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The Occurrence of Polycyclic Aromatic Hydrocarbons in Fish Meat and Their Impact on Food Quality

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REVIEW

Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds known to be potentially toxic for human health. Humans are exposed mostly through ingestion, but also through inhalation and skin contact. Thus, their concentrations in foods should be as low as possible. Fish products are appreciated as functional food, with high nutritional properties and organoleptic attributes. Despite these qualities, fish products may also contain PAHs. Humans are exposed to PAHs by eating fish meat, which can be contaminated from environmental sources, from industrial food processing methods, and from some home cooking practices. The main aspects concerning PAHs contamination of fish meat are briefly discussed: analysis and occurrence in some fish products, main factors and sources of contaminations, methodology of the determination of PAHs and current legislation on PAHs. The contamination of fish meat with PAHs has an impact on food quality. Mainly, topical studies from the last 20 years were taken into account, and also some studies that were the basis of the research in PAHs. The following platforms were used in the search: Web of Science and Scopus.

Keywords: Benzo[a]pyrene, contamination, human health risks, meat products, PAHs.

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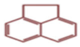
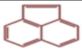
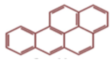
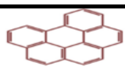
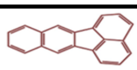
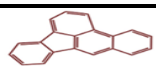
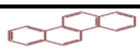
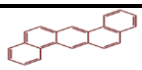
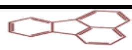
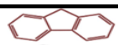
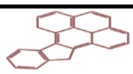
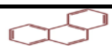
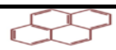
INTRODUCTION

Fish brings an important contribution to the human diet because of its high nutritional quality (Maurya et al., 2018). Due to increased knowledge of its benefits for human health, there is a growing demand for fish. According to Lefevre (2008), the concept of quality, both for fish and for other food products, can be characterized in terms of biological quality (species and age), technological quality (growth system and primarily to final processing), nutritional quality (micro and macro nutrients), and organoleptic qualities (the sensory perception of products including texture, appearance, aroma and color). In addition to these features, according to FAO (2003), product safety is the intrinsic factor of quality that decisively influences its value and availability to the consumer. An important aspect of food safety is studying the occurrence of toxic compounds for human health. Throughout the production and supply chain, some hazards of biological, chemical or environmental origin can be introduced into aquaculture (Vergis et al., 2021). Despite many advantages of fish consumption, a wide variety of chemical contaminants may be present in fish meat, like acrolein, acrylamide, furan, heterocyclic amines, monochloropropanediol (MCPD), nitrosamine and polycyclic aromatic hydrocarbons (PAHs) (Iko et al., 2021). PAHs came to the attention of the public because of their wide distribution and their effects on human health (Ahmed, 1991). PAHs are organic compounds consisting mainly of carbon and hydrogen, with two or more fused aromatic rings in various structural configurations (Francis et al., 2014; Lawal, 2017; Ejike et al., 2022). Two major mechanisms leading to PAHs formations are pyrosynthesis and pyrolysis, promoted by saturated hydrocarbon under oxygen-deficient conditions (Dat and Chang, 2017). They are formed in fish meat from the incomplete combustion of the organic matter during processing methods like smoking, grilling and roasting, or tend to bioaccumulate during the growth period (Iko et al., 2021; Recabarren-Villalón et al., 2021). Although there are several hundred chemically related compounds, most studies and regulations focus on a limited number of PAHs, generally up to a maximum of 20 individual PAH compounds (Abdel-Shafy and Mansour, 2016). These compose a large group of chemical agents that have been demonstrated to be carcinogenic, mutagenic and potent immunosuppressants by several studies (Baird et al., 2005; Jägerstad and Skog, 2005; Audeberd et al., 2012; Da Silva Junior et al., 2021; Sleight et al., 2021).

Table 1. Molecular structure and toxic-carcinogenic action of PAHs (NRC (1983); Nisbet and LaGoy (1992); Nikolau et al., 2009)

PAH Name	CAS No.	Molecular structure	Molecular formula	Toxicology		
				TEF[a]	**IARC[b]**	**EPA[c]**
Acenaphthene	83-32-9		$C_{12}H_{10}$	0.001	3	D
Acenaphthylene	208–96–8		$C_{12}H_{8}$	0.001	n.c	D
Anthracene	120–12–7		$C_{14}H_{10}$	0.01	3	D
Benzo[a]anthracene	56–55–3		$C_{18}H_{12}$	0.1	2B	B2
Benzo[a]pyrene	50–32–8		$C_{20}H_{12}$	1	1	B2
Benzo[g,h,i]perylene	203–12–3		$C_{22}H_{12}$	0.01	3	D
Benzo[b]fluoranthene	205–99–2		$C_{20}H_{12}$	0.1	2B	B2
Benzo[k]fluoranthene	207–08–9		$C_{20}H_{12}$	0.1	2B	B2
Chrysene	218–01–9		$C_{18}H_{12}$	0.01	2B	B2
Dibenzo[a,h]anthracene	53-70-3		$C_{22}H_{14}$	5	2A	B2
Fluoranthene	206–44–0		$C_{16}H_{10}$	0.001	3	D
Fluorene	86–73–7		$C_{13}H_{10}$	0.001	3	D
Indeno[1,2,3-c,d]pyrene	193–39–5		$C_{22}H_{12}$	0.1	2B	B2
Phenanthrene	85–01–8		$C_{14}H_{10}$	0.001	3	D
Pyrene	129–00–0		$C_{16}H_{10}$	0.001	3	D
Naphthalene	91–20–3		$C_{10}H_{8}$	0.001	2B	C

[a]Toxic equivalent factor relatively congener to that of BaP (Nisbet and LaGoy, 1992)

[b]IARC Monographs on the identification of carcinogenic hazards to humans; https://monographs.iarc.who.int/list-of-classifications; (1, carcinogenic to humans; 2A, probably; 2B, possibly; 3, not classifiable as carcinogenic to humans; n.c., not classified)

[c]EPA carcinogenic classification: A, human carcinogenic; B1 and B2: probable; C, possible; D, not Classifiable as to human carcinogenicity; E, evidence of non-carcinogenicity for humans (EPA/600/R-93/089)

The United States Environmental Protection Agency (USEPA) comprised a priority list of 17 PAHs, using four criteria: toxicity, potential for human exposure, frequency of occurrence at National Priorities List (NPL) hazardous waste sites and extent of information available (ATSDR, 1995). In the European Union (EU), 33 PAHs were selected by the Scientific Committee on Food (SCF, 2002) as presenting risks to human health. Among these, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that 16 PAHs are clearly genotoxic and carcinogenic: benzo[a]anthracene (BaA), benzo[b]-, benzo[j]- and benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BghiP), benzo[a]pyrene (BaP), chrysene (CHR), cyclopenta[cd]pyrene, dibenz[a,h]anthracene (DBahA), dibenzo[a,e]-, dibenzo[a,h]-, dibenzo[a,i]-, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. Some of these PAHs are presented in Table 1 and to depict their chemical structures we used Adobe Photoshop 2020 (v21.2.10.118), adapted by NRC, 1983.

MAIN FACTORS AND SOURCES OF CONTAMINATION

PAHs accumulate as toxic compounds in different food categories including cereals, vegetables, fruits, water and meat (Stumpe et al, 2008). However, special attention has been paid to fish meat products, as they are frequent in the human diet and because of their high levels of PAHs detected. Generally, PAHs have origins in environmental sources, industrial food processing methods, packing materials, and home cooking practices (Zhang and Zhang, 2021). These pathways of PAHs contamination in fish meat are pointed in Figure 1.

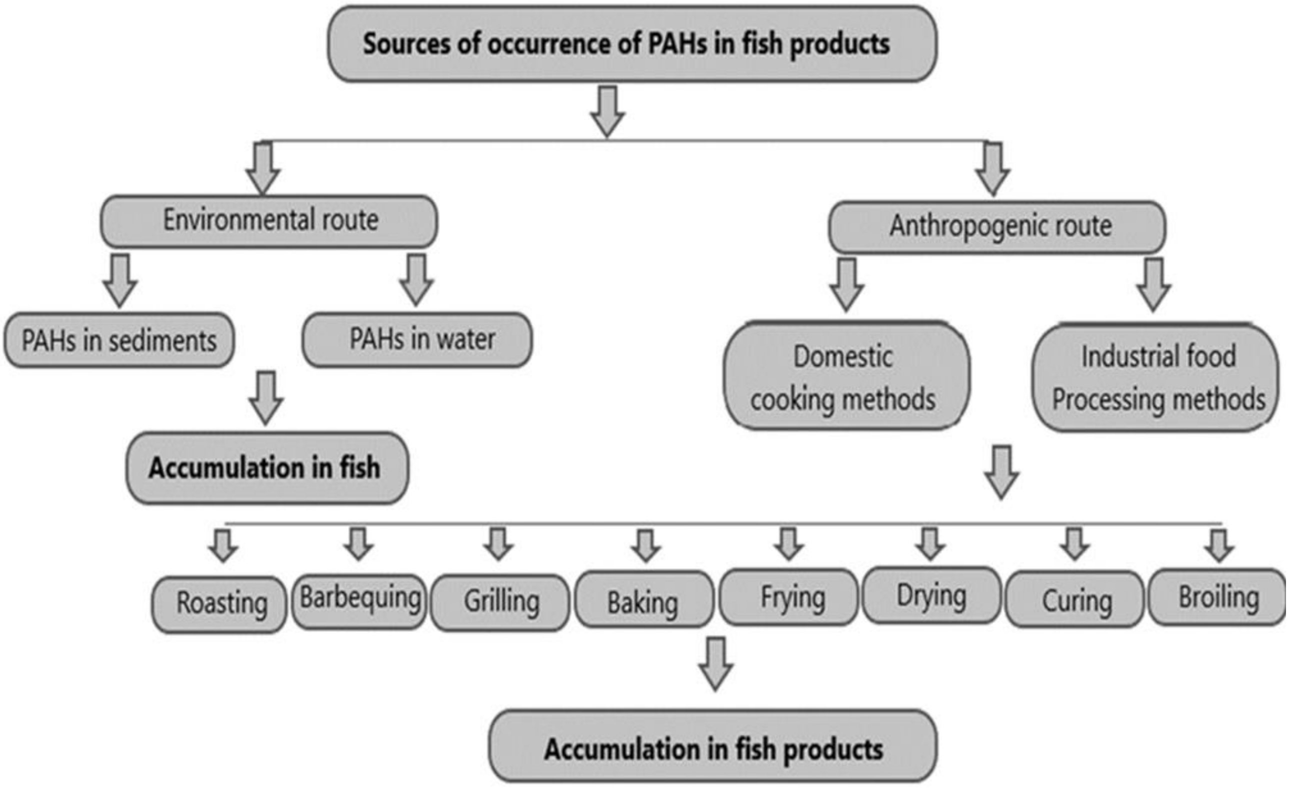


Figure 1. Diagram pointing the pathways of PAHs contamination in fish meat (Adapted by Bansal and Kim, 2015)

The most frequent pathway by which PAHs contaminate fish meat is related to food processing methods, such as drying (Okenyi et al., 2016) and smoking (Stołyhwo and Sikorski, 2005), or thermal cooking methods, such as grilling, roasting and frying (Dutta et al., 2022). Generally, there are some major factors that influenced the formation of PAHs in fish products during these procedures and practices include the heat source type, duration, temperature, fat content of fish, fuel and wood type, smoke generator, the distance between the food and the heat source, cleanliness and maintenance of equipment (Onopiuk et al., 2021; Sampaio et al., 2021). There have been some studies examining the relationship between fish products and different cooking practices for the determination of PAH compounds. Rose et al (2015) evaluated the formation of different PAHs in food prepared by various cooking methods.

In the diversified offer of the European food market, reasonable quantity of fish is available as cold or hot smoked products (Stołyhwo & Sikorski, 2005). Smoking is an ancient way of food preparation, basically representing a set of chemicals, thermal, diffusive, and biochemical processes in which smoke is produced by combustion (sometimes incomplete combustion) applied to a preliminary salted product (Adeyeye, 2018). The smoking process is used to improve the organoleptic qualities of food (flavour, taste, colour, appearance), but also to extend the preservation period. Smoke is a set of particles, solid or liquid, in dispersion in a gaseous medium produced by combustion with a complex composition of chemical compounds identified in wood smoke or smoke flavor from a number of sources:

40 acids, 22 alcohols, 131 carbonyls, 22 esters, 46 furans, 16 lactones, and 75 phenols (Toledo, 2008).

Over time, from the basic smoking by hanging the meat over the fire, new methods of smoking were discovered and improved. Therefore, there are three types of smoking methods: (a) cold-smoking, (b) warm-smoking, and (c) liquid smoking. Smoking is the processing method which generates most PAHs in fish meat products. Codex Alimentarius Commission code of practice CAC/RCP 68/2009 mentioned 10 variables that may influence the PAH content of smoked foods: 1) the process of smoke generation direct influenced by the temperature of pyrolysis and smoke generator(liquid smoke, smouldering, friction, thermostated plates); 2) the direct or indirect smokingmethod; 3) position of the food in chamber; 4) the distance between the food and the heat source; 5) type of fuel (wood species and other plant materials, diesel, liquid/solid waste, gases); 6) temperature of smoking; 7) duration of smoking; 8) fat content of the food; 9) the smoking chamber design and the other equipment used; 10) cleanliness and maintenance of equipment. Ledesma et al (2016) focused on analyzing these variables and concluded that the generation of PAHs in meat largely depends on 3 out of 10 variables recommended by CAC/RCP 68/2009: the temperature of smoke generation, the type of casing and the smoking method (direct or indirect).

Frying is a fast-cooking practice that provides desired texture and taste. It can be classified into four classes: (a) pan frying having no oil or fat, (b) stir frying with minimum oil or fat, (c) deep frying having maximum oil or fat, (d) air frying (Siddique et al., 2021). Usually, all frying classes require temperatures between 145 and 200 °C (Iwegbue et al., 2020). These temperatures promote oil intake, crust formation, modifying the molecular structure of a macronutrients, and improves organoleptic properties, but reduce its nutritional value (Lee et al, 2020). However, despite the number of studies conducted to evaluate PAHs compounds formation in fried fish products, most have concluded that PAHs content in fried fish is present without exceeding the limits (Hafez et al, 2018; Wang et al, 2021).

Cooking fish with direct or indirect heat are methods often used when grilling or roasting, being used more and more often both at home and in restaurants. Fish meat cooked at temperatures above 200 °C or cooked for a long time tend to form more PAHs. Although there are few studies on the PAHs concentration of grilled fish, numerous studies on other types of meat can be considered. Lee et al. (2016) studied the effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats and concluded that the most important factor contributing to the production of PAHs in grilled meats was the incomplete combustion of fatty meat drippings, excepting the incomplete combustion in the first period. Thus, the authors detailed how PAHs are formed when fat and juices from grilled meat drip directly over a heated surface or open fire, causing flames and smoke. The results of other studies suggested that charcoal grilling increase accumulation of PAHs and the amount of the produced PAHs might be related to the time of grilling, with a longer grilling time possibly leading to more PAHs (Gao et al., 2022). The PAHs content of grilled fish depends on some characteristics such as the barbecue design, the type of fuel, the temperature, the fish species and the pretreatment of the meat (Duedahl-Olesen and Ionas, 2021).

Aquatic ecosystems bioaccumulate PAHs during the incomplete combustion of organic matter and during the slow maturation of organic matter accumulated in deep sedimentary environments, having a pyrolytic, petrogenic or biogenic origin (Colier et al., 2013). Aquatic toxicities of environmental PAH contamination have been demonstrated by several authors (Celino et al., 2010; Jesus et al., 2022). Baali and Yahyaoui (2020) concluded that rivers and lagoons contain PAH amounts much higher than coastal waters due to low water exchange. Contamination of fish with PAHs can occur during the entire period of active growth and accumulate in lipid-rich tissues (Jidansa et al., 2020). Part of the bioaccumulation of PAHs in fish is directly correlated with PAHs concentration in aquatic plants and phytoplankton, rather than the PAHs concentration from water and sediments (Zhang et al., 2015). However, it was noticed that the concentrations of PAHs followed a reverse order with the increasing trophic level, meaning that fish have the lowest concentration of PAHs in the food chain (Takeuchi et al., 2009; Behera et al., 2018). This is possible through the tendency of fish to metabolize PAHs because of the cytochrome P450 enzymes in their tissues that oxidatively biotransform PAHs to hydroxylated metabolites (Baali and Yahyaoui, 2020). As a result of fast metabolism in fish, PAHs concentrations are low in muscle and other tissues.

Direct food contact with packaging materials, contaminated additives and supplements can lead to contamination by the migration of some toxic substances into foods. Plastic packaging is widely used in the food industry, even for fish meat products, due to low cost, flexibility and its light weight. Thus, PAHs can also contaminate packaged fish products through packaging processes. According to some studies (Rochman et al., 2013; Li et al., 2017; Schweighuber et al., 2019), PAHs may be present in polystyrene (PS) food contact materials (FCMs) due to production methods. They noticed that PAH levels were higher in expanded PS products compared to the extruded ones. There were not found high-ring PAHs, but low-ring PAHs, probably because more chemical steps are required for formation of high-ring PAHs compared to low-ring PAHs. However, in these studies there are considerable variations of simulated migration values of PAHs from PS FCMs to food, exhibiting enormous opportunities for research.

ANALYSIS AND OCCURRENCE of PAHs IN SOME FISH PRODUCTS

The occurrence of PAHs in fish meat is promoted by several physicochemical characteristics that determine their accumulation and localization. Table 2 presents the basic physical and chemical characteristics for 16 PAHs. The general features and functions of PAHs are high melting and boiling points, low vapor pressure, low aqueous solubility, highly lipophilic, light sensitivity, heat resistance, conductivity, emittability, and resistance to corrosion (Rengaranjan et al., 2015; Zhao et al., 2017). The persistence, volatility, solubility and carcinogenicity of PAHs depends on their molecular weight (Alaekwe and Abba, 2022). PAHs can be divided in two categories: high molecular weight (HMW) with four or more benzene rings fused together, or low molecular weight (LMW) with two or more fused benzene rings (Amodu et al, 2013). These characteristics showed in Table 2 influence biological activity and metabolic activation, as well as their deposition and disposition (Jameson, 2019).

Table 2. General characteristics and functions of PAHs (Ifemeje et al., 2014; Ghosal et al., 2016)

PAH name	Molecular weight	Boiling point(°C)	Melting point(°C)	Vapor pressure (mmHg 25°C)	Solubility (mg/l)
Acenaphthene	154.21	279	93.4	2.5×10^{-3}	3.93
Acenaphthylene	154.20	280	91.8	6.68×10^{-3}	1.93
Anthracene	178.20	342	216.4	6.53×10^{-6}	0.076
Benzo[a]anthracene	228.89	438	158	4.11×10^{-3}	0.010
Benzo[a]pyrene	251.30	495	179	5.49×10^{-9}	3.8×10^{-3}
Benzo[g,h,i]perylene	276.30	500	277	1.0×10^{-10}	2.6×10^{-5}
Benzo[b]fluoranthene	251.30	481	168.3	5.0×10^{-7}	0.0012
Benzo[k]fluoranthene	251.30	480	215.7	9.7×10^{-10}	7.6×10^{-4}
Chrysene	228.30	448	254	6.23×10^{-9}	1.5×10^{-3}
Dibenzo[a,h]anthracene	278.35	524	262	9.55×10^{-10}	5.0×10^{-4}
Fluoranthene	202.26	375	108.8	9.22×10^{-6}	0.20–0.26
Fluorene	166.20	295	116.7	6.0×10^{-4}	1.68–1.98
Indeno[1,2,3-c,d]pyrene	276.30	536	161.3	1.25×10^{-3}	0.062
Phenanthrene	178.20	340	106.5	1.2×10^{-4}	1.20
Pyrene	202.30	393	150.4	4.5×10^{-6}	0.132
Naphthalene	128.00	218	80.02	8.5×10^{-2}	31

Many studies have evaluated the presence of PAHs in fish products and the data reported are highly variable because of different procedures used for evaluation and the difference in meat composition and processing methods. Generally, PAHs profiles are investigated in terms of ∑16PAHs, ∑2PAHs or only one compound, like BaP, which is the most representative of PAHs. Thus, it is recognized and confirmed that most of the PAHs in fish meat come from the processing methods.

Rascón et al. (2019) analyzed 32 meat and fish samples from European markets, 30 of which contained the target PAHs at levels from 11 to 6900 ng/kg. Four types of smoked fish studied (*Salmo salar* (salmon*), Brama brama* (pomfret), *Gadus morhua* (cod) and *Clupea pallasii* (kipper) presented concentrations of PAHs higher than non-smoked samples. They contained high concentrations of Nap, Flu, BaA, Chr (580–6900 ng/kg) and also BaP (46–330 ng/kg), or ∑ 16 PAHs for smoked salmon 8267 ng/kg, smoked pomfret 17303 ng/kg, smoked cod 22881 ng/kg and smoked kipper 19433 ng/kg.

Olatunji et al. (2015) compared the content of BaP and BkF in different fish species including *Merluccius poli* (hake), *Tyrsites atun* (snoek), *Seriola lalandi* (yellow-tail) and pomfret from fish shops in Western Cape, South Africa. Fish were processed by frying, grilling and boiling. The sum of the two PAHs (∑2PAH) ranged between 0.56 and 1460 ng/kg in all boiled, grilled and fried fish species. The grilled and boiled fish indicated considerably lower levels of ∑2PAH than fried fish ($p < 0.05$). The results indicated that the fish products were safe for consumers as these ∑2PAH levels varied over a significant field between fish species and between different processing methods and within all samples that did not exceed the recommended 5000 ng/kg value for BaP in food substances.

There are several cases like smoked fish in canned oil, in which the contamination was influenced by the smoking process (Drabova et al., 2013), or presumably by vegetable oil (Stołyhwo & Sikorski, 2005). PAHs concentrations in smoked sprats in oil were investigated by Ciecierska & Obiedziński (2007). Both fish and oils were investigated and the conclusions were somewhat predictable considering the lipophilic character of PAHs. Hence, it was concluded that oils had between 7-times and 11-times higher total content of PAHs than the sprat itself. Total PAHs concentration in all oils in study ranged from 94.79 ng/kg to 562.03ng/kg, whereas for sprats were in the 12.68- - 85.55 ng/kg range.

Naturally, fish should not usually contain high levels of PAHs, yet in many cases, the concentrations of PAHs are oberved in unprocessed fish due to the environmental background contamination. In general, the concentrations are below the maximum limits currently allowed by worldwide regulations, but, nevertheless, they must be taken into account because they are cumulative with other sources of contamination. In a study conducted in West Africa, along the coast of the Gulf of Guinea (Bandowe et al., 2014), the concentrations of ∑28PAHs in muscle tissues averaged 192 ng/kg and were not statistically different between locations. The findings of other similar studies from Cotonou's lagoon and Benin's continental region (Soclo et al., 2008) or from the Niger Delta (Nwaichi et al., 2016) showed that the values of ∑16PAHs were up to 171.900 ng/kg for fish species like *Periophthalmus koeleuter* (mudskipper).

METHODOLOGY OF THE DETERMINATION OF PAHs

The methods for the determination of PAHs in fish have undergone changes from the first attempts in 1970s-1980s (Dunn and Fee, 1979; Lawrence and Weber, 1984; Douabul et al., 1987). However, nowadays, there are some issues associated with extract clean-up, separation of all individual PAHs, separation of all individual PAHs from matrices, detection of the separated components and quantification of the identified compounds. According to Akinpelu et al (2019), PAHs are nonpolar compounds, that accumulate in matrices with high lipid content or other nonpolar components. Among these, there are some factors that can affect their quantification such as pH, temperature, ionic strength and solubility in their matrix of origin (Akinpelu et al., 2019). PAHs are compounds with a varied physical and chemical characteristic. Due to this their quantification is often difficult from a technical and financial point of view, being no official procedure accepted and utilized by all researchers and laboratories. Legal regulations do not indicate a suitable method for the qualitative and quantitative analyses of PAHs, defining only the requirements for the methods used (Onopiuk et al., 2022). ISO 15753:2006 is an international standard that has been provided only for BaP in fats and oils. There are methods for benzo[a]pyrene analysis, as a general requirement and used for food control purposes. It must comply with the provisions of points 1 and 2 of Annex III (which refers to characterisation of methods of analysis) to Regulation (EC) No 882/2004 of the European Parliament and of the Council of on official controls in order to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (EFSA, 2008).

Sample preparation is a main and critical step in the analysis of PAHs from different fish product matrices. Several methods have been applied for the isolation of PAHs like solid-phase microextraction (SPME) which is a single-step, solventless and green sample pretreatment approach for the sampling of PAHs (Jalili et al., 2022). This type of sampling was applied to determine PAHs levels in fish samples (Yuan et al., 2019). Under optimized conditions, recoveries were 80.2–101% and RSDs were less than 6%. The LODs and LOQs were calculated in the range of 0.11–1.40 ng/kg−1 and 0.36–4.61 ngkg−1, respectively. The coating showed satisfying reproducibility and repeatability (RSD D <8.6%), with only 10 mL of 10% (v/v) acetone in water used as the extraction phase. Authors had concluded that the method is simple, sensitive, and robust. Particularly, other methods used are : Soxhlet extraction (Birkholz et al., 1988; Ajai et al., 2012), batch extraction enhanced by sonication (Jánská et al., 2006), alkaline saponification followed by re-extraction (Lee et al., 2015; Onopiuk et al., 2022), supercritical fluid extraction (SFE) (Järvenpää et al., 1996; Ali and Cole, 2002), pressurized liquid extraction (PLE) (Lund et al., 2009; Pinheiiro et al., 2021), microwave-assisted extraction (MAE) (Pena et al, 2006; Ramalhosa et al., 2012) and QuEChERS extraction (Ramalhosa et al., 2009; Forsberg et al., 2011). For the performance of the extraction step, organic solvents such as methanol, acetone, chloroform, dichloromethane, hexane and cyclohexane, or mixtures such as hexane-acetone, dichloromethane-acetone, hexaneacetone-toluene and hexanedichloromethane are used (Jánská et al., 2006). Certainly, each method has advantages and disadvantages and the choice of extraction depends on several factors including capital cost, operating cost, sample matrix, simplicity of operation and the availability of a standardized method (Oluseyi et al., 2011).

After sample preparation, the cleaning process follows in which the separation between target PAHs and other compounds takes place, and also the removal of any interferences that can challenge the determination of PAHs (Peng and Lim, 2022). Based on literature reviews, only a few methods have been studied extensively, namely column chromatography (CC), gel permeation chromatography (GPC), and thin-layer chromatography (TLC). Viegas et al. (2012) had compared two different methods of cleanup and pre-concentration steps. In the first approach, the eluted PAHs fraction was cleaned by SPE SiO_2 column and in the second, through a column with 17 ml of a n-hexane/dichloromethane (70:30)(v/v) mixture. In order to minimize volatile losses, the collected fraction was evaporated to dryness under nitrogen stream at room temperature. The residue was dissolved in 100 ml of acetonitrile and injected into the HPLC/FLD.

Generally, depending on the nature of food matrix the methods for the extraction of PAHs is chosen. In fish products, there are two methos widely used for determining the quantity of PAHs: high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). Peng and Lim (2022) state that HPLC with fluorescence detection (HPLC-FLD) is a highly recommended method for the routine screening purpose of PAHS due to its selectivity and sensitivity, being also used for the quantification of PAH isomers. Zachara et al. (2017) measured PAHs qualitatively and quantitatively using HPLC-FLD. In canned smoked sprats in oil were found the highest levels of Bap content (36.51 ng/kg) and ∑4PAHs (73.01 25 ng/kg). Another study investigated 12 PAHs in aquatic products by using the HPLC-FLD method (Zhang et al., 2010). The microwave-assisted extraction and neutral Al2O3 solid phase purification were the basics for the preliminary treatment procedure, followed by a selective isolation of PAHs from aquatic samples. In the 35 aquatic samples, researchers have detected variable levels of summed PAHs, ranging from 0.0 to 57.76 ng/kg.

The GC-MS methods have been used in the last decade due to the the selectivity of the MS-detector, the use of mass spectrum data for reliable confirmation of PAHs, and the possibility to use isotope labelled PAHs as internal standards (EFSA, 2008). It is considered that GC has a better resolution efficiency compared to HPLC and MS, providing high selectivity and sensitivity as well as the structural information of PAHs (Peng and Lim, 2022). BS EN 16619:2015 is a well-known European Standard that states a method for the evaluation of 4 of the 16 EU priority polycyclic aromatic hydrocarbons (PAHs), identified as target PAHs. Several researchers have investigated the levels of PAHs in fish meat with the use of GC-MS (Varlet et al., 2007; Tan et al., 2019; Tareq et al., 2022), and analysed both contaminated and spiked samples, ranging from 500 ng/kg to 1190 μg/kg. Li et al. (2013) developed a new method to determine the contents of PAHs in fish by gas chromatography–triple quadrupole mass spectrometry (GC–QqQ-MS/MS) with SRM mode. In this study, the limits of detection (LODs) ranged from 0.024–0.06 μg/kg and the limits of quantitation (LOQs) ranged from 80–20 μg/kg. By using GC-MS in a total of 32 meat and fish samples from European markets, Rascón et al. (2019) obtained low limits of detection (3–70 ng/kg), good accuracy (recoveries of 85–105%) and relative standard deviations below 7.5%. In grilled, smoked and roasted fish were present higher concentrations of PAHs than raw samples. Most of samples contained the target PAHs at levels from 11 to 6900 ng/kg with naphthalene, acenaphthene, fluoranthene and pyrene at the highest concentration.

CURRENT LEGISLATION ON PAHs

As previously described, PAHs are known as contaminants present in human life. Most studies dealing with PAHs exposure have focused on populations with a high occupational risk of contamination, which live near a potential pollutant source or subjects that are long-term smokers (Ibanez et al, 2005). Although people are exposed to PAHs through inhalation and skin absorption, the majority of contaminations occur through food (Wenzl et al., 2006). Thus, a regular daily diet (DDI) may contain high levels of PAHs. Several studies have estimated usual food intake analyzing the total PAHs content or only B[a]P. Ibanez et al. (2005) included a large sample sized of 40690 subjects from five geographic areas of Spain in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Spain cohort. The mean intake of B[a]P in the population was 14000 ng/day, and the mean intake of total PAHs was 85700 mg/day. Another study on 100 Chinese urban residents evaluated daily dietary intakes (DDI) of PAHs, using a "duplicate plate method" (Duan et al., 2016) and concluded that the DDI of B[a]P ranged from 60 ng per day to 13500 μg per day. Kazerouni et al. (2001) have estimated higher levels of B[a]P intake from several food products in 228 subjects in the Washington, DC metropolitan area. Authors have found that about 80% of subjects had a B[a]P intake within the range of 20 – 80 ng/day.

As can be seen, daily intake of PAHs may vary largely across geographical areas and subjects due to differences between food choices. Worldwide, there are several organizations that evaluate the relevance of PAHs in human life: the European Food Safety Authority (EFSA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Scientific Committee on Food (SCF), the International Agency for Research on Cancer (IARC), the United States Environmental Protection Agency (EPA U.S.) and the Agency for Toxic Substances and Disease Registry (ATSDR).

In the EU, SCF reviewed the presence and toxicity of PAHs in food and determined that a number of 15 PAHs represent a potential toxic hazard for human health, and also concluded that BaP may be used as an indicator of occurrence and effect of the carcinogenic PAHs in foods (SCF, 2002). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) confirmed these findings 3 years later and proposed adding benzo[c]fluorene to the group of PAHs of concern in foods. On the basis of the SCF findings, the Commission introduced maximum levels for BaP for some foods that are relevant for human diet intake, and in which high PAH levels were found (oils and fats, smoked meats and meat products, smoked fish and fish products, muscle meat of fish other than smoked, crustaceans, cephalopods, bivalve molluscs and infant foods). The current Regulation setting maximum levels for PAHs in foodstuffs is Commission Regulation (EC) No. 1881/20065. EFSA's CONTAM Panel (2008) has evaluated different PAHs and issued that a sum of either 4 or 8 PAHs would be a better indicator of both the occurrence and toxicity of the genotoxic and carcinogenic PAHs than B[a]P or PAH 2 alone.

According to these statements, the legislation was amended in the Commission Regulation (EU) No 835/2011 by setting maximum limits for benzo[a]pyrene and PAH4, which are presented in Table 3.

Table 3. Maximum limits for benzo[a]pyrene and PAH4 in the Commission Regulation (EU) No 835/2011

ENTRY NO.	FOODSTUFFS	MAXIMUM LEVELS (ng/kg)	
		Benzo(a)pyrene	Sum of benzo(a)pyrene, benz(a) anthracene, benzo(b)fluoranthene and chrysene
1	Smoked meat and smoked meat products	5000 until 31.8.2014 2,0 as from 1.9.2014	30000 as from 1.9.2012 until 31.8.2014 12,0 as from 1.9.2014
2	Muscle meat of smoked fish and smoked fishery products, without food products listed in points 3 and 4. The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans (*Brachyura and Anomura*) it applies to muscle meat from appendages.	5000 until 31.8.2014 2,0 as from 1.9.2014	30000 as from 1.9.2012 until 31.8.2014 12,0 as from 1.9.2014
3	Smoked sprats and canned smoked sprats (*Sprattus sprattus*); Smoked Baltic herring ≤ 14 cm length and canned smoked Baltic herring ≤ 14 cm length (*Clupea harengus membras*); Katsuobushi (dried bonito, *Katsuwonus pelamis*); bivalve molluscs (fresh, chilled or frozen); heat treated meat and heat-treated meat products sold to the final consumer	5000	30000
4	Bivalve molluscs (smoked)	6000	35000

CONCLUSIONS

This study aimed to synthesize existing knowledge, drawing from the examples presented in different studies on the subject. Quantitative and qualitative determination of PAHs in fish products has already a long history and it has been widely demonstrated that PAHs are present in the entire food chain of fish products, from raw products to industrial or domestic processed fish. The results of this finding imply that the majority of studies reported high variable levels of PAHs in fish products. This is due to the lack of standards and regulations. For a better understanding of this issue, which is a component of the global problem, it is necessary to consistently monitor the levels of PAHs and other contaminants in the aquatic environment. Thus, the premises of mitigation strategies that will effectively minimize any potential risks to both humans and aquatic creatures are created. Reducing the risk associated with the consumption of fish products contaminated with PAHs is a priority that requires a well-planned approach. Even if food security is a complex concept, periodically updated regulations on PAHs in fish products are needed, and one of them is an improved HACCP for fish products known to contain high levels of PAHs contamination. To improve food quality, monitoring systems for toxic compounds found in fish meat and proper response protocols to warn and protect public health may be developed. Therefore, further studies are needed to evaluate the impacts of PAHs from fish products on human health and methods to reduce the contamination.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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