PhD THESIS

Assessment of the quality of traditional smoked trout

(SUMMARY OF Ph.D. THESIS)

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Introduction

Global aquaculture is experiencing continuously increasing production levels, with the demand for fish, seafood, and products associated with this sector growing significantly. According to the Food and Agriculture Organization of the United Nations (FAO), in 2020, global aquaculture production reached 122.6 million tons, indicating a considerable increase compared to previous years. The total value of this production is estimated at approximately 281.5 billion US dollars (FAO, 2022).

Trout consumption is popular worldwide, and trout meat is of high nutritional quality, being a rich source of essential nutrients in human diets (Simopoulos, 2002). Methods for preserving fish meat are essential for maintaining its availability, nutritional quality, and safety. Key techniques include refrigeration, freezing, salting, drying, smoking, and canning. Refrigeration and freezing are widely used to slow microbial growth and enzymatic activity, thus extending shelf life. For example, freezing fish at -18°C or lower can preserve it for several months (Tirado et al., 2010).

Smoking is a traditional fish preservation method that combines dehydration, the antibacterial properties of smoke, and cooking to extend shelf life and enhance flavor. Smoking improves the sensory qualities of fish while providing a barrier against spoilage-causing microorganisms and oxidative rancidity. This method remains popular worldwide, from traditional practices to large-scale industrial operations.

Through this doctoral thesis, we aimed to make contributions to ensure the safety and quality of traditionally smoked trout by attempting to evaluate the physicochemical, microbiological parameters, and polycyclic aromatic hydrocarbon content that directly influence the product's safety and quality.

SCOPE AND RESEARCH OBJECTIVES

THE PURPOSE of the doctoral thesis entitled "Evaluation of the quality of Traditional Smoked Trout" was to analyze the technological process and the influence of different types of packaging used after traditional smoking in order to ensure the quality of the finished product.

The objectives of the thesis are represented by:

1. Summarizing current knowledge related to the traditional smoking preservation of rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis);
2. Organizing the experimental setup in a farm with an intensive farming system and the necessary facilities for traditional fish smoking;

3. Researching the physicochemical parameters of traditionally smoked trout to determine quality, safety, and shelf life, in different types of packaging;
4. Researching the microbiological parameters of traditionally smoked trout to determine quality, safety, and shelf life, in different types of packaging;
5. Researching the polycyclic aromatic hydrocarbons (PAHs) content of traditionally smoked trout to determine quality, safety, and shelf life, in different types of packaging.

DOCTORATE THESIS STRUCTURE

The PhD thesis titled "Evaluation of the Safety and Quality of Traditionally Smoked Trout Processed in Different Types of Packaging " comprises a total of 120 pages and is structured into two parts, respectively Part I – "Current stage of knowledge" and Part II – „Personal Contribution".

1. The current stage of knowledge is structured into four chapters
2. The personal contribution is structured in six chapters

PART I - CURRENT STAGE OF KNOWLEDGE

Part I comprises 4 chapters and represents a summary of the current knowledge related to the current status of the Salmonidae family, production systems in salmon farming, preservation methods, and traditional smoking preservation.

Chapter I is entitled "General Considerations Regarding the Salmonidae Family" and includes 3 subchapters: Salmon farming at global, European, and national levels; Salmon farming systems; and Main salmonid species exploited in Romania.

Chapter II is entitled "Nutritional Quality and Chemical Composition of Trout Meat." This chapter includes 2 subchapters: Protein content of trout meat; Lipid content of trout meat.

Chapter III is entitled "Food Preservation: Fish Preservation Methods" and includes 3 subchapters: Refrigeration and freezing; Salting and drying; Smoking fish; Fish canning; Methods for extending the shelf life of fish products.

Chapter IV is entitled "Technology of Smoking Fish Products" and includes 5 subchapters: Types of smoking; Wood materials for generating smoke for smoking; Physical and chemical properties of smoke; Smoke generation; Effects of smoking.

PART II - PERSONAL CONTRIBUTION

Part II comprises six chapters and represents the personal contribution. Within these chapters, the goals and objectives of the research, the biological material

and methods used, the results of the studies and related discussions, general conclusions and recommendations, as well as the elements of originality and innovative contributions of the thesis, are presented.

Chapter V is entitled "Goals and Objectives of the Research," presenting the 5 previously mentioned objectives and the experimental design.

Chapter VI is entitled "Description and Characterization of the Salmon Farm," including the characteristics of the environment (salmon farm) where the research was conducted.

Chapter VII is entitled "Material and Methods," where the following aspects are presented: biological material, the technological process used to obtain traditionally smoked trout, methods used for each specific objective, as well as statistical methods used for advanced data analysis.

Chapter VIII is entitled "Results and Discussions" and includes 4 subchapters presenting all research results and the discussions based on them.

Chapter IX is entitled "General Conclusions and Recommendations" and includes the general conclusions and recommendations derived from the research results.

Chapter X is entitled "Originality and Innovative Contributions of the Thesis" and includes the elements of originality and the contributions of the thesis to the scientific community.

RESEARCH RESULTS

Organization of the experimental device

The biological material analyzed in the study consists of 100 specimens of Rainbow trout (*Oncorhynchus mykiss*) (Fig. 1) and 100 specimens of Brook trout (*Salvelinus fontinalis*) (Fig. 1), sourced from the Trecătoarea Ursului salmonid farm in Braşov County. The number of specimens analyzed in the study was 100 individuals per batch, with a total of 200 specimens being collected, prepared, and traditionally smoked. The average weight of the rainbow trout was 231.3 ± 0.73 g, and the average total length was 27.3 ± 0.58 cm. For the brook trout, the average weight was 216.77 ± 0.34 g, and the average total length was 26.88 ± 0.5 cm.

Fig. 1 Rainbow trout (Oncorhynchus mykiss) and Brook trout (Salvelinus fontinalis)

All experiments and procedures applied to animals complied with current Romanian legislation (PR 43/2014) and European legislation (EC 2010/63/EU). The study received approval from the Bioethics Committee of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (No. 145/2019) and

adhered to all relevant bioethical guidelines for animal studies established by the Institutional Animal Bioethics Committee.

The Trecătoarea Ursului salmon farm is located within the territorial area of Râşnov city, Braşov County, approximately 7 km from the city center, in the Valea Glăjeriei area, near the DN73A Râşnov-Predeal road. The nature of its activities does not produce a significant negative impact on the biodiversity of the area, as the aquaculture technological processes harmoniously fit the specifics of the mountainous region in which it is located. It is an intensive aquaculture unit for raising salmonids and is situated at the foot of the Bucegi Massif (Fig. 2).

Fig. 2 General view of Trecătoarea Ursului salmonid farm (original)

Results Regarding the Evaluation of the Physicochemical Parameters of Traditionally Smoked Trout

The physicochemical parameters of the products are influenced by species, diet, age, processing method, and other factors. The protein content in smoked fish showed a decrease in the first 20 days (from 23.38% to 22.05%), followed by a slight increase in the next 10 days (up to 22.26%) (Fig. 3). The nitrogen content of the two species studied varied during the experimental period. In the case of rainbow trout, it fluctuated between 3.53% and 3.69%, with the maximum value at T0. Brook trout had a nitrogen content between 3.52% and 3.66%.

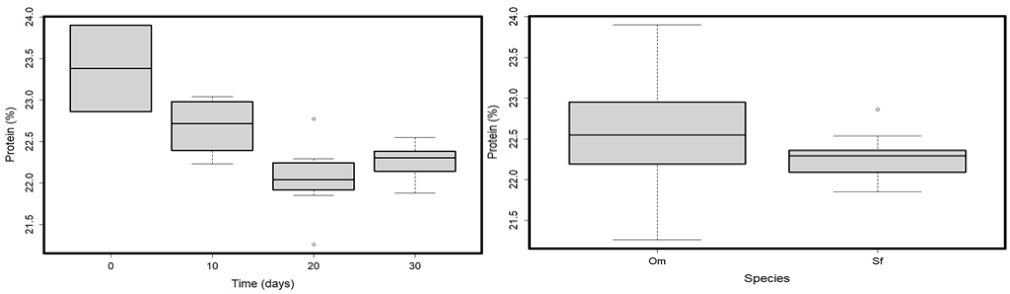

Fig. 3 (a) Protein content values variation over time; (b) Protein content values in the two analyzed species (O. mykiss – Om and S. fontinalis – Sf). Plots represent median (line inside the box) values, 25-75 percent quartiles (boxes), and minimal and maximal values, shown with short horizontal lines ("whiskers"). Outliers are represented with an open circle sign.

The lipid content determined from smoked fish meat in this study showed a decreasing trend over the sampling periods. Lipids are essential as they are involved in a variety of metabolic, cellular, and signaling processes, including cellular membrane construction, selective permeability, lipid rafts, and steroid hormone synthesis (van Meer et al., 2008).

The pH value in the samples studied was slightly acidic, ranging between 6.22 and 6.39. Çoban et al., 2014, observed a pH of 6.48 in fresh samples of rainbow trout, which decreased to 5.75-5.91 after smoking. Kiczorowska et al., 2019, also found a decrease in pH of rainbow trout, from 5.9 in fresh samples to 5.7 in smoked ones.

TVB-N (Total Volatile Basic Nitrogen) is a parameter used to assess the degree of food spoilage, especially for fish. Typically, the TVB-N value for freshwater species ranges from 10-20 mg/100 g (Fıcıcılar and Genccelep, 2017). According to EC regulations 2074/2005 (Commission Regulation (EC), 2005), the TVB-N content should not exceed 25-35 mg/100 g, depending on the fish species. TVB-N is strongly influenced by storage time and temperature.

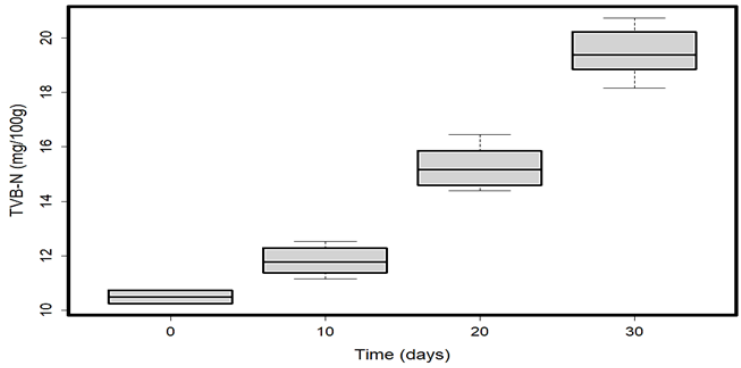

Fig. 4. TVB-N values variation in time. Plots represent median (line inside the box) values, 25-75 297 percent quartiles (boxes), and minimal and maximal values, shown with short horizontal lines ("whiskers").

We observed a significant effect of time on TVB-N levels (Fig. 4). The most significant differences were observed between T0 (mean = 10.51 mg/100g, SD = 0.35) and T20 (mean = 15.26 mg/100g, SD = 0.76), as well as T30 (mean = 19.47 mg/100g, SD = 0.90). Significant differences were also recorded between T10 (mean = 11.82 mg/100g, SD = 0.52) compared to T20 and T30, as well as between T20 and T30. The results obtained were below the maximum allowable threshold. Bienkiewicz et al. also observed an increase in TVB-N value after hot smoking of rainbow trout, from 7 to 9 mg/100 g.

Results on the Microbiological Parameters of Traditional Smoked Trout

In the context of consumers, efficient management of the fish smoking process is crucial for ensuring the quality and safety of the product. This involves the complex management of stages such as harvesting, processing (including smoking), transportation, storage, and the type of packaging used, all of which are critical factors that can influence multiple parameters of the product (Wiernasz et al., 2021; Mei and Xie, 2019; Maillet et al., 2021).

In this research, microbiological analyses included the detection of Salmonella spp., E. coli, Yersinia spp., L. monocytogenes, and Total Aerobic Mesophilic Bacteria (TAMB). Among these five microbiological parameters investigated, only detectable levels of E. coli and TAMB were identified (Fig. 4).

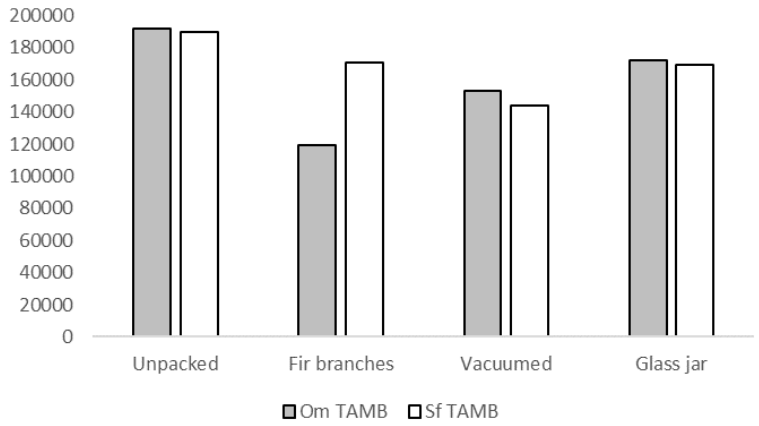

Fig. 4. Values of Total Aerobic Mesophilic Bacteria (TAMB, CFU/g) in the two analyzed species (O. mykiss – Om and S. fontinalis – Sf) after 30 days of storage (T30) with different packaging types

In our study, the microbiological load remained below limit values. The observation of bacterial growth suppression during storage, regardless of the type of

packaging and storage conditions (2-4°C), highlighted the effectiveness of hot smoking treatment in preventing bacterial proliferation.

According to specialized literature, accepted values for E. coli immediately after the completion of the production process should be <10 CFU/g, with a maximum acceptable value of 10^3 CFU/g throughout the shelf life of the food product (Medonca et al., 2020). Numerous studies have shown that hot smoking has inhibitory effects on foodborne pathogens such as L. monocytogenes, Aeromonas hydrophila, Yersinia enterocolitica, E. coli, applicable to various smoked products [Zaki et al., 2021; Abdul-Baten et al., 2020; Rana et al., 2021].

Results on the Polycyclic Aromatic Hydrocarbons (PAHs) Content in Traditional Smoked Trout

PAHs are aromatic compounds composed of carbon and hydrogen atoms, with two or more fused benzene rings, and they are ubiquitous in the environment. They are primarily formed as a result of human activities and can be found in foods exposed to incomplete combustion of organic matter. Contamination with PAHs in food occurs both through exposure to polluted environments and during food processing such as smoking, drying, or grilling (Chen, 1997; Zelinkova and Wenzl, 2015).

Biological monitoring of PAH exposure is of paramount interest given the widespread presence of these compounds and their toxicological relevance. Seventeen PAHs have been identified as particularly concerning in terms of potential exposure and adverse health effects on humans (Husein et al., 2016). Some of these compounds are known or suspected human carcinogens, including Benzo[a]pyrene, Naphthalene, Chrysene, Benzo[a]anthracene, Benzo[k]fluoranthene, and Benzo[b]fluoranthene.

In our study, despite using the traditional smoking method involving high temperatures and direct exposure to smoke, out of the fifteen PAHs analyzed, compounds such as Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene, and Indeno[1,2,3-cd]pyrene were found at undetectable levels in samples. The other PAHs showed variable proportions depending on the type of packaging after 30 days of storage. However, all determinations complied with the values identified in the specialized literature regarding PAH content in smoked meat products [Bogdanović et al., 2019; Duedhal-Olsen et al., 2010], as well as in regulations No. 1881/2006/EC and No. 1255/2020/EC (European Commission (EC), 2006; Commission Regulation (EU), 2020).

Conclusions

This doctoral thesis aimed to evaluate the safety and quality of traditionally smoked rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis), focusing on the influence of physico-chemical parameters, microbiological aspects, and polycyclic aromatic hydrocarbons (PAHs) content.

The traditional smoking process involves several critical stages, from harvesting the biological material and stunning the fish to evisceration, washing, salting, desalting, drying, hot smoking, and finally, cooling and storing the finished product. Each stage was meticulously executed to ensure the quality and preservation of the fish, adhering to traditional methods that involve high temperatures and direct exposure to smoke.

In this study, 15 PAH compounds were analyzed, with results indicating that compounds such as Benzo[a]pyrene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene, and Indeno[1,2,3-cd]pyrene were undetectable in the samples tested. The remaining PAHs showed varying proportions depending on the type of packaging used after a 30-day storage period.

These findings highlight the effective application of traditional smoking methods in reducing PAH content, thereby maintaining the safety and quality of the smoked product. The study contributes to understanding traditional smoking processes and developing recommendations to enhance these processes for the protection of consumer health and the assurance of superior product quality.

Recommendation

It is recommended to use fish species with high lipid content, such as salmon, trout, mackerel, herring, etc., for traditional smoking, as they absorb smoke well and maintain high moisture content in the finished product. It is advised that the fish used for traditional smoking be freshly harvested to ensure firm muscle texture and a pleasant aroma and appearance.

During the salting stage, it is recommended to either use the dry method (dry salting) or a brine solution (composed of water, salt, sugar, and optional spices) for a minimum of 12 hours to enhance flavor, texture, and prevent the growth of pathogenic microorganisms. Smoking should ideally be conducted at temperatures ranging from 65°C to 85°C, with an optimal smoking temperature around 80°C. It is recommended to use hardwoods such as oak, beech, apple, cherry for smoking, while softwoods should be avoided due to their resin content, which can impart a bitter taste to the final product.

Regarding storage, it is recommended to refrigerate the smoked fish for up to three to four weeks or vacuum-seal and freeze it for long-term storage.

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