $NaHCO_3$ (saturated solution), 50 μL of NaOH 2 N, 0.5 mL of dansyl chloride solution (10 mg mL^{-1} in acetone), and 0.5 mL of acetone. The mixture was then left at 65 °C for 40 min. After cooling it at room temperature, 1 mL of the derivatized extract was filtered, using a 0.45 μm syringe filter (Sartorius, Goettingen, Germany), into a 2 mL autosampler vial. An HPLC Jasco quaternary pump (Ishikawa-cho, Japan), furnished with an autosampler, was used for biogenic amines separations. A Spherisorb ODS-2 reverse-phase column (5 μm, 125 mm × 4 mm; Waters Corporation, Milford, MA, USA) was employed. The separation of analytes was obtained by a gradient based on acetonitrile (Phase A) and ammonium formate 9 mM (pH = 3.4, Phase B) with a flow rate of 1 mL min^{-1}. The gradient initiated with 60% of Phase A and was maintained for 10 min. At the 12th min, it raised to 80% of A and was kept in this condition until the 30th min. Whitin 1 min, the initial conditions were re-established and re-equilibrated for 10 min. The volume of injection was 40 μL and the detection was achieved by an UV–visible detector (Ishikawa-cho, Japan), working at 254 nm. For each compound, the limit of quantification (LOQ) was defined as 0.05 mg/kg^{-1}.

2.4. Statistical analyses

Microbiological determinations, product features measurements, and chemical analyses were performed in triplicate at each experiment and data were expressed as average ± standard deviation. To detect significant differences between the two experiments performed (E1 and E2) at each time of sampling, a two-way ANOVA followed by a Šidák's multiple comparison test ($p < 0.05$) was used. Statistical analyses and graphing were performed with GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, California, USA).

3. Results

At each sampling point and prior the sample processing, the trout specimens were photographed to highlight macroscopic changes occurred on the skin and external parts during dry aging. As visible in Fig. 2, a drying of the skin and external parts of fish was noticeable from day 3 to the end of curing. Alongside skin dehydration, a posterior displacement of the ocular bulb was evident at day 10 and especially at day 14 of dry aging. Information extracted from the data loggers indicated that the temperature and relative humidity values slightly exceeded the expected and designated levels within the dry aging cabinet. The average temperatures recorded were 3.14 °C in E1 and 3.57 °C in E2, with a mean environmental relative humidity of 77.65% in E1 and 78.58% in E2.

3.1. Microbiological analyses

Outcomes of quantitative microbiological analyses are reported in Table 2. The loads observed in dorsal muscle samples during the two experiments were low and often below the limit of quantification (LOQ) of the method. At the end of dry aging (14 days), total mesophilic bacteria and total psychrophilic bacteria were quantifiable, with levels of 2.8 ± 0.2 (E1) and 3.2 ± 0.8 (E2) log_{10} CFU/g, 3.0 ± 0.5 (E1) and 3.4 ± 0.1(E2) log_{10} CFU/g, respectively. The other quantifiable population at day 14 was represented by *Pseudomonas* spp. and a significant difference ($p < 0.05$) was observed among the two experiments: 3.1 ± 0.2 (E1) and 3.9 ± 0.1 (E2) log_{10} CFU/g. In ventral muscle samples, higher microbial loads were observed especially from day 6 of dry aging. At day 14, yeasts and moulds exceeded the acceptable level (3.3 ± 0.4 log_{10} CFU/g) and higher loads were detected for *Pseudomonas* spp., with values of 6.4 ± 0.5 (E1) and 6.1 ± 0.8 (E2) log_{10} CFU/g. Well represented also total mesophilic and psychrophilic bacteria, with loads of 4.4 ± 0.3 (E1) and 5.3 ± 0.7 (E2) log_{10} CFU/g, 5.8 ± 0.3 (E1) and 5.4 ± 0.1 (E2) log_{10} CFU/g, respectively. The comparison of obtained outcomes with benchmark limits considered (Table 1) highlighted that microbiological indicators in dorsal and ventral muscle were almost always within a fully satisfactory level. The situation for skin samples was quite different, with other bacterial populations, including *Enterobacteriaceae* and coagulase-positive staphylococci, quantifiable from the first day of process and persisting until day 14. A higher variability among the two experiments for skin compared to muscle samples was observed but, considered the benchmark limits applied, only in two cases (*Enterobacteriaceae* in E1 at day 0, yeasts and moulds in E1 at day 3) we observed not satisfactory microbial loads. The detection of *L. monocytogenes* and *S. aureus*, performed at each sampling point on muscle and skin samples, was always negative during both experiments.

Fig. 2. Changes of external appearance of rainbow trout specimens during the dry aging process.

3.2. Product features and chemical analyses

3.2.1. Water activity and pH

Data on a_w and pH changes during the two experiments are reported in Fig. 3 and Table 1SM. A decreasing trend for a_w was observed in both experiments, with final values significantly ($p < 0.05$) lower in E1 (0.968 ± 0.010) compared to E2 (0.986 ± 0.001). The pH was significantly different ($p < 0.05$) among the two experiments but in both cases the pattern was the same, with relatively stable values and a slight decrease observed at the end of storage. In E1, an initial value of 6.60 ± 0.02 was observed, with a maximum of 6.74 ± 0.03 on day 6 and a finale value of 6.47 ± 0.04 on day 14 of dry aging. In E2, the initial level was 6.48 ± 0.00 and a constant decrease was observed (final value of 6.39 ± 0.03 at day 14).

Table 2
Average and standard deviation of three replicates of quantitative microbiological analyses (log_{10} CFU/g) during dry aging of rainbow trout in Experiment 1 (E1; T: 3.14 °C; RH: 77.65%) and Experiment 2 (E2; T: 3.57 °C; RH: 78.58%).

Sampling area	Microbiological indicators	Day 0		Day 3		Day 6		Day 10		Day 14	
		E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
Dorsal muscle	Total mesophilic bacteria	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.0 ± 1.0 [a]	< LOQ [b]	2.8 ± 0.2	3.2 ± 0.8
	Total psychrophilic bacteria	< LOQ	< LOQ	< LOQ	< LOQ	1.1 ± 1.0 [a]	< LOQ [b]	< LOQ	< LOQ	3.0 ± 0.5	3.4 ± 0.1
	Pseudomonas spp.	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.1 ± 0.2 [a]	3.9 ± 0.1 [b]
	Enterobacteriaceae	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Lactic acid bacteria	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Coagulase-positive staphylococci	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Yeasts and moulds	< LOQ	< LOQ	1.5 ± 1.3	1.4 ± 1.3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ventral muscle	Total mesophilic bacteria	1.1 ± 1.0	1.4 ± 0.7	< LOQ	< LOQ	3.6 ± 0.3	3.6 ± 0.2	4.2 ± 0.3 [a]	2.8 ± 0.1 [b]	4.4 ± 0.3	5.3 ± 0.7
	Total psychrophilic bacteria	< LOQ	< LOQ	< LOQ	< LOQ	3.7 ± 0.3	3.8 ± 0.4	4.2 ± 0.3 [a]	3.0 ± 0.2 [b]	5.8 ± 0.3	5.4 ± 0.1
	Pseudomonas spp.	< LOQ	< LOQ	< LOQ	< LOQ	2.3 ± 0.5 [a]	3.5 ± 0.3 [b]	2.4 ± 0.6 [a]	4.4 ± 0.0 [b]	6.4 ± 0.5	6.1 ± 0.8
	Enterobacteriaceae	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ [a]	1.5 ± 0.2 [b]	< LOQ	< LOQ	2.8 ± 0.2 [a]	< LOQ [b]
	Lactic acid bacteria	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.1 ± 1.1 [a]	< LOQ [b]
	Coagulase-positive staphylococci	< LOQ	< LOQ	< LOQ	< LOQ	1.5 ± 1.3 [a]	< LOQ [b]	< LOQ	< LOQ	1.8 ± 1.7 [a]	< LOQ [b]
	Yeasts and moulds	< LOQ	< LOQ	< LOQ [a]	1.5 ± 1.3 [b]	< LOQ	< LOQ	< LOQ	< LOQ	3.3 ± 0.4 [a]	< LOQ [b]
Skin	Total mesophilic bacteria	5.4 ± 1.5 [a]	2.5 ± 2.2 [b]	3.4 ± 0.4	3.2 ± 0.3	4.6 ± 0.0	4.5 ± 0.1	3.9 ± 0.3	3.7 ± 0.3	4.4 ± 0.3	5.3 ± 0.9
	Total psychrophilic bacteria	4.9 ± 1.9 [a]	2.0 ± 1.7 [b]	3.6 ± 0.6	3.1 ± 0.2	5.0 ± 0.1	3.9 ± 0.1	3.0 ± 0.0	3.0 ± 0.0	3.7 ± 0.6	5.7 ± 0.7
	Pseudomonas spp.	4.7 ± 0.6	2.8 ± 2.5	4.6 ± 0.7 [a]	< LOQ [b]	2.3 ± 0.4	3.3 ± 0.5	3.3 ± 0.1	2.6 ± 0.6	3.8 ± 1.0	5.2 ± 0.1
	Enterobacteriaceae	4.5 ± 0.2	3.0 ± 0.9	3.3 ± 0.4	2.0 ± 1.8	1.1 ± 0.2	1.7 ± 1.4	1.4 ± 0.4	1.1 ± 0.2	1.7 ± 0.1	1.9 ± 1.1
	Lactic acid bacteria	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.3 ± 0.4 [a]	< LOQ [b]	< LOQ	< LOQ
	Coagulase-positive staphylococci	2.6 ± 0.1	2.3 ± 0.5	< LOQ [a]	1.4 ± 1.3 [b]	3.3 ± 0.4 [a]	1.6 ± 1.4 [b]	2.3 ± 0.3 [a]	< LOQ [b]	3.1 ± 0.2	2.5 ± 0.8
	Yeasts and moulds	< LOQ	< LOQ	3.0 ± 0.1 [a]	2.2 ± 0.2 [b]	< LOQ	< LOQ	2.3 ± 0.2 [a]	< LOQ [b]	2.0 ± 0.0 [a]	< LOQ [b]

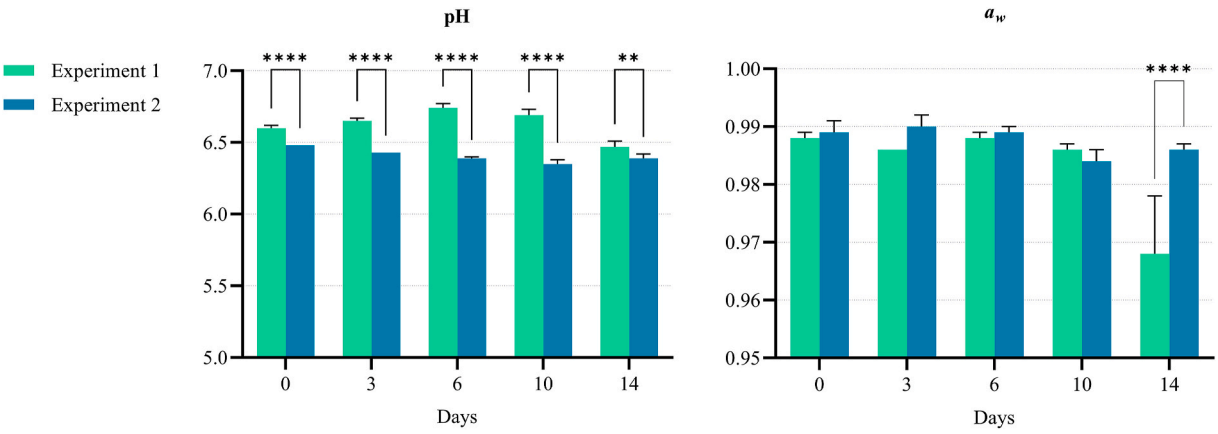

Fig. 3. Changes in pH values and water activity (a_w) in rainbow trout muscle during dry aging (T: 3.14 °C in E1, 3.57 °C in E2; RH: 77.65% in E1, 78.58% in E2). Asterisks indicate the significant differences among the two experiments according to the Šidák's multiple comparison (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001).

3.2.2. Colour

The data presented in Figure 1SM proved a relatively slight modification of colour during dry aging. Measurements of L* (lightness) were stable during the maturing of fish, with highest values observed at day 10 in both experiments (66.07 ± 4.86 in E1, 62.88 ± 1.65 in E2) and significant ($p < 0.05$) differences among the trials only at day 6 of aging. In contrast, the a* (redness) value was less constant in both trials, and the significant ($p < 0.05$) differences between the two experiments at days 3, 10, and 14 highlighted the greater variability of this parameter. The b* (yellowness) indicator followed a similar pattern in both trials, with highest values at day 6 (17.14 ± 2.25 in E1, 17.36 ± 4.11 in E2).

3.2.3. Biogenic amines

Outcomes on biogenic amines are reported in Table 3. Only putrescine was detectable with concentrations ≥ 0.5 mg/kg. The levels were low in both experiments and relatively stable until the end of dry aging, with the highest concentration of 2.05 ± 0.02 mg/kg detected at day 14 in E1 and no significant ($p < 0.05$) differences observed among the two experiments.

4. Discussion

Drying techniques for fish preservation are traditionally applied from many years in several parts of the world, but the employment of fish aging under monitored temperature and humidity conditions at foodservice level is a novelty of the recent years (Fitri et al., 2022; Food and Wine, 2019; Food and Wine Italia, 2021; Identità Golose, 2020; Identità Golose, 2021; LA Eater, 2022; San Francisco Chronicle, 2023). The innovation resides not only in the method itself, but also in the application and management of this practice directly at restaurants. In

Table 3
Average and standard deviation of three replicates of concentrations of biogenic amines (mg/kg) in fish muscle during dry aging in Experiment 1 (E1; T: 3.14 °C; RH: 77.65%) and Experiment 2 (E2; T: 3.57 °C; RH: 78.58%).

Biogenic amines	Day 0		Day 3		Day 6		Day 10		Day 14	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
Putrescine	1.67 ± 0.01	0.93 ± 0.04	1.60 ± 0.01	1.33 ± 0.62	1.56 ± 0.05	1.00 ± 0.02	1.61 ± 0.76	1.12 ± 0.06	2.05 ± 0.02	1.16 ± 0.01
Cadaverine	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Histamine	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Spermidine	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Spermine	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

[1] Values followed by different superscript letters in the same row at each sampling day are significantly different according to the Šidák's multiple comparison ($p < 0.05$).
[2] LOQ = Limit of quantification (0.5 mg/kg).

this context, it is crucial to understand the initial features of fish and the changes in shelf-life and safety indicators during dry aging, in order to assess the overall quality of final products and protect consumers' health. To the best of our knowledge, there are no studies on the safety and quality of whole freshwater fish subjected to dry aging and served in restaurants. To be in line with conditions of foodservice environments and to acquire reliable data considering all variables, we decided to perform the experiments at a local restaurant. To confirm the robustness of data, the experiment was completely replicated twice in each part and with the same conditions. Our approach was based on a comprehensive hazard assessment, including the monitoring of microbiological and chemical indicators, in order to provide preliminary data about the possible impact of dry aging on freshwater fish.

Microbial growth is undoubtedly one of the main causes of the high perishability and reduced shelf-life of fish. The majority of alterative phenomena during fish storage are due to the increase of spoilage bacteria and subsequent formation of specific metabolites (Gram & Dalgaard, 2002; Zhuang et al., 2021). In the present study, we decided to perform analyses differentiating among dorsal and ventral muscle of specimens, as microbial communities and subsequent contamination may significantly differ in these two areas of fish (Wang et al., 2021). To date, there are no defined regulations and microbiological boundaries for dry-aged fishery products. In the absence of precise benchmarks, we selected microbiological indicators widely used to assess quality and shelf-life of fish and which have been previously considered for trout (Atamanalp et al., 2021). The thresholds were determined in accordance with the recommendations of regional organisations (CeIRSA, https://www.ceirsa.org/matrice_alim.php#inizio) and with respect to the scientific literature (Civera et al., 2011; Lyhs, 2002; Mol et al., 2007; Nguyen et al., 2018). Compared to the ranges indicated in Table 1, microbial levels defined as "satisfactory" were identified as more desirable. However, considering that the product underwent a cooking process before being served, microbial levels within the "acceptable" range were also tolerated for determining the shelf-life. Reassuring findings were obtained from both type of samples. Levels often below the quantification limits were observed in dorsal muscle for all populations considered until day 10 of dry aging (see 3.1 and Table 2). Sporadic quantifications occurred at day 3, 6, and 10 for yeasts and moulds, psychrophilic and mesophilic bacteria, but loads were always very low. The higher levels observed at day 14 for mesophilic, psychrophilic bacteria, and *Pseudomonas* spp. were not worrying, since loads were always widely below the satisfactory baseline boundaries considered (see Table 1). A slightly different situation was observed in ventral muscle samples, where higher but still satisfactory microbial loads were detected from day 6 of dry aging. At day 10, the value for mesophilic bacteria in E1 was in the acceptability range considered, while levels for the other populations quantified (psychrophilic bacteria and *Pseudomonas* spp.) were still completely satisfactory. At day 14, a non-satisfactory value was detected for yeasts and moulds (3.3 ± 0.4 $\log_{10}$ CFU/g). Loads were instead still acceptable for the other populations, but it is evident the approaching to critical levels, especially for mesophilic, psychrophilic bacteria, and *Pseudomonas* spp. These microbiological indices upward and close to the maximum considered threshold levels indicated the approaching of spoilage phases in ventral muscle, suggesting that a dry aging time of 10 days is probably the most appropriate for whole rainbow trout at these temperature and relative humidity conditions. Overall, these results firstly highlighted the high-quality level of fish provided by the local farm, since initial loads of microbial populations considered were often under the limit of quantification or below levels previously observed for farmed rainbow trout (Arashisar et al., 2004; Del Torre et al., 2023). In healthy live fish and freshly caught individuals, the muscle is sterile (Huss, 1995). After death, bacteria present on the skin, gills, and intestine can colonize the flesh. The time necessary for bacteria to reach the muscle is variable, but is obviously limited by maintaining temperatures close to 0 °C during transport and storage. The trout used in the present study were slaughtered, vacuum packaged, and transported to the restaurant within 24–48 h keeping a temperature of 0–1 °C. The strict adherence to good hygienic practices during fish slaughtering and initial handling, along with the promptness of transportation and respect of the cold chain, probably allowed the fish muscles to remain almost sterile in the initial stages of the experiments. Furthermore, our outcomes emphasized the potential of dry aging as a valid tool to keep acceptable microbial levels in fish during maturing. Results, in fact, were satisfying not only in relation to selected threshold limits but also in comparison with other surveys performed in standard storage conditions. Chytiri et al. (2004) observed mesophilic counts of ~7.0 $\log_{10}$ CFU/cm^2 after 18 days for whole ungutted trout stored in ice, while *Pseudomonas* reached ~7.0 $\log_{10}$ CFU/cm^2 in filleted trout after 10 days and 6.0 $\log_{10}$ CFU/cm^2 in whole fish. *Enterobacteriaceae* counts in whole ungutted trout samples reached 4.2 $\log_{10}$ CFU/cm^2, while in our study a maximum level of 2.8 ± 0.2 $\log_{10}$ CFU/g was observed at day 14 in ventral muscle. Significant in our experiments was the quantification of lactic acid bacteria only at day 14 in low levels (1.1 ± 1.1 $\log_{10}$ CFU/g), since some of them, including *Latilactobacillus sakei*, *L. curvatus*, and *Carnobacterium piscicola*, are typical spoilers of rainbow trout (Lyhs et al., 2002). It was not surprising that microbiological levels observed on skin were generally higher than in muscle samples in the early stages of the experiments and were extremely variable between trials and various temporal stages of the curing process. Skin was the part of fish exposed to handling and external atmosphere during aging, so this major variability was basically expected. However, these findings do not appear worrying as no significant increases in microbial loads were observed in time, probably due to the drying of skin and the reduction of superficial humidity during aging. Even the indicators that initially exceeded the critical level (*Enterobacteriaceae* in E1 at day 0, yeasts and moulds in E1 at day 3), indeed, fell within an acceptable range from the day 6 of dry aging onwards. In addition, we should emphasize that in this restaurant fish are cooked from the skin side before being served, thereby reducing the risk for consumers. For pathogens detection, we selected *L. monocytogenes* and *S. aureus*. Fish and seafood products, in fact, can often be cause of human listeriosis (Zakrzewski et al., 2024). In addition,

L. monocytogenes is able to persist in several food handling and retail environments, including foodservice and restaurants (Liggans et al., 2019; Toro et al., 2022). *S. aureus*, on the other hand, represents a hazard linked to inappropriate hygienic conditions and manipulations by operators. This bacterium is usual commensal in skin, nares, and gastrointestinal tract of humans. Asymptomatic operators can contaminate food by handling or respiratory secretions (Bencardino et al., 2021). The absence of considered pathogens during the entire duration of both experiments was significant in muscle but especially in skin samples, as external parts can often go in contact with surfaces, operators, and air. The findings of quantitative and qualitative microbiological analyses are in agreement with the only available study on dry-cured fish, in which the process, conducted under controlled time, temperature, relative humidity, and ventilation conditions, did not negatively influence the microbiological safety of salmon, swordfish, and yellowfin tuna (Indio et al., 2024). However, we must mention another biological hazard not considered during our preliminary experiments, but potentially significant for these types of products: non-proteolytic *C. botulinum* and its toxins. Spores of these bacteria, in fact, can be found in aquatic environment and fishery products. The rare outbreaks due to non-proteolytic *C. botulinum* type B, E or F are often associated with consumption of preserved products with extended shelf-life, such as smoked fish, fermented fish, and fish packaged in modified atmosphere (Koukou et al., 2021, 2022; Rawson et al., 2023). Growth of these bacteria and toxin production usually require extended storage time with temperatures comparable to those applied in our study. Graham et al. (1997) observed growth and toxin production from spores of *C. botulinum* types B, E, and F after 5 weeks with 3 °C, 3–4 weeks with 4 °C, and 2–3 weeks with 5 °C in an anaerobic medium. Garren et al. (1995) studied growth and toxin production of *C. botulinum* type E in rainbow trout packaged with either oxygen-barrier or oxygen-permeable films. Toxin was detected after 6 days at 10 °C, but in samples stored at 4 °C no toxins were revealed until 21 days in both types of packaging. In our experiment, the optimal dry aging time was 10 days and the average temperature remained consistently below 4 °C (3.14 °C in E1 and 3.57 °C in E2). Furthermore, the entire process was conducted under strict operators' control and there was no chance of significant temperature fluctuations or thermal abuse, as also proved by the values recorded by the data loggers. Finally, we must mention that products were cooked at a core temperature of 75–85 °C, with possible effect on *Clostridium* toxins and further reduced risks. Woodburn et al. (1979), indeed, recommended 20 min at 79 °C or 5 min at 85 °C as the minimum heat treatment for the inactivation of 10^3 LD_{50} botulinum toxins per gram of different foods. In conclusion, considering temperature conditions, ideal dry aging time, and cooking temperatures used for these products, *C. botulinum* and its toxins were not quantified in our trials since the risk was assessed as low. However, this is a preliminary study and the hazard posed by non-proteolytic *C. botulinum* strains needs to be addressed in future fish curing processes. Product characteristics and dry aging parameters must be properly selected and controlled to prevent the growth and toxin production by these bacteria. The use of specific probes inside the fish flesh to monitor and control all key parameters, such as temperature, pH, a_w, etc., during dry aging could be an extremely useful tool to minimize *C. botulinum* risk. Product characteristics analyses highlighted a gradual decrease of a_w and a relative stability of pH during dry aging (see 3.2.1, Fig. 3, and Table 1SM), even if with some differences among experiments. These fluctuations may be linked to several factors, such as shape and size of fish, as well as slight differences in the parameters maintained during the maturation process. Despite this variability, the observed pattern was the same in both trials, as largely expected considered the relative humidity conditions respected during the process. Similarity in colour evolution was observed in both trials (see 3.2.2 and Figure 1SM). While L* (lightness) value remained relatively stable during the process, a* (redness) parameter showed constant increase until day 6 but decreasing values at day 10 and 14 of dry aging. Regarding b* (yellowness), a little increase at day 6 of dry aging and a subsequent slight decrease until day 14 was observed. A similar evolution of colour parameters was observed by Shen et al. (2015) on rainbow trout fillets packed in polyvinyl chloride bags and stored at 3 °C. Authors, in fact, reported relative stability for L* value until day 15 of storage, while a* reached the higher value (11.78 ± 0.65) at 6 days of storage and decreased slightly until 8.75 ± 0.67 at 15 days. Conversely to our experiments, instead, b* showed a constant deceasing pattern from day 0 to day 15.

Levels of biogenic amines are widely used to evaluate quality, freshness, and safety of fishery products (Atmaca et al., 2023; Costa et al., 2018). More attention is for histamine, as limits for its content in risky fish families are imposed by the Commission Regulation (EC) No. 2073/2005 (Anonymous, 2005) and amendments. No specific tolerable levels are established by the European Legislation for histamine or other amines in low-risk species. However, EFSA emphasized the lack of data on content of other biogenic amines in free histidine-poor fish species (EFSA, 2015). Regarding salmonids, a joint assessment by FAO and WHO in 2018 concluded that the available literature did not support the inclusion of Salmonidae in the same risk category for *Scombrotoxin Fish Poisoning* as other more commonly implicated fish families. However, the review also indicated that, under certain conditions, histamine development could occur in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorynchus mykiss*). Studies on these species, in fact, demonstrated that free histidine in their muscle can be enough to led to histamine formation (FAO & WHO, 2018). Atmaca et al. (2023) proposed putrescine content as freshness indicator for rainbow trout. In this study, in fact, putrescine formation increased significantly in muscle samples stored at 0, 2, and 4 °C. Fluctuating levels of tyramine, spermidine, and spermine with a positive correlation with putrescine concentration were observed, while histamine, tryptamine, and cadaverine were absent or inconsistent. In our study, only putrescine was present in quantifiable levels during dry aging (see 3.2.3 and Table 3). We did not observe a particular increasing trend in concentrations, and the highest putrescine level (2.05 ± 0.02 mg/kg in E1 at day 14) was significantly lower than concentrations observed by Atmaca et al. (2023) (4.76 ± 0.87 mg/kg after 14 days at 0 °C). In summary, dry aging conducted at the curing time, temperature, and humidity conditions of this study did not increase biogenic amine levels in rainbow trout. Similarly, Indio et al. (2024) reported that biogenic amines, including histamine, were under the limit of quantification (3 mg/kg) in dry-cured salmon, swordfish, and tuna. Further data are needed for the assessment of biogenic amines risk in dry-aged fish belonging to other species.

5. Conclusion

In the present study we evaluated the quality and safety of dry-aged rainbow trout ripened at restaurant level. Results of microbiological and chemical analyses were satisfactory, since low microbial loads, absence of foodborne pathogens, and negligible levels of putrescine were detected in dorsal and ventral muscle during storage. The inconstant microbial loads observed on the skin were not worrying, considered that dry-aged trout were cooked on the skin side before consumption. Our approach led to a definition of 10 days as maximum ideal time for dry aging of whole rainbow trout at 3 °C and 78% of relative humidity. Further research involving various fish species and different dry aging conditions is necessary to thoroughly assess the potential risks linked to this emerging and expanding practice within the restaurant sector.

CRediT authorship contribution statement

Felice Panebianco: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Maria Nobile:** Writing – original draft, Visualization, Methodology, Investigation. **Giorgio Pasinetti:** Methodology, Investigation. **Daniele Pattono:** Writing – review & editing, Methodology, Investigation. **Sara Panseri:** Writing – review & editing, Methodology, Investigation.

Tiziana Civera: Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing 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.

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Appendix A. Supplementary data

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