Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN FISH AFTER HEAT TREATMENT

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are predominant pollutants in the aquatic environment that can cause a variety of potentially toxic effects, affecting the entire ecosystem. PAHs were evaluated following the application of different heat treatments to fish meat by liquid chromatography (HPLC). The following polycyclic aromatic hydrocarbons were determined: acenaphthene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(gy)pyrylene, chrysene, dibenzo(a,h)anthracene, phenanthrene, fluorene, fluoranthene, indeno(1,2,3-cd)pyrene, naphthalene and pyrene. The samples analyzed were from raw trout (Salvelinus fontinalis); fried in sunflower oil; fried in olive oil; fried with rapeseed oil; baked in the oven and fried in lard. The brook trout (Salvelinus fontinalis) was harvested from the Mărișel area, Cluj county. The highest values for the hydrocarbons analyzed from raw fish were for naphthalene (5.51±0.21) and benzo(a)anthracene (2.47±0.06). The trout fried in sunflower oil showed the highest values for anthracene (10.34±0.09) and pyrene (10.24±0.30) and Indeno(1,2,3-cd)pyrene was <0.05. Trout fried with rapeseed oil showed the highest values for anthracene (10.34±0.09), followed by pyrene (10.24±0.30) and indeno(1,2,3-cd)pyrene was below <0.05. Trout fried in olive oil showed very high values for pyrene (51.37±0.63) and fluoranthene (20.36±0.89) and dibenzo(a,h)anthracene was <0.05. According to this study, fish meat has the highest percentage of PAHs following the frying process. These compounds play a very important role in the ecosystem, therefore the most affected is the consumer due to the carcinogenic effect they can cause on the body.

Key words: *heat treatments, olive, PAHs, rapeseed, Salvelinus fontinalis, sunflower oil.*

INTRODUCTION

Polycyclic aromatic hydrocarbons are a major class of hydrophobic organic compounds, which are made up of two or more joined aromatic nuclei and are found in both freshwater and aquatic environments (Grimmer et al 1968, 1983; Nielsen et al., 1996; Neff et al., 2005). During processes of incomplete combustion of organic matter or pyrolysis processes, hundreds of PAHs can form and release into the environment (Fetzer, 2000; Llobet et al., 2006). Regarding the natural formation process of polycyclic aromatic hydrocarbons, it is achieved by carbonization of organic matter, and a significant percentage that reach the environment come from incomplete combustion of fossil fuels, used in internal combustion engines, but also from the pyrolysis of organic matter (Adonis, 2003; Grimmer et al., 1979; Nielsen et al., 1996; Wretling et al., 2010). Polycyclic aromatic hydrocarbons, from a biochemical point of view, are lipophilic, with affinity for fatty substances, and their solubility and volatility leads to percentage decrease with increasing molecular weight (Grimmer et al., 1983; Ake, 2012). Therefore, this group of organic compounds, due to their characteristics, are considered pollutants with a large spread in the ecosystem (Grimmer et al., 1983; Boca et al., 1988). PAHs are spread right in the air of the room where fish is prepared through the roasting process, and this process removes most PAHs into the air compared to boiling or other processes, according to a study by Michiko et al. (2001) in Japan. According to the European Communities (2001), following Research has highlighted certain PAHs as substances with the ability to damage DNA in cells, giving them the character of genotoxicity, carcinogenic, and there is no minimum level of safety. The process of contamination of food with PAH can also be done by applying thermal processes: frying, boiling and smoking. Their level (PAH), according to studies, increases significantly in processed foods or foods that have gone through the cooking process (European Communities, 2001; Farhadian et al., 2010; US EPA, 1984; ATSDR, 1999; 2004; 2007; 2012). Fish meat subjected to heat treatments or processes leads to the appearance of carcinogenic, mutagenic and teratogenic lipophilic products, given by the cooking process and increased temperature, according to Ake et al. (2012). In terms of toxicity, PAHs are considered ecosystem contaminants due to their encounters in the food chain, through their accumulation and concentration in air, water or soil. They have action that reflects on the quality of human life (European Communities, 2001; Laslo, 1995). PAHs are also called universal environmental contaminants (air, water, soil), therefore their presence in the food chain is inevitable, the human body being the most affected (Tofană, 2011). As a result of thermal processes for processing and preparing meat, by certain methods either properly applied or abusively performed at high temperature, using traditional procedures (roasting, grilling, smoking) several harmful components are eventually formed, including various mutagens and carcinogens, including PAH in the final product (Jagerstad & Skog, 2005, cited by Aaslyng et al., 2013). Studies conducted on laboratory animals are a confirmation that exemplifies their carcinogenic effect, and from the point of view of chemical analyzes, these substances also result from isolation from certain compounds. To date, more than 500 PAHs have been isolated, only a small percentage has been assessed for toxicity (Banu, 2007; Laslo, 1995). From an epidemiological point of view, there is an increased percentage of cancer risk in various organs (intestines, breast, bladder, prostate and pancreas) after an increased consumption of thermally prepared meat, either through the frying process or through grilling (Kazerouni et al.; Aaslyng et al., 2013). According to Dutta et al. (2022) and other updated studies (Wang et al., 2021), the major PAH exposures of the population are through a diet based on various types of meat, including fish meat subjected to certain heat treatments.

According to the studies carried out by Perelló et al. (2009), the highest concentrations of PAHs were discovered after the frying process of various foods, including fish. The aim of the study is to analyze the content of PAHs in fish according to the thermal treatments applied and the vegetable and animal source applied for cooking.

MATERIALS AND METHODS

PAH analysis was performed by high-performance liquid chromatography (HPLC) with fluorescence detection after solid-liquid extraction.

In this study, the aim was to evaluate 15 PAHs (acenaphthene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)pyrylene, chrysene, dibenzo(a,h)anthracene, phenanthrene, fluorene, fluoranthene, indeno(1,2,3-cd)pyrene, naphthalene, pyrene) in brook trout (*Salvelinus fontinalis*).

For analysis, the Perkin Elmer 200 series High Performance Liquid Chromatograph (HPLC) with fluorescence detector and data processing system with FLD detector was used.We used 10 grams of properly ground and homogenized sample, saponified with 100 ml KOH solution in ethanol, then extracted 25 ml Hexane at the ultrasonic bath for about 20 minutes. When evaporation took place, on the rotary evaporator, the samples were returned with 1 ml of acetonitrile and finally injected into the HPLC. The 15 PAHs mentioned above were run on an Inertsil ODS-P 5 pm, 4.6x150mm which was maintained at a temperature of 24°C. Regarding the injection volume, it was 50 pl. Regarding the mobile phase, a water gradient was used, and the substance used was acetonitrile and a time-programmed FLD detector for detection. The samples analyzed for hydrocarbons, were raw Brook trout (*Salvelinus fontinalis*); fried in sunflower oil; fried in olive oil; fried with rapeseed oil; baked in the oven and fried in lard. Brook trout (*Salvelinus fontinalis*) was harvested from the

Mărișel area, Cluj county. Five samples were used for each category analysed. The fish samples were stored in the Faculty of Veterinary Medicine Cluj-Napoca, in optimal conditions, at a temperature of -2ºC in a device equipped with BioFreshPlus technology so that the fish preserves its properties. The weight of the fish was 240-280 grams and a temperature of 180-190ºC was used for cooking, with a duration of 20-25 minutes. The oils and lard used were purchased commercially. Analysis of fish fat and protein was performed by the Soxhlet and Kjeldahl method and minerals by calcination.

RESULTS AND DISCUSSIONS

PAHs are compounds released by various cooking processes. Depending on the cooking process applied and the vegetable and animal source used, PAHs were released in different amounts, much higher compared to the raw fish samples analyzed. Tables 1 to 6 show mean values and variability for polycyclic aromatic hydrocarbons from raw fish compared to thermal baking and roasting processes. Another factor considered is the type of vegetable or animal fat used and the impact on the concentration of polycyclic aromatic hydrocarbons in fish meat.

Table 1. Mean values and variability for polycyclic aromatic hydrocarbons in raw brook trout (*Salvelinus fontinalis*)

Parameter	Raw brook trout (*Salvelinus fontinalis*)	
	X±sx	V%
Naphthalene	5.51±0.21	8.43
Acenaften	<0.05	-
Fluorene	0.67±0.07	22.21
Phenanthrene	1.03±0.02	3.96
Anthracene	2.03±0.12	13.69
Fluoranthene	2.34±0.13	12.68
Pyrene	1.09±0.03	6.01
Benzo(a)anthracene	2.47±0.06	5.36
Crisen	<0.05	-
Benzo(b)fluoranthene	<0.05	-
Benzo(k)fluoranthene	0.87±0.01	2.98
Benzo(a)pyrene	1.04±0.01	3.00
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	<0.05	-
Indeno(1,2,3-cd) pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

For the raw fish sample, high values were recorded for the following polycyclic aromatic hydrocarbons: naphthalene 5.51±0.21 ng/g, followed by benzo(a)anthracene with a value of 2.47±0.06 ng/g and fluoranthene with a value of 2.34±0.13 ng/g (Table 1). Following the analysis of the sample of fish fried in sunflower oil, high values were observed for PAHs, such as anthracene which has the highest value, which is 10.34±0.09 ng/g. It was followed by pyrene with a value of 10.24±0.30 ng/g. The third type of high PAH is naphthalene with a value of 10.23±0.18 ng/g (Table 2).

Table 2. Average values and variability for hydrocarbons in brook trout (*Salvelinus fontinalis*) fried with sunflower oil

Parameter	Brook trout (*Salvelinus fontinalis*) fried with sunflower oil	
	X±sx	V%
Naphthalene	10.23±0.18	3.93
Acenaften	0.94±0.03	6.50
Fluorene	2.17±0.04	4.55
Phenanthrene	4.18±0.04	2.22
Anthracene	10.34±0.09	1.90
Fluoranthene	9.31±0.13	3.08
Pyrene	10.24±0.30	6.50
Benzo(a)anthracene	6.94±0.04	1.36
Crisen	0.31±0.02	15.26
Benzo(b)fluoranthene	0.16±0.01	14.76
Benzo(k)fluoranthene	2.27±0.04	3.90
Benzo(a)pyrene	4.04±0.19	10.72
Dibenzo(a,h)anthracene	0.51±0.03	13.84
Benzo(ghi)perylene	1.12±0.02	4.90
Indeno(1,2,3-cd) pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

Table 3. Mean values and variability for hydrocarbons in brook trout (*Salvelinus fontinalis*) fried with rapeseed oil

Parameter	Brook trout (*Salvelinus fontinalis*) fried with rapeseed oil	
	X±sx	V%
Naphthalene	16.78±0.16	2.11
Acenaften	1.24±0.08	14.18
Fluorene	5.22±0.06	2.50
Phenanthrene	25.18±0.98	8.63
Anthracene	24.95±0.41	3.64
Fluoranthene	22.36±0.44	4.38
Pyrene	32.17±0.84	5.87
Benzo(a)anthracene	12.57±0.98	17.55
Crisen	0.42±0.08	14.71
Benzo(b)fluoranthene	0.96±0.09	20.87
Benzo(k)fluoranthene	3.04±0.02	1.30
Benzo(a)pyrene	7.76±0.25	7.27
Dibenzo(a,h)anthracene	0.79±0.06	18.12
Benzo(ghi)perylene	3.07±0.39	28.74
Indeno(1,2,3-cd)pyrene	0.87±0.03	7.33

v-variability; x-mean value; sx-standard deviation; n=5

For fish fried in rapeseed oil, four types of polycyclic aromatic hydrocarbons have been observed to have higher values than the others. These were pyrene with the highest concentration of 32.17±0.84 ng/g, followed by phenanthrene with 25.18±0.98 ng/g. Anthracene had a concentration of 24.95±0.41 ng/g and fluoranthene had a concentration of 22.36±0.44

ng/g (Table 3). In the case of fish fried in olive oil, high values for polycyclic aromatic hydrocarbons were observed for pyrene, with a concentration of 51.37±0.63 ng/g, the second high value was for fluoranthene with 20.36±0.89 ng/g, followed by naphthalene with 15.75±12.19 ng/g and anthracene with 15.398±0.27 ng/g (Table 4).

Table 4. Mean values and variability for hydrocarbons in brook trout (*Salvelinus fontinalis*) fried with olive oil

Parameter	Fried brook trout (*Salvelinus fontinalis*) with olive oil	
	X±sx	V%
Naphthalene	15.75±12.19	0.86
Acenaften	0.48±0.01	4.28
Fluorene	4.03±0.18	9.11
Phenanthrene	3.72±0.16	9.91
Anthracene	15.398±0.27	3.87
Fluoranthene	20.36±0.89	9.73
Pyrene	51.37±0.63	2.74
Benzo(a)anthracene	10.56±0.26	5.44
Crisen	0.25±0.07	16.13
Benzo(b)fluoranthene	0.53±0.05	21.69
Benzo(k)fluoranthene	2.128±0.01	1.22
Benzo(a)pyrene	6.72±0.20	6.71
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	2.15±0.05	4.86
Indeno(1,2,3-cd) pyrene	0.07±0.01	16.62

v-variability; x-mean value; sx-standard deviation; n=5

In the sample of fish fried in pork lard, high values of the following polycyclic aromatic hydrocarbons were observed, namely fluoranthene, pyrene and naphthalene, with values 16.65±0.43 ng/g, 12.42±0.16 ng/g and 12.35 ±0.33 ng/g respectively (Table 5).

Table 5. Average values and variability for hydrocarbons from fried fish with pork lard

Parameter	Fried fish with pork lard (*Salvelinus fontinalis)*	
	X±sx	V%
Naphthalene	12.35 ±0.33	5.97
Acenaften	<0.05	-
Fluorene	3.11±0.22	1.78
Phenanthrene	4.07±0.21	11.56
Anthracene	9.76±0.36	8.28
Fluoranthene	16.65±0.43	5.73
Pyrene	12.42±0.16	2.97
Benzo(a)anthracene	9.02±0.10	2.57
Crisen	<0.05	-
Benzo(b)fluoranthene	<0.05	-
Benzo(k)fluoranthene	1.97±0.12	13.62
Benzo(a)pyrene	1.98±0.24	16.57
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	<0.05	-
Indeno(1,2,3-cd)pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

In samples of baked fish, high levels were reported for the following PAHs: pyrene, anthracene and naphthalene, which had the following values: 14.75±-0.50 ng/g, 11.58±0.75 ng/g and 9.24±0.09 ng/g respectively (Table 6).

Table 6. Average values and variability for hydrocarbons in baked brook trout (*Salvelinus fontinalis*)

Parameter	Baked brook trout (*Salvelinus fontinalis*) in the oven	
	X±sx	V%
Naphthalene	9.24±0.09	2.23
Acenaften	<0.05	-
Fluorene	1.17±0.04	7.20
Phenanthrene	1.06±0.02	5.08
Anthracene	11.58±0.75	14.51
Fluoranthene	2.47±0.07	6.23
Pyrene	14.75±-0.50	7.53
Benzo(a)anthracene	4.01±0.34	18.98
Crisen	<0.05	-
Benzo(b)fluoranthene	<0.05	-
Benzo(k)fluoranthene	1.974±0.04	4.23
Benzo(a)pyrene	2.67±0.07	6.07
Dibenzo(a,h)anthracene	<0.05	-
Benzo(ghi)perylene	<0.05	-
Indeno(1,2,3-cd)pyrene	<0.05	-

v-variability; x-mean value; sx-standard deviation; n=5

Following the analyzes we notice for samples from raw fish, which has not been subjected to any heat treatment, it still presents polycyclic aromatic hydrocarbons in its composition, but in much smaller quantities compared to those subjected to frying or baking treatment. PAHs with the highest levels in fish meat were observed to be pyrene, fluoranthene, anthracene and naphthalene. In food, PAHs tend to be formed during processing and preparation by different methods: grill use, smoke use, drying, frying, baking or roasting. We can see that rapeseed oil and olive oil used in the thermal roasting process produce the highest amounts of polycyclic aromatic hydrocarbons.The PAHs that were below the value of 0.05 are different according to the thermal process applied and according to the source of vegetable and animal origin applied for cooking. For raw fish, most PAHs (acenaphthene, chrysene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, benzo(gy)perylene, indeno(1,2,3-cd)pyrene) were observed which were below the value of 0.05. In fish fried with sunflower oil, only one hydrocarbon was observed below the value of 0.05, namely indeno(1,2,3-cd)pyrene. Frying fish in rapeseed oil did not identify any hydrocarbons below 0.05, and for fish fried in olive oil, dibenzo(a,h)anthracene was below this value.

For fish cooked in lard and cooked in the oven, the following PAHs (acenaphthene, chrysene, benzo(b)fluoranthene, dibenzo(a,h)anthracene, benzo(ghi)perylene, indeno(1,2,3-cd)pyrene), were below the value of 0.05. The lowest average values are for fluorene, ranging from 3.62 (ng/g) when frying to 1.18 (ng/g) when baking. Benzo(k)fluoranthene has the lowest average values: 2.34 ng/g when frying and 1.98 ng/g when baking. According to the study conducted by Aaslyng et al (2013), on meat of different animal species (pork, beef, chicken), for the determination of PAH and amines, they pointed out that the time and temperature to which meat is subjected is the essential. The study by Farhadian et al (2012) highlights the influence of cooking oil (a component of the meat marinating recipe ingredients) leading to the formation of several PAHs following the thermal process to which the meat is subjected and the introduction. of lemon juice in the basic marinade recipe leads to a significant decrease in PAH concentrations. To confirm the degree of danger to human health, Wang et al. (2021) determined 15 PAHs, from Shandong area, China, and from the obtained results they highlighted that both fried fish and grilled fish represent a danger to the body human, due to concentrations of PAHs and other compounds. Updated studies lead to increased attention for careful monitoring of these potentially toxic compounds. In the process of cooking at high temperature (PAH) is formed. In the study conducted by Sahin et al. (2020), the following samples were analyzed (meat doner, chicken doner, meatballs, grilled chicken and fish), the total PAH contamination was 6.08, 4.42, 4.45, 4.91 and 7.26 μg/kg. Benzo(a)pyrene (BaP) in meatballs and grilled fish samples had a level of 0.70 and 0.73 μg/kg. The physico-chemical parameters from the raw brook trout (*Salvelinus fontinalis*) samples analyzed showed the following average values: water (%) 73.80, fat (%) 4.55, protein (%) 22.66, minerals (%) 1.28. These results are in agreement with the average values reported in the literature for the specific physico-chemical composition for brook trout (*Salvelinus fontinalis*).

CONCLUSIONS

For samples analysed from fish meat, the highest level of PAHs is when roasting in rapeseed oil. The lowest values were recorded in the case of chrysenic, regardless of the type of fat used and regardless of the thermal process applied. Fish meat showed the highest content in PAHs in the roasting process. The lowest values were recorded for the fish sample fried in sunflower oil and in the oven fish sample. Regardless of the thermal process applied, the highest level is represented by: fluoranthene, anthracene, pyrene and naphthalene.

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