PhD THESIS

Risk assessment and management of fish meat intended for public consumption in a regional context

(SUMMARY OF PhD THESIS)

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Introduction

The consumption of fish and fish-derived products has increased significantly in recent decades, driven both by the recognition of their exceptional nutritional value and by international dietary recommendations. Fish constitutes an important source of high-quality proteins, polyunsaturated fatty acids essential for cardiovascular health and cognitive functions, as well as fat-soluble vitamins and minerals. At the same time, the physiological and biochemical characteristics of fish muscle tissues make this type of food extremely perishable and highly sensitive to environmental, technological, and microbiological factors.

Against the backdrop of climate change, the intensification of aquatic pollution, and the diversification of processing and preservation methods, the assessment of fish quality and safety has become a priority both for fundamental research and for the applied domain of veterinary and human public health. In this context, the present doctoral thesis aims to provide an integrated analysis of the factors that influence fish quality, with a focus on chemical composition, freshness, microbiological contamination, the presence of chemical contaminants, and the impact of preservation processes on product stability.

The structure of the thesis was designed to capture both the fundamental dimension—by exploring the mechanisms of spoilage and contamination—and the applied dimension, through its relevance to food safety, public health, and the food industry.

Current state of knowledge

Chapter 1 – Importance of fish consumption and characteristics of its chemical composition

The scientific literature highlights significant variations in the chemical composition of different fish species, as well as within the same species, depending on habitat, diet, season, or exposure to pollutants. The essential role of proteins and omega-3 polyunsaturated fatty acids in defining the nutritional value of fish is consistently confirmed, alongside the vulnerability of lipids to oxidative processes, which affect both sensory stability and product safety. Consequently, the assessment of chemical composition remains a fundamental element in characterizing fish quality.

Chapter 2 – Spoilage mechanisms of fish meat

Freshness is a key criterion for consumer acceptance and food safety. The most frequently used biochemical and physicochemical indicators are pH, total volatile basic nitrogen (TVB-N), and ammonia, which reflect post-mortem metabolic changes. Recent literature emphasizes that these parameters must be correlated with microbiological factors and with strict compliance with the cold chain, as the accelerated spoilage of fish products is strongly influenced by storage and handling conditions.

Chapter 3 – Pathogenic microorganisms in fish meat

Fish constitutes a favorable substrate for the development of a diverse microflora, including spoilage bacteria (*Pseudomonas, Aeromonas, Shewanella*), as well as species with pathogenic potential. Numerous studies have reported the presence of Enterobacteriaceae, *E. coli*, and even zoonotic agents in fishery products, highlighting the importance of continuous monitoring. Freezing and refrigeration contribute to reducing microbiological risk; however, they do not eliminate it completely and do not prevent oxidative spoilage processes. At the same time, the literature draws attention to the role of opportunistic pathogenic species (*Pseudomonas, Acinetobacter*) as indicators of hygiene and processing quality.

Chapter 4 – Chemical contaminants in fish meat

Particular attention is paid to chemical contaminants, especially heavy metals and polycyclic aromatic hydrocarbons (PAHs). The bioaccumulation of heavy metals depends on the characteristics of the aquatic environment and can significantly affect the ecotoxicological quality of fish, while also posing a risk to consumer health. In parallel, the smoking process generates PAHs, compounds with carcinogenic potential that are strictly regulated at the European level. The scientific literature confirms that PAH levels depend on the intensity of the smoking process, the type of fuel used, and the technological conditions, which necessitates rigorous standards to ensure compliance and the safety of smoked products**.**

Personal research

Working hypothesis and research objectives

The working hypothesis of this thesis is based on the premise that the compositional, microbiological, and chemical parameters of fish are influenced both by intrinsic species-related factors and by extrinsic factors related to the environment and to processing and preservation technologies. It is assumed that aquatic pollution, storage and processing methods, as well as hygienic conditions throughout the food chain, exert a cumulative impact on the quality and safety of fish products. Accordingly, the thesis aims to demonstrate that an integrated approach—combining chemical, biochemical, microbiological, and toxicological analyses—enables a comprehensive characterization of fish quality and the formulation of practical recommendations for consumer health protection.

The general objectives of the research were as follows:

- To perform a comparative evaluation of compositional quality, microbiological status, and heavy metal contamination in rainbow trout (*Oncorhynchus mykiss*) and crucian carp (*Carassius carassius*) originating from the north-western region of Romania.
- To assess oxidative spoilage processes and their correlation with the level of microbial contamination in frozen fish.
- To analyze the dynamics of microbial load and histamine production in rainbow trout (*Oncorhynchus mykiss*) and mackerel (*Scomber scombrus*) stored on ice flakes.
- To evaluate the compositional quality, microbial contamination, and polycyclic aromatic hydrocarbon (PAH) levels in various cold-smoked fish products intended for public consumption.

Study 1 – comparative evaluation of compositional quality, microbiological status, and heavy metal contamination in rainbow trout (*Oncorhynchus mykiss*) and crucian carp (*Carassius carassius*) from northwestern romania

The first study focused on the characterization of fresh fish, with particular emphasis on rainbow trout (*Oncorhynchus mykiss*) and crucian carp (*Carassius carassius*), two representative freshwater species widely consumed in Romania. The research compared samples originating from areas with different levels of pollution in order to evaluate the impact of environmental factors on nutritional quality and product safety.

The compositional analysis revealed a high protein content, confirming the dietary value of these species. However, specimens collected from contaminated areas showed a significant reduction in protein levels, suggesting a negative influence of pollution on nitrogen metabolism. In parallel, lipid levels were higher in fish from polluted zones, a phenomenon that may be explained by adaptive mechanisms to environmental stress or by differences in the availability of trophic resources. Moisture content varied inversely with lipid levels, while collagen values remained relatively stable, indicating a low sensitivity of this parameter to environmental conditions.

The assessment of freshness, based on biochemical parameters (NH_3, TVB-N, and pH), showed values generally within the acceptable limits for fresh or relatively fresh fish. Nevertheless, some samples from contaminated areas exceeded the critical thresholds for TVB-N and pH, indicating an increased susceptibility to spoilage. These results are consistent with findings reported in the literature, which emphasize that environmental factors can accelerate post-mortem degradation and compromise the sensory quality of fish.

From a microbiological perspective, the overall bacterial load was moderate and fell within acceptable limits, attesting to a satisfactory quality of the analyzed fish. However, opportunistic pathogenic bacteria were detected, including Listeria innocua, a species generally considered non-pathogenic but currently recognized as a hygiene indicator with potential zoonotic implications, as suggested in recent studies. The absence of *Salmonella* spp. was a favorable outcome, indicating a low risk of transmission of this major zoonotic agent through consumption.

The determination of heavy metals showed low levels of lead and copper and the absence of cadmium, suggesting a reduced toxicological risk for consumers. Zinc, however, exhibited an increasing trend in samples from contaminated areas, confirming its bioaccumulation capacity and its role as a sensitive biomarker of aquatic pollution.

In conclusion, this study demonstrated that rainbow trout and crucian carp are valuable nutritional sources of proteins and minerals, yet their quality may be influenced by the degree of environmental pollution in their habitat. The integration of chemical, biochemical, and microbiological results highlights the importance of strict monitoring of aquatic environments, as well as rigorous hygienic control along the production chain, in order to ensure consumer safety and preserve the nutritional value of fresh fish.

Study 2 – Assessment of oxidative spoilage processes and their correlation with microbial contamination in frozen fish

The second study investigated the dynamics of oxidative and microbiological processes in rainbow trout fillets stored at −20 °C for 12 months. The aim was to assess

the effectiveness of freezing as a long-term preservation method while also highlighting its limitations in maintaining product quality.

The lipid oxidation assessment showed a constant progression during storage. The concentration of malondialdehyde (MDA), used as a marker of lipid peroxidation, increased from 0.13 mg/kg at the beginning to 1.07 mg/kg after 12 months. Exceeding the threshold of 1.0 mg/kg—considered in the literature as the limit for sensory acceptability—indicates that autoxidation processes continue even at subzero temperatures. The mechanisms involved include the action of residual oxygen, the presence of metal ions, and hem pigments, which catalyze oxidative reactions even in the absence of significant microbial activity.

The microbial configuration revealed progressive changes throughout freezing. At the initial stage, the microbiota was diverse, including spoilage bacteria such as *Pseudomonas, Aeromonas*, and *Shewanella*, as well as potentially pathogenic species such *as E. coli* and *Vibrio parahaemolyticus*. After 6 months of storage, diversity decreased significantly, with psychrotrophic bacteria capable of surviving low temperatures being selectively retained, particularly *Pseudomonas, Psychrobacter*, and *Acinetobacter*. At the same time, a drastic reduction of E. coli was observed. After 12 months, the microbiota became even more simplified, being dominated by *Pseudomonas* (42%), *Psychrobacter* (32%), and *Shewanella* (10%), while enteric and pathogenic species disappeared completely.

The correlation between oxidative and microbiological parameters revealed a strong inverse relationship ($r \approx -0.91$) between MDA levels and the total microbial load. This association confirms that oxidative processes were not stimulated by bacterial metabolism but by non-biological factors, which is plausible at –20 °C where microbial activity is minimal.

From a food safety perspective, the results demonstrate that long-term freezing reduces microbiological risk by gradually eliminating enteric and pathogenic species, thus keeping the product safe for consumption even after one year of storage. However, lipid oxidation limits sensory stability and product acceptability, indicating that microbiological safety does not necessarily overlap with organoleptic stability.

These findings highlight the need for complementary technological strategies to preserve the quality of frozen fish over extended storage periods. Such measures include vacuum or modified-atmosphere packaging, the use of high-barrier materials against oxygen, and the application of natural antioxidants. Therefore, freezing at –20 °C is confirmed as an effective method for microbiological safety, but insufficient to prevent oxidative deterioration, making it necessary to integrate additional technologies to ensure both safety and sensory acceptability.

Study 3 – Analysis of microbial load dynamics and histamine production in rainbow trout (*Oncorhynchus mykiss*) and mackerel (*Scomber scombrus*) stored on ice flakes

The third study evaluated the biochemical and microbiological changes occurring in fish stored on ice flakes, using rainbow trout (*Oncorhynchus mykiss*), a freshwater species, and mackerel (*Scomber scombrus*), a marine species, as experimental models. The objective was to determine the dynamics of spoilage during the shelf life, with emphasis on freshness, microbiological profile, and toxicological potential.

The microbiological analysis showed a constant increase in the total bacterial load from the first days until the end of the storage period. Although the values remained below the maximum thresholds set by European regulations, it was observed that the final days of shelf life were characterized by a much higher bacterial density compared to the first three days. This confirms that the risk of spoilage increases exponentially with storage time. The isolated microbiota was dominated by Gram-negative bacilli, including *Pseudomonas fluorescens, Acinetobacter lwoffii*, and *Stenotrophomonas maltophilia*, species known for their capacity to induce organoleptic deterioration. In addition, zoonotic bacteria such as *Escherichia coli, Yersinia enterocolitica*, and *Francisella tularensis* were identified, highlighting the importance of proper thermal preparation of fish products.

The biochemical determinations included pH and total volatile basic nitrogen (TVB-N), both recognized indicators of freshness. The pH values showed a progressive increase, correlated with microbial development, confirming the literature which states that bacterial activity generates the accumulation of alkaline compounds. TVB-N values also increased gradually, reaching the critical limits established for fresh fish toward the end of storage.

Another parameter of interest was the histamine level, implicated in foodborne intoxications. The results showed the absence of histamine during the first days of storage and low values thereafter, well below the toxic threshold of 200 mg/kg. These findings indicate a reduced risk of histamine poisoning when proper ice refrigeration conditions are maintained. However, the progressive accumulation of histamine confirms that species such as mackerel may become hazardous if the cold chain is interrupted or if the storage period is excessively prolonged.

From a food safety perspective, the results suggest that rainbow trout and mackerel stored on ice can be safely consumed for up to nine days, a period that ensures a balance between sensory acceptability and microbiological safety. Exceeding this storage interval increases the risk of spoilage and reduces the nutritional and sensory quality of the products.

In conclusion, the study confirms that ice refrigeration is an effective method for maintaining fish quality in the short term but requires careful monitoring of biochemical and microbiological indicators. Integrating these parameters into quality control programs allows for the rapid identification of the optimal consumption window and reduces the risk of foodborne illnesses.

Study 4 – Evaluation of compositional quality, microbial contamination, and polycyclic aromatic hydrocarbons (PAHs) in cold-smoked fish products for public consumption

The fourth study focused on the characterization of cold-smoked fish, analyzing four species of relevance to the consumer market: rainbow trout (*Oncorhynchus mykiss*), salmon (*Salmo salar*), mackerel (*Scomber scombrus*), seasoned mackerel (*Scomber scombrus*), and sprat (*Sprattus sprattus*). The main objective was to evaluate nutritional characteristics, freshness, microbiological quality, and contamination with polycyclic aromatic hydrocarbons (PAHs), compounds specifically generated by the smoking process.

The compositional analysis revealed notable differences among species. Trout and salmon showed a favorable nutritional profile, characterized by high protein content and low lipid levels, making them suitable for a balanced diet with reduced fat intake. In contrast, mackerel and seasoned mackerel exhibited high lipid concentrations, which provide superior energy value but also greater susceptibility to oxidative processes. Sprat had the lowest protein content but stood out for its higher collagen level, directly influencing texture and sensory acceptability.

The freshness assessment revealed interspecies variability. Salmon and sprat exceeded the maximum admissible values for TVB-N and, in the case of salmon, also for NH_3, indicating a loss of freshness before the expiry date. This suggests deficiencies in maintaining the cold chain during storage and distribution. By contrast, trout, mackerel, and seasoned mackerel showed values within the standard limits for all parameters, confirming acceptable freshness throughout the recommended consumption period.

The microbiological results also reflected significant differences. Salmon and sprat showed high total bacterial counts (6 log CFU/g), accompanied by the presence of Enterobacteriaceae and E. coli, which correlated with unfavorable freshness indicators and pointed to possible hygiene deficiencies after processing. Mackerel and seasoned mackerel showed intermediate levels (5.2 log CFU/g), consistent with an acceptable hygienic status. Trout displayed the best microbiological profile, with low total viable counts (2.6 log CFU/g), suggesting effective control of the smoking process and proper cold storage. The absence of Salmonella spp. was a favorable finding; however, the detection of *Listeria welshimeri* indicated the presence of an environmental reservoir, relevant for the potential risk posed by Listeria monocytogenes.

The chemical contaminant analysis focused on PAHs, compounds strictly regulated at the European level. Salmon and trout had the lowest ΣPAH values, while sprat and mackerel showed intermediate levels. Seasoned mackerel presented higher concentrations, though dominated by 2–3 ring compounds, which are considered less toxicologically relevant. All products complied with the limit for benzo[a]pyrene (≤2.0 µg/kg), but plain smoked mackerel slightly exceeded the PAH4 limit of 12 µg/kg. This finding confirms that PAH4 is the decisive indicator for consumer protection, in line with European regulations.

In conclusion, this study demonstrated that cold-smoked fish has a variable nutritional value, influenced by both species and processing technology. Trout and salmon emerged as products with superior dietary value, whereas mackerel and seasoned mackerel require increased attention with respect to oxidative stability and PAH levels. Salmon and sprat proved more vulnerable to freshness loss, underlining the importance of strict compliance with the cold chain. Overall, the correlation of compositional, microbiological, and toxicological results underscores the crucial role of technological control and official monitoring in ensuring the quality and safety of cold-smoked fish products.

General conclusions

The research carried out within this thesis confirmed the high nutritional value of fish, while also highlighting its vulnerability to environmental factors, technological conditions, and microbiological and chemical contaminants. The results allowed the formulation of integrated conclusions, structured along the main directions of analysis.

With regard to chemical composition, trout and salmon were distinguished by a favorable protein-to-lipid ratio, confirming their dietary value. By contrast, mackerel and seasoned mackerel exhibited high lipid levels, which provide greater energy value but expose them to increased oxidation risks. Crucian carp showed low values for heavy metals, below the admissible limits, confirming a low toxicological risk for consumers. At the same time, zinc emerged as a sensitive biomarker of aquatic environmental pollution.

The freshness assessment demonstrated that biochemical parameters (pH, TVB-N, NH_3) and microbiological dynamics are reliable indicators for evaluating the quality of fish products. Trout and crucian carp from contaminated areas, as well as smoked salmon and sprat, showed increased susceptibility to spoilage, underscoring the importance of strict compliance with the cold chain during handling and storage.

From a microbiological perspective, both fresh and smoked fish presented a diverse flora, including spoilage bacteria and opportunistic pathogenic species. Freezing proved effective in reducing microbial diversity and eliminating enteric and pathogenic

species after 12 months, confirming the role of this method in reducing zoonotic risk. However, the persistence of psychrotrophic bacteria in refrigerated and smoked products indicates the need for strict hygienic control.

Regarding oxidative processes and specific contaminants, the findings confirmed that lipid oxidation progresses even at subzero temperatures, exceeding sensory acceptability thresholds after prolonged storage. Histamine, monitored in fish refrigerated on ice, remained below toxic levels, indicating a low risk when refrigeration conditions are properly maintained. In the case of smoked fish, polycyclic aromatic hydrocarbon (PAH) levels generally complied with legal limits, with only one minor exceedance of PAH4, confirming that adherence to technological parameters is decisive for product safety.

Overall, the results support the necessity of an integrated One Health approach that correlates compositional, microbiological, and chemical data for a rigorous evaluation of fish quality and safety. The thesis demonstrates that preventive and control measures, applied consistently throughout the production and distribution chain, are essential for safeguarding public health and preserving the nutritional value of fish products.

Recommendations

Based on the results obtained, a series of scientific and practical recommendations can be formulated, addressed to the food industry, control authorities, and consumers:

Monitoring of aquatic environments and raw materials:

- Regular evaluation of water quality and pollutant levels in aquaculture areas is recommended, with particular attention to heavy metals, in order to prevent bioaccumulation and its impact on the nutritional value of fish.
- The identification of zinc as a sensitive biomarker calls for its inclusion in ecotoxicological monitoring programs.

Maintenance of the cold chain:

- Strict compliance with optimal storage and transport temperatures is necessary for both fresh and smoked fish to reduce the risk of premature spoilage.
- For fish stored on ice, consumption is recommended up to the ninth day of storage, avoiding consumption at the upper limit of commercial shelf life.

Complementary preservation technologies:

- Freezing ensures microbiological safety but does not prevent lipid oxidation; therefore, the use of vacuum packaging, modified atmosphere packaging, and natural antioxidants is recommended to maintain sensory quality.

- In the case of smoked fish, strict control of smoking intensity, wood type, and spice additives is essential to keep PAH levels within legal limits.

Hygiene in processing and handling:

- The presence of opportunistic pathogenic bacteria and species with zoonotic potential underscores the importance of strict adherence to hygiene standards at all stages of the production chain.
- Staff training and the implementation of HACCP programs are recommended to reduce the risk of contamination.

Consumer information and education:

- Fish products should be accompanied by clear labeling regarding shelf life, storage conditions, and preparation recommendations.
- Consumers should be made aware of the necessity of proper cooking, particularly in the case of fresh or cold-smoked fish.
- These recommendations aim to support the implementation of integrated control measures that guarantee the maintenance of fish quality and safety throughout the entire chain, from producer to consumer.

Originality and innovative contributions of the thesis

The originality of this thesis lies in its integrated and comparative approach to several fish species—rainbow trout, crucian carp, mackerel, salmon, and sprat—analyzed in different forms of presentation (fresh, frozen, refrigerated on ice, and cold-smoked). The novelty of the scientific endeavor derives from the complex character of the research, which combines chemical, biochemical, microbiological, and toxicological evaluations with the aim of obtaining a comprehensive picture of fish quality and food safety.

A distinctive element of originality is the correlation of oxidative spoilage processes with microbiological parameters in frozen fish. The results demonstrated that lipid oxidative degradation follows a dynamic independent of bacterial activity at subzero temperatures, representing a novel contribution to the understanding of spoilage mechanisms under long-term storage conditions. This aspect, relatively underexplored in the scientific literature, supports the necessity of introducing complementary technological measures to maintain the quality of frozen products.

The thesis also provides original data regarding the influence of aquatic pollution on the chemical composition and microbiological profile of freshwater fish. The identification of zinc bioaccumulation trends in contaminated areas and the decrease of protein content in exposed species constitute significant contributions to the assessment of the impact of pollution on the nutritional value of fish.

The study of cold-smoked fish generated new insights into the relationship between nutritional profile, oxidative stability, freshness, and contamination with polycyclic aromatic hydrocarbons (PAHs). The correlation of compositional results with PAH levels and microbiological profiles represents an important practical contribution to the foundation of food safety measures and the optimization of technological processes.

Another original aspect is the creation of a comprehensive database resulting from the application of standardized methods to a diversified biological material, allowing the formulation of generalized conclusions for multiple species and processing types. The integration of these data with European regulations on chemical and microbiological contaminants adds applied value and practical relevance to the research.

Through its applied character, this thesis contributes both to the advancement of scientific knowledge and to the improvement of strategies for fish quality and safety control, in accordance with the principles of the One Health approach. The work demonstrates that the integrated evaluation of biochemical, microbiological, and chemical factors is an essential prerequisite for protecting public health and ensuring the quality of fish products throughout the entire food chain.