Quantitative and Comparative Assessment of Smoked and Unsmoked Fish for Polycyclic Aromatic Hydrocarbons (PAHs)

David Emmanuel[a] and Francis Olumide Oladapo[a,#]
[a]Department of Chemical Sciences, Tai Solarin University of Education, Ijagun, Ijebu-Ode, Ogun State, Nigeria.
[#]Correspondence email: address: oladapofo@tasued.edu.ng, Tel. No: +2348032426294

Abstract

This study was aimed to profile and compare polycyclic aromatic hydrocarbons (PAHs) in different brands of raw and smoked fish. Fish samples were homogenized in anhydrous sodium sulphate and the homogenate was extracted using soxhlet extractor in a temperature-controlled mantle. The extract was purified using silica gel and analyzed using Gas Chromatography coupled with Flame Ionization Detector (GC-FID). The results from the analysis confirmed the presence of 15 PAHs in both the smoked and unsmoked fish samples. The results further showed that smoking increased PAHs in all fish samples analyzed but some levels of reduction were observed in herring. Naphthalene and acenaphthene were not detected in the three fish samples. Herring rarely acquired PAHs during smoking except for the concentration of benzo(a)anthracene and benzo(g,h,i)perylene which was 556.6% and 266.7% respectively after the smoking process. Additionally, this study revealed the biosorption potential of variety of fish to bioaccumulate environmental PAHs, ranked in the order of herring<catfish<mackerel. The sum of PAH4 detected in all fish samples exceeded the European Union safe permissible limit of 12µg/kg. The prevalence of high molecular weight PAHs was an indicator of likely carcinogenic health risks their consumption could elicit.

Keywords: Polycyclic Aromatic Hydrocarbons, Smoked Fish, Wood, carcinogens

Introduction

Fish constitutes a major component of human dietary menu up to a third of the global population. It serves as a cheap source of protein and, is rich in vitamins, minerals and, omega-three fatty acids linked with the amelioration of coronary heart risks, cancer and high blood pressure (Carvalho *et al*., 2005). Processing techniques such as boiling, cooking, frying, sun-drying, oven-drying, smoking and grilling are widely employed to preserve raw fish for consumption. In the last decades, research focus has shifted towards human exposure to polycyclic aromatic hydrocarbon (PAH) via a consumption of smoked fish. Traditional fish preservation technology elicits incomplete combustion with wood and charcoal which potentially raises the PAH levels in fish (Silva *et al*., 2011). Consumption of smoked fish has been recognized as one of the food exposure pathways by humans to PAHs (Cheung *et al*., 2007).

PAHs are a class of toxic organic chemicals formed from the fusion of two or more benzene rings and, are prevalent in the global environment. Hundreds of PAHs exist in nature but only sixteen have been considered by the United States Environmental Pollution Agency (USEPA) as priority contaminants (Yan *et al*., 2004). Amongst these sixteen, only naphthalene belongs to the two-ring family; five (acenaphthene, acenaphthylene, phenanthrene, fluorene and anthracene) to the three-ring family, four (benzo(a)anthracene, chrysene, pyrene and fluoranthene) belongs to the four-ring family, four (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene) to the five-ring family whilst the other two belong to the six-ring family. PAHs with high molecular weight easily bioaccumulate in soil particles (Tao *et al*., 2002; Duan *et al*., 2015), in benthic sediments (Palmer-Fleming *et al*., 2004, Wang *et al*., 2009; Leung *et al*., 2015), in air samples (Zhang *et al*., 2011) and in invertebrates (Bordajandi *et al*., 2004) in the environment. The transport pathways of PAHs in the biosphere are pointedly responsible for their occurrence in the biological food chain. Cheng *et al* (2016) reported PAHs in Napier grass and remnants of food materials integrated into fish meals. A study has reported 0.184-0.51 mg/kg of PAHs in tilapia fish (dry weight) from Mai Po, Hong Kong (Liang *et al.,* 2007) and 0.002-0.118 mg/kg in fish samples available in Hong Kong

markets in China (Cheung *et al*., 2007). In Nigeria, studies have confirmed the occurrence of PAHs in varying degrees in fish samples from Lagos Lagoon (Sogbanmu *et al*., 2019; Rose *et al*., 2012) and Warri Coastal fish (Asagbra et al., 2015)

PAHs have received significant attention due to the potential adverse health problems associated with their occurrence in fish, aquatic invertebrates and, human beings at elevated concentrations (Zedeck and MS, 1980; White, 1986). Fish exposed to PAHs, from either petrogenic or pyrogenic sources, can develop adverse physiological and developmental effects which include DNA damage, endocrine distortions, immunosuppression, growth reduction, cardiotoxicity, hepatic and epidermal lesions, osmoregulatory imbalance, disrupted cell membrane, reduced fecundity and poor elongated development to maturity (Tuvikene, 1995, Murawski *et al*., 2014, Hicken *et al*., 2011, Heintz *et al*., 2007, White *et al*., 1999, Brette *et al*., 2014). It has been reported that PAHs may potentially result in carcinogenicity and mutagenicity in fish and may pose carcinogenic risks to human beings (White, 1986, IARC, 2010). Sogbanmu *et al.* (2016) has published the development of embryotoxicity and genotoxicity by zebrafish (*Danio rerio*) embryos when exposed to sediment extracts contaminated with PAHs.

This study was conducted to profile, quantify, and compare the levels of PAHs in raw and smoked fish samples and evaluate the potential carcinogenic health risks these PAHs may pose if smoked fish samples were consumed.

Materials and Methods

Materials

A certified PAH mixture comprising naphthalene, acenaphtylene, acenaphthene, flourene, phenanthrene, anthracene, chrysene, flouranthene, pyrene, benzo(a)anthracene, benzo(b)flouranthene, benzo(k)flouranthene, benzo(a)pyrene, benzo(g,h,i)perylene dibenzo(a,h)anthracene,and indo(1,2,3-cd)perylene was purchased from Sigma-Aldrich, United Kingdom. GC-FID was purchased from Agilent Technologies, USA.

Methods

Sample Collection

Raw samples of catfish (*Clarias gariepinus*), herring (*Clupea harengus*), and mackerel (*Scomber scrombrus*) were bought at Oke-Aje and New Market in Ijebu-Ode, Nigeria. The length and weight of raw were measured and each fish triplicate was divided into two equal half with a stainless steel knife. One-half of each fish species was smoked with firewood whilst the other halves were kept raw in the refrigerator at -20^{o}C for further analysis. Triplicate samples of each fish species of similar weights were isolated for analysis.

Sample Preparation

The descaled mackerel, catfish and herring were thoroughly washed with clean tap water to remove dirt and sand. They were subsequently rinsed with distilled water and brined with 10% NaCl solution before placing on wire gauze fitted to a drum-type smoking kiln. Wood served as a fuel and a distance of 30 cm was maintained between fish and the flame. The smoking was carried out for 6 hours after which the fish was allowed to cool for 1 h and then wrapped in foil paper before PAH analysis. The smoked fish samples were kept at -20^{o}C.

Soxhlet Extraction and Purification

A homogenized fish muscle sample (20 g) was weighed and mixed thoroughly with 5 g of anhydrous sodium sulphate in a laboratory crucible until a complete homogenate was obtained. The extraction was carried out using a Soxhlet extractor apparatus which consists of a 250 cm^3 round-bottomed flask, a condenser and an extractor tube, seated in a temperature-controlled heating mantle. The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of the Soxhlet extraction unit. The extraction was carried out as recommended by USEPA 3540 method, using 150 cm^3 dichloromethane for 16 hours. The extract was concentrated to 2 cm^3 using a Fischer brand rotary

evaporator in a water-bath that was pre-set to a temperature of 35 °C and was stored in an amber bottle and kept in a refrigerator to avoid oxidation of the extract before its clean-up. The same procedure was used for all the fish samples collected. The extracted samples were purified using a column chromatography technique. The column was prepared by loading 10 g of activated silica gel (100-200 Mesh) into a chromatographic column (1 cm internal diameter) to 5 cm and was topped with 1cm of anhydrous Na_2SO_4. The column was then conditioned with dichloromethane. 2 cm^3 of the concentrated extract was loaded and eluted with 20 cm^3 of dichloromethane. The polar lipids from the extract was completely removed using this method. Before GC/FID analysis, the extracts obtained were preserved in an amber bottle to avoid oxidation.

Gas Chromatographic Analysis

Samples were analyzed using Agilent 7890B Gas chromatograph equipped with a flame ionization detector (FID), fitted with a DB-1 capillary column coated with 5% Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25 µm film thickness) (Agilent Technologies). 1 µL of the samples was injected in a split-less mode at an injection temperature of 220 °C, at a pressure of 14.861psi and a total flow of 21.364 mL/min. Purge flow to split vent was set at 15 mL/min at 0.75 min. The oven was initially programmed at 100 °C (2 min) then ramped-up at 10 °C/min to 280 °C (4 min) and then ramped to 300 °C at 10 °C/min. The FID temperature was 300 °C with hydrogen air-flow at 30mL/min. Nitrogen was used as a complementary gas at a flow-rate of 18 mL/min.After calibration, the samples were analyzed and corresponding concentrations calculated. The labeled chromatograms were also extracted and reported.

Results and Discussion

The results of the fish analyses show different build-up patterns of PAHs in three different kinds of fish samples (Table 1.0). The occurrence of PAHs in unsmoked fish samples is a confirmation that the aquatic environment constitutes a sink for runoffs, treated domestic and industrial wastewaters, crude wastes, and atmospheric condensate, which are potential sources of PAHs. The capacity for bioaccumulation of PAHs in fishes is subject to their general attributes and behaviours, which include ability of the gills to ingest food and sediments, tropic levels in the marine and fresh water environments, fat contents and metabolic capability (Cheung *et al*., 2007; Meador *et al*., 1995; Van der Oost *et al*., 2003). Acephthylene, fluorene and anthracene were not detected in unsmoked catfish but 5.55µg/kg, 1.23 µg/kg and 18.46 µg/kg respectively were found in smoked catfish fish. The total PAHs recorded in smoked catfish, herring and mackerel fish were 832.16 µg/kg, 488.04 µg/kg and 1612.79 µg/kg respectively. The process of smoking raised the total PAH contents in catfish, mackerel and herring fish correspondingly by 524.79%, 576.93% and 63.97%. Shi *et al* (2016) documented a total PAH content of 1177µg/kg in chub mackerel used for their work. The levels of PAHs bioaccumulation in catfish, herring and mackerel fish during smoking are subject to their lipid contents. Previous studies have reported a strong correlation between fat contents in fish and the concentrations of PAHs (Cheung *et al*., 2007, Silva *et al*., 2011).

In mackerel fish, the prevalence of high molecular weight benzo(a)anthracene, fluoranthene, pyrene and chrysene, classified as PAHs with 4-6 rings (ATSDR, 2000), strongly suggests high carcinogenic risks associated with their consumption. Eight of such high molecular weight PAHs are classified to be carcinogenic because of their known potentials to induce carcinogenicity (IARC, 2010)

Table 1.0: Polycyclic Aromatic Hydrocarbons in Different Fish Classifications (n = 3)

PAHs	Catfish(mg/kg)		Herring Fish(mg/kg)		Mackerel Fish(mg/kg)	
	Unsmoked	Smoked	Unsmoked	Smoked	Unsmoked	Smoked
Acenaphthylene	-	5.55±0.10	1.81±0.24	1.42±1.32	0.88±0.21	67.47±7.34
Fluorene	-	1.23±0.31	1.21±0.05	2.66±1.3	1.64±0.62	5.33±2.2
Phenanthrene	1.34±0.01	6.69±0.82	2.01±0.95	1.94±1.1	2.20±0.12	370.14±40.22
Anthracene	-	18.46±2.21	5.04±1.58	5.15±2.60	3.08±1.12	63.96±6.42
Fluoranthene	3.83±0.05	59.08±2.13	10.70±2.47	15.38±2.79	9.44±2.1	284.50±30.28
Pyrene	1.09±0.01	9.26±1.41	1.79±0.94	0.62±0.02	0.97±0.14	132.84±14.4
Benzo(a)anthracene	61.23±11.14	398.70±9.35	38.55±3.44	253.12±28.7	21.63±2.24	366.99±38.6
Chrysene	18.81±0.2	196.84±20.7	17.19±4.20	8.37±2.22	4.54±1.10	124.50±12.8
Benzo(b)fluoranthene	-	51.05±6.7	1.41±1.04	3.93±0.62	7.74±1.02	62.78±6.44
Dibenzo(a,h)anthracene	7.98±0.06	9.38±2.2	12.82±2.14	9.65±1.55	3.32±0.64	15.74±2.46
Benzo(k)fluoranthene	8.18±2.01	26.54±3.22	18.22±2.32	7.22±2.13	16.01±2.1	67.06±6.48
Benzo(a)pyrene	1.56±0.12	5.03±2.14	6.22±1.44	12.84±3.24	3.81±1.0	7.90±2.24
Indeno(1,2,3-cd)perylene	1.86±1.05	24.40±2.6	5.22±2.06	6.34±1.11	5.29±1.43	20.03±2.62
Dibenzo(a,h)anthracene	7.98±1.03	9.38±3.04	12.82±2.55	9.65±2.02	3.32±0.05	15.74±3.22
Benzo(g,h,i)perylene	19.33±2.18	10.57±2.32	40.83±4.21	149.75±50.2	154.38±16.82	7.81±2.55
Total PAHs	133.19±17.86	832.16	175.84±29.63	488.04±29.63	238.25±30.71	1612.74±178.27

Comparatively, the PAH contents in catfish, herring and mackerel are clearly expressed in Figures 1.0, 1.1 and 1.2. For the catfish, the low molecular weight PAHs were prominent in unsmoked samples but the high molecular weight PAHs were more dominant in smoked fish. Herring acquired high proportions of benzo(a)anthracene and benzo(g,h,i)perylene during smoking similar to catfish (Figure 1.1). The levels of PAH biosorption in mackerel were high across the low molecular weight to the high molecular weight PAHs. These results strongly suggest the occurrence of high lipid content in mackerel fish compare to the other two (Cheung *et al*., 2007).

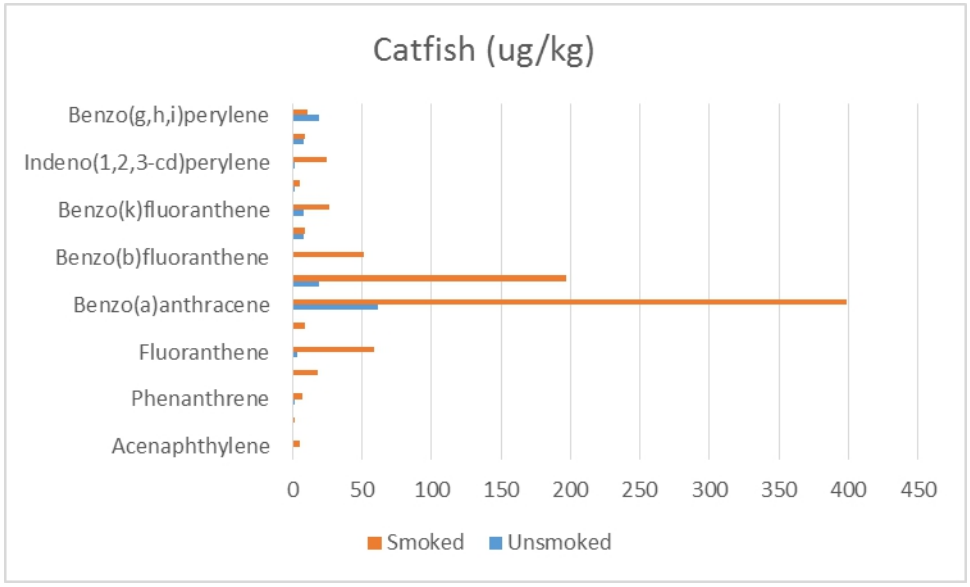

Figure 1.0: Concentrations of PAHs in smoked and unsmoked catfish

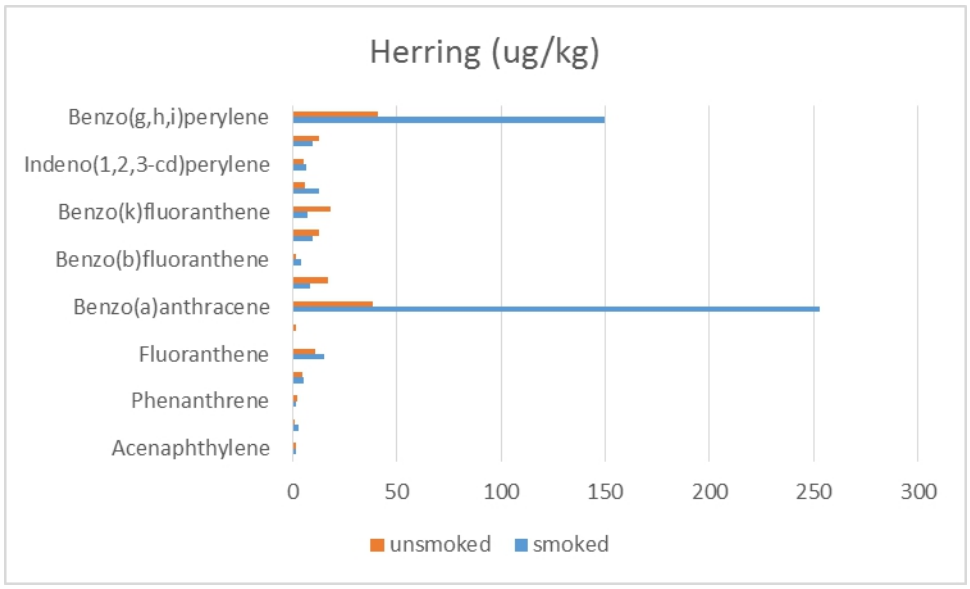

Figure 1.1: Concentrations of PAHs in smoked and unsmoked herring fish

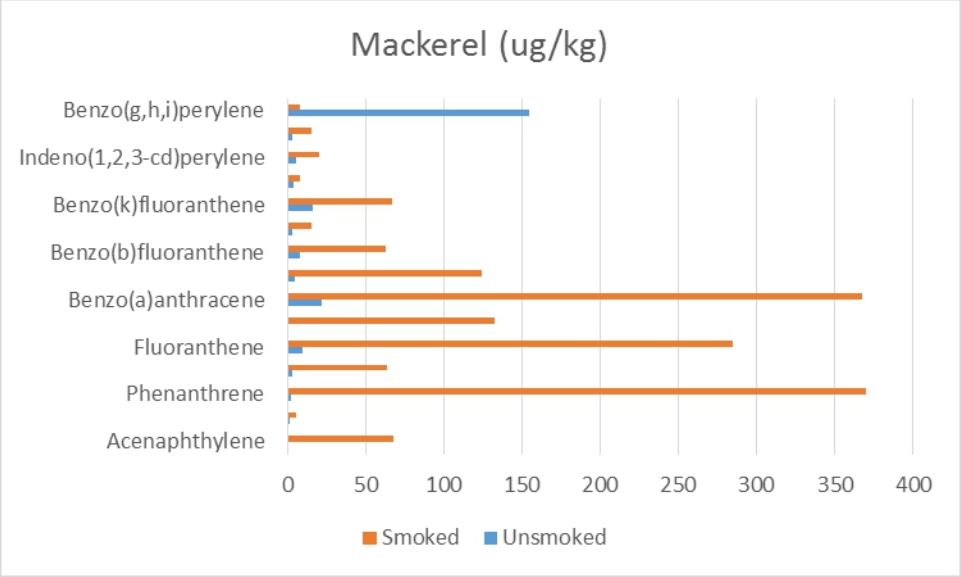

Figure 1.2: Concentrations of PAHs in smoked and unsmoked mackerel fish.

Given that eight PAHs, ranked amongst the high molecular weight PAHs, are known and/or potential carcinogens, consumption of mackerel may elicit serious health risks. The concentrations of the sum of PAH4 (namely, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(a)pyrene) in all the three fish samples were 653.62 µg/kg, 278.26 µg/kg and 562.17 µg/kg respectively which exceeded the recommended European Union safe permissible limit of 12µg/kg (EC No:1881/2006) established in December 19th, 2006.

Conclusion

This study revealed that PAHs may be acquired by fishes in the aquatic environment. Biosorption of PAHs into the fish tissues may occur during fish smoking process and the levels of such biosorption is predicated on the lipid contents of the fish. Naphthalene and acenaphthene were not detected in the three fish samples examined. The total levels of PAHs recorded after smoking vary in ascending order of herring <catfish< mackerel. The sum of PAH4 found in all the fish samples exceeded the European Union safe permissible limit. A significant amount of high molecular weight PAHs found in mackerel raises serious concern given that it may constitute potential health risks to fish consumers.

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