Ph.D. THESIS

Evaluation of the morphological status and the effect of some contaminants on the composition and quality of fish meat

(SUMMARY OF Ph.D. THESIS)

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INTRODUCTION

The choice of the present research topic highlights the importance of fish consumption, which has become a food with a very important role in the human lifestyle, thanks to high-quality proteins, vitamins, mineral substances and polyunsaturated fatty acids. The high degree of digestibility of fish is an essential factor compared to other proteins of animal origin. In Romania, preferences for fish meat have gradually increased, from one year to another, according to statistics. Fish is valued for its low level of sodium (Na), methionine and threonine, showing an excess of lysine. Fish-based products can be found in different forms: live fish, fresh fish, frozen fish or subjected to various thermal or smoking treatments.

The approach of this study had the main purpose of bringing more scientific information from another perspective, aiming to determine the morphological characteristics, the influence of some external factors on the composition and quality of fish meat and the impact of some contaminants (heavy metals and polycyclic aromatic hydrocarbons), on trout from Transylvania, Romania.

Looking at the continuous development at the global level, in all fields - industrial, agricultural, food, as well as the massive growth of the population, this has led to heavy metal pollution, which has become a major, very topical problem regarding the health of the environment. Heavy metals are found in different concentrations in soil, water, air, from where they end up in animal and vegetable products. These are a category of toxic pollutants and at the same time, stable, but of particular importance in the ecosystem.

Non-compliance with food safety conditions can lead to major risks regarding the health of the consumer, due to the fact that fish is a highly perishable food.

The perishability is given by the high-water content in the body, the content of easily oxidizable fats and the almost neutral pH - aspects that accentuate the deterioration of the quality of the fish.

The use of different heat treatments applied to fish meat leads to the formation of compounds that can become toxic to the human body. The thermal processes of food processing, either through correctly performed methods, most of the time, or through the application of "abusive" methods, form polycyclic aromatic hydrocarbons in the final product.

THESIS STRUCTURE

The doctoral thesis entitled "Evaluation of the morphological status and the impact of some contaminants on the composition and quality of fish meat", includes a number of 194 pages and presents a specific iconography, with a number of 104 images and 32 tables.

The first part of the thesis - the current state of knowledge, comprises a number of 62 pages and is structured in 6 chapters.

Chapter I - "***General considerations regarding the Salmoniformes Order***", contains information about the species of the Salmonidae family, the evolutionary process and the taxonomic and systematic classification of the Salmoniformes Order.

Chapter II, titled "***General notions regarding fish morphology***", includes basic anatomical aspects, shapes and body segments of fish.

Chapter III - "***Morphological peculiarities in trout***", presents notions regarding the morphology, coloration, habitat, dimensions, body mass and reproduction peculiarities, in brook trout and rainbow trout.

Chapter IV - "***Biometrics of fish***", provides information on somatic measurements and body indices in fish.

Chapter V - "***Notions regarding the concept of quality and freshness of fish***", captures the aspects regarding the characteristics of fresh fish and the chemical composition of fish meat.

Chapter VI - "***Contaminants and toxic effects on fish***", presents data on the contamination of fish meat with heavy metals and polycyclic aromatic hydrocarbons.

The second part of the thesis presents the personal contribution. It contains a number of 111 pages and is structured in 7 chapters. The content of this part refers to information about the working hypothesis; the purpose and objectives of the investigations; material and methods used; results obtained and discussions - compared with the specialized literature; evaluation of the physico-chemical properties regarding the quality of water and trout meat from the fish farms studied; morphometric investigations on rainbow trout and brook trout; performing histological examinations on trout meat; assessment of heavy metals in trout meat; as well as the evaluation of the level of polycyclic aromatic hydrocarbons depending on the heat treatment applied and the type of fat used.

The work ends with a chapter of general conclusions and one on the originality of the thesis.

RESEARCH OBJECTIVES

1. The morphological peculiarities of some fish species and morphometric investigations.

2. Characterization of the physico-chemical composition of water and fish meat, depending on the area of origin.

3. Evaluation of heavy metal content in fish meat and establishment of histological impact on organs.

4. The influence of the thermal process and the type of fat (vegetable/animal) on fish meat and highlighting the level of polycyclic aromatic hydrocarbons.

RESULTS AND DISCUSSION

Chapter VIII of the thesis, entitled "***Materials and methods***", contains information on the biological material, the chemicals, the laboratory equipment used, but also the instruments used.

Within **chapter IX** - ***Study 1***-„***Evaluation of the physico-chemical parameters of water and trout meat***'' we pursued, as main objectives: the analysis of the water quality parameters, from the two trout (nitrogens, nitrates, ammonium, chlorides , total hardness, permanganate index, pH, conductivity and turbidity), evaluation of production performance for rainbow and brook trout, physicochemical composition (fat, protein, water, mineral salts and dry matter) of trout meat, such as and the physico-chemical composition of fish meat under the influence of applied thermal treatments.

The results of the slaughter yield for the two fish units studied show us that the rainbow trout presented higher values regarding body mass, meat obtained, morphology of the head and fins, skin, scales, intestinal mass and bones.

The data obtained regarding the weight - in the case of the trout taken in the study, confirm the value of the superior biological material from the farms studied, but also the corresponding feeding and maintenance conditions. According to the physico-chemical composition of the trout meat, we recall: the water content (%) of the rainbow trout had a higher value than that of the brook trout, and the dry substance value of the spring trout was lower than the rainbow trout. Protein is of significant value in rainbow trout.

Another objective in this chapter was the evaluation of the physico-chemical composition of fish meat (carp, rainbow trout and brook trout) under the influence of some thermal treatments used.

The thermal treatments used were boiling, frying in sunflower oil and palm oil, but also baking in the oven. These processes improve the sensory characteristics of the fish. Physico-chemical parameters analyzed were: fats, proteins, water content and mineral substances.

The following conclusions were drawn from the study: frying in sunflower oil and palm oil has the greatest influence on the fat content of carp (Cyprinus carpio), then rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis), showing the highest values for the samples in which oil was used, compared to the

samples collected following the baking and boiling processes. The protein from carp (Cyprinus carpio), rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis) was positively influenced by baking and boiling processes, showing the highest values, compared to frying in palm oil and sunflower oil- the sun, where the values were lower. The fat content of rainbow trout, for all applied thermal processes, has lower values than that of spring trout and carp.

Chapter X - ***Study 2***- "***Morphometric investigations***" in which we followed the systematic and morphological characteristics of the brook trout (Salvelinus fontinalis) and the rainbow trout (Oncorhynchus mykiss) from the Salmonidae family. As for the color of the trout, it was within the species standard for both brook trout and rainbow trout.

According to the morphology, the brook trout has a more rounded body than the indigenous trout, laterally flattened, covered with small scales, with a slightly pronounced lateral line, olive green, furrowed with wavy stripes of orange color, on the dorsal side, silver, orange and red, on the flanks, with rounded-cornered orange or carmine-red stars, some of them surrounded by bluish rings.

The brook trout has red ventral, anal and caudal fins and presents a milky-white stripe at the edge, terminally furrowed with a black stripe; the caudal fin is hollowed out at a younger age. Regarding the body mass growth of the brook trout, it depends on the feed they consume, but it is faster than the indigenous and rainbow trout.

The rainbow trout has a body similar to that of the native trout, but with larger scales and a more pronounced lateral line than in other salmonids. It has a gray-green color, sometimes bluish, on the dorsal side, silvery on the flanks, whitish on the ventral side. On the flanks it also has a stripe with iridescence in the colors of the rainbow, which is where it gets its name, being easy to recognize. The dorsal side, flanks and caudal fin have black dots. Morphologically, the skull is proportional to the body. The brood, in the first summer, has oval transverse stripes on the flanks (they disappear in the second summer).

Chapter XI - ***Study 3***- „ ***Evaluation of histological changes in trout***'' sought to highlight the damage caused by heavy metals, in certain tissues harvested from trout. In the case of this study, we followed the lesions caused at the tissue level, in order to certify the quality of the fish, but also of the surrounding environment.

The results regarding the impact on consumers is favorable if there are no major histological changes. Tissue samples were collected from skin, muscle, gill, kidney, intestine, hepato-pancreas and spleen. After examining the samples, the gills and skin did not show significant histological changes - degenerative or necrotic lesions in the examined sections.

At the liver level, a discrete congestion with diffuse, moderate lipid vacuolar degeneration was observed. The spleen showed diffuse congestion.

Chapter XII - **Study 4** - "***Evaluation of heavy metals in fish***" pursued two major objectives, regarding the contamination of fish with heavy metals:

- comparative analysis of heavy metals and the profile of some elements in trout species from two different areas;
- assessment of heavy metal contamination of certain types of fish meat.

In the first objective, the aim was to highlight the importance of contaminants on fish, for the two species of trout (rainbow and brook trout). The concentrations of macro- and microelements evaluated showed different results from one species to

another. In the presence of heavy metals, rainbow trout from unit 1 showed reduced concentrations of As, Rb and Sn. In contrast, Pb levels were substantially elevated in rainbow trout compared to brook trout harvested from Unit 2. In addition, Cr concentrations were slightly higher in rainbow trout, but not statistically different, from the group of brook trout. Quantitative differences were also observed in the macroelement profiles of the two trout species. In particular, Na, Mg, K and Ca concentrations showed statistically significant increasing trends in brook trout, indicating higher nutritional value, compared to rainbow trout groups.

Differences in Cu, Fe and I trace element profiles between the two trout species from different locations were also notable. Specifically, Cu levels were higher in rainbow trout samples, while Fe and I concentrations were higher in brook trout samples. In addition, the levels of Mn, Mo, Ni and Zn showed minor variations between the experimental groups, although these differences were not statistically significant.

The second objective aims to assess the heavy metal contamination of certain types of fish meat. In this study, the lowest values were for Hg, in all fish samples analyzed: carp (Cyprinus carpio), brown trout (Salmo trutta), phytophagus (Hypophthalmichtys molitrix) and the highest values were observed for Cu. The values obtained for the four elements analyzed are consistent with those in the specialized literature and within the limits allowed by the legislation.

Chapter XIII - ***Study 5*** - "***Evaluation of the level of polycyclic aromatic hydrocarbons according to the heat treatment applied and the type of fat used***" follows:

- food contamination with polycyclic aromatic hydrocarbons through the application of thermal processes: frying, boiling and smoking;

- assessment of polycyclic aromatic hydrocarbons (PAH) for trout meat, depending on the type of fat used.

Contamination of food with polycyclic aromatic hydrocarbons through the application of thermal processes aimed at establishing the level of PAHs in fish meat, after the application of boiling, frying and baking processes, using four types of fat (sunflower oil, rapeseed oil, of extra virgin olives and lard). To determine the level of PAHs, the chromatographic method was applied, using Perkin-Elmer HPLC equipment.

The following conclusions were drawn from the study: in the case of the fish meat samples analyzed, the highest level of PAHs is when applying the frying process in rapeseed oil. The lowest values were recorded in the case of horseradish, regardless of the type of fat used and regardless of the thermal process applied. Fish meat presented the highest content of PAHs, in the case of the frying process.

The lowest values were recorded for the fish sample fried in sunflower oil and in the fish sample subjected to the oven baking process.

Regardless of the thermal process applied, the highest level is represented by: fluoranthene, anthracene, pyrene and naphthalene. The lowest mean values are for fluorene and benzo(k)fluoranthene in the frying and baking processes.

The evaluation of polycyclic aromatic hydrocarbons (PAH), depending on the type of fat used in the frying process, was carried out on samples represented by brook trout (Salvelinus fontinalis), taken from the Transylvania area. The results obtained from the study can clarify the aspects regarding the choice of the right type of fat for the process of frying fish meat. A number of several samples were subjected to the frying step in 5 types of fat (ostrich, duck, badger, small ruminant and large ruminant).

Ostrich and duck fat were obtained from a facility in Romania, sheep and beef fat were purchased from Turkey, and badger fat was purchased from a commercial pharmaceutical facility in Ukraine.

The results highlighted the fact that:

- naphthalene shows the highest value in the case of frying trout meat in badger lard followed by sheep lard;
- phenanthrene had the highest value when beef lard was used;
- the lowest detectable average value was in the case of duck fat for benzo(gy)perylene;
- ostrich and duck lard have the best values for PAHs;
- badger lard is not the most suitable for the frying process, it leads to the formation of large amounts of PAHs, following its use in the process of frying fish;
- some types of polycyclic aromatic hydrocarbons, de - benzo(b)fluorants, were not formed or were not detectable following the frying process of trout meat, in ostrich, duck and sheep lard.

GENERAL CONCLUSIONS

1. The physico-chemical and microbiological parameters of the water from the studied units correspond to the sanitary and veterinary norms imposed by the legislation in force;

2. Following the physico-chemical determinations, carried out on the two species of trout (spring and rainbow trout), the results regarding the content of water, dry matter, fat, protein and mineral substances did not show significant differences;

3. The thermal process applied and the source used - of plant and animal origin, influenced the physical-chemical composition of the analyzed fish meat;

4. The morphological characteristics of the studied specimens fall within the intervals given in the specialized literature, for rainbow trout and spring trout.

5. From a histological point of view, the tissue samples did not show significant lesions;

6. Among the heavy metals analyzed in trout, copper showed the highest values in rainbow trout, and iron and iodine showed the highest values in brook trout;

7. For the evaluation of polycyclic aromatic hydrocarbons, we took into account the thermal process applied and the source of fat used (vegetable/animal), which determined the increase in the content of polycyclic aromatic hydrocarbons and the change in the quality of the meat;

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The innovative contributions brought by the investigations carried out refer to the physico-chemical studies, regarding the quality of water and trout meat subjected or not subjected to thermal processes, as well as morphometric/morphological ones, for rainbow and spring trout.

The research undertaken also highlighted the impact of some contaminants (heavy metals and polycyclic aromatic hydrocarbons) on the quality of trout meat.

The originality of the thesis is given by the evaluation of the level of polycyclic aromatic hydrocarbons under the influence of thermal processes applied to the fish meat and the influence of the fat source used (vegetable/animal).