Characterization of PAHs Concentration in Fresh and Raosted Catfish Samples from Esan West L.G.A

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Abstract

The characterization of PAHs in fresh and roasted Catfish samples was the objective of these studies. PAHs has been partly identified by scientist amongst the many factors attributed to cancerous effect. Sixteen (16) polycyclic aromatic hydrocarbons (PAHs) in fresh and roasted fish samples were evaluated in this study. Extraction was done using mix solvent of cyclohexane and acetone in ratio 50:50. Analysis was performed by GC-MS. The procedure involved extraction, and adsorption column for clean up. The cleaned fluid samples were concentrated using rotary evaporator and compressed air. The resulting solution were stored in vial bottles ready for PAH's determination. The various samples were run through G.C-MS chromatography column. Results of each sample were obtained graphically. Our findings revealed that of all 16 PAH's targeted, only 6 PAH's were detected. These are Pyrene, Fluoranthene, Florene, Phenanthrene, Anthracene and Benzo(a)anthracene. Bulk of the PAH's were below detectable limits. The roasted fish samples had greater PAH's concentration(2.899mg/kg maximum). In general the smoked fish samples had higher PAH's contents (an average of 1.945mg/kg). Benzo(a)anthracene was only detected in FRFH 3. From the findings the concentration of PAHs was above recommended level: 5.0ug/kg BaP in smoked fishery product. It was also discovered that when fresh fish are roasted, the amount of PAHs increases and new PAHs can be formed in the process.

Keywords: *Polycyclic Aromatic Compound, Cats fish, Clarias gariepinus*

INTRODUCTION

Polycyclic aromatic hydrocarbons are hydrocarbons-organic compounds containing only carbon and hydrogen- that are composed of multiple aromatic rings (organic rings in which the electrons are delocalized). PAHs are uncharged, non-polar molecules found in coal and in tar deposits. Studies suggest that PAHs account for a significant percentage of all carbon in the universe.

They are also produced by the thermal decomposition of organic matter. Kirk K.B. and Logan, M. B. (2015)

Polycyclic aromatic hydrocarbon, PAHs are non-polar and lipophilic. Larger PAHs are generally insoluble in water, although some smaller PAHs are soluble and known contaminants in drinking water (Fetzer, 2000).

Polycyclic aromatic hydrocarbons are discussed as possible starting materials for abiotic syntheses of materials required by the earliest forms of life. The tricyclic species phenanthrene and anthracene represent the starting members of the PAHs(Hoover, 2014). Two-ringed PAHs, and to a lesser extent three-ringed PAHs, dissolve in water, making them more available for biological uptake and degradation(Walker, MacAskill, Rushton, Thalheimer and Weaver, 2013). In solid state, these compounds are less accessible for biological uptake or degradation, increasing their persistence in the environment. Human exposure to PAHs does not occur singly, it is encountered in complex mixtures of PAHs (Amal, Murad, Helaleh, Nisar, Ibtisan, and Zainab, 2010). Foodstuffs can be contaminated by PAHs in several ways, such as either direct or indirect contact with smoke. The routes through which PAHs travel include food, air, and water. The highest levels of PAHs are found in foodstuffs; both processed and unprocessed (Amal et al., 2010). European Union legislation (EUL) sets a maximum allowed concentration for benzo[a]pyrene B[a]P in different food product in the range 1–10 ug/kg and for benzo[a]anthracene (B[a]A) and Benzo[a]pyrene (B[a]P) in liquid smoke flavoring of 20 ug/kg. Recommended allowable daily intakes range from 0.04 to 0.42 ug/day in Italy (Amal et al., 2010). A wood-burning open-air cooking stove, Smoke from solid fuels like wood is a large source of PAHs globally. Burning solid fuels such as coal and biofuels in the home for cooking and heating is a dominant global source of PAH emissions that in developing countries leads to high levels of exposure to indoor particulate air pollution containing PAHs, particularly for women and children who spend more time in the home or cooking (Srogi, 2007). For the general population in developed countries, the diet is otherwise the dominant source of PAH exposure, particularly from smoking or grilling meat or consuming PAHs deposited on plant foods, especially broad-leafed vegetables, during growth (Haritash and Kaushik, 2009).

Emissions from vehicles such as cars and trucks can be a substantial outdoor source of PAHs in particulate air pollution. Geographically, major roadways are thus sources of PAHs, which may distribute in the atmosphere or deposit nearby (Choi, Harrison, Komulainen, Delgado and Saborit, 2010). PAHs transform slowly to a wide range of degradation products. Biological degradation by microbes is a dominant form of PAH transformation in the environment (Hylland, 2006). PAHs are rich in carbon and therefore they are lipophilic, a property which facilitates their accumulation in lipid tissues (Chen and Liu, 2011). The presence of PAHs in foodstuffs has been found to vary depending on the type and fat content of the food, cooking process (fried, grilled, roasted, boiled and smoked), temperature and duration of cooking, type of fuel used (electrical, gas, wood and charcoal), proximity and direct contact with heat source (Elliyana, Parvanch, Jinap, and Ahmad, 2016).

There are a number of proposed possible mechanisms which explain how PAHs are generated during cooking. Perhaps, the most significant mechanism for formation of PAHs is when fat drips directly over flame or heat surface. Besides, cooking food at high temperature also leads to endogenous formation of PAHs by pyrolysis of organic matter such as fats, proteins and carbohydrates, and the greatest concentrations PAHs have been shown to a rise from pyrolysis of fat (Elliyana et al., 2016). Further, incomplete combustion of fuel can generate PAHs that are brought onto the surface of the food (Akpambang, Puraco, Ladije, Amoo, Conte and Moret, 2009).

Amal et al., 2010 determined the Levels of Polycyclic Aromatic Hydrocarbons in Toasted Bread Using Gas Chromatography Mass Spectrometry. The effects of toasting procedures on the polycyclic aromatic hydrocarbons (PAHs) levels in bread was tested, and they reported that PAH levels are up to 350 ug/kg in toasted samples when using a wood flame. Several researchers have looked at the concentration of PAHs in staple foods viz; grilled meat, fish, plantain, yam etc.

Tajudeen, Aderibigbe, and Olasupo, (2017) studied Polycyclic Aromatic Hydrocarbon (PAHs) in some smoked foodstuffs in Lagos state, southwest Nigeria. The results of sixteen PAHs in the studied smoked food showed that PAHs of low molecular weight such as acenaphthene and anthracene were detected in all the food samples analyzed viz: smoked fish, roasted yam, shawama, suya, roasted plantain and roasted corn. Higher molecular weights PAHs such as fluoranthene, pyrene and benzo(a) anthracene were also detected. Amos-Tauta, Inengite, Abasi, and Amirize, (2013) did analysis of raw and roasted ready-eat food namely; Atlantic mackerel, suya beef and plantain sold and consumed in Amassoma town. These foods were screened for 15 polycyclic aromatic hydrocarbons (PAHs). Appreciable amount of benzo(a)pyrene and benzo(b)fluorantehene were found present in roasted mackerel fish, while a mean concentration of 7.23ug/g benzo(a)anthracene were detected in suya beef.

In a research carried out by Akpoghelie (2018), on assessment of Polycyclic Aromatic Hydrocarbon (PAHs) on smoked fish and suya meat consumed in Warri, Nigeria, the result obtained revealed that the PAHs values for grilled meat/smoked fish are far higher than those obtained with those soaked in boiled water. Okoronkwo, Mba, and Ajuonuma (2014); Akpanbang, Puraco, Lajide, Amoo, Conte and Moret (2009) all have results which aligned with initial researches. However, there was a deviation in the amount of PAH present in raw and roasted plantain (0.19ppm and 40.33ppm respectively) in a study carried out by Adetunde, Oluseyi, Olayinka, Oyeyiola and Alo (2012). Amos Tauta *et al.*, (2013) recorded that no PAH was detected in roasted plantain. They surprisingly saw that benzo(a)pyrene was not detected at all in suya beef. This is contrary to result obtained by Akpambang *et al.*, (2009) who reported BaP at levels ranging from 2.4 to 31.2ug/kg weights smoked fish and meat samples.

Materials and Methods

Location:

Ekpoma is a town in Edo State, Nigeria. It is the administrative headquarters of the Esan West Local Government Area. Ekpoma lies on the geographical coordinate of latitude 6°45′N 6°08′E *Longitude: 6° 08' 25.04" E* at a distance of about 78km from Benin City Main town . The town has an official Post Office, and it is home to the Ambrose Alli University. Currently Ekpoma town is developing with major infrastructures, hospitals, schools, modern eateries and roads. The town is also secured. It has a population of over 290,000 people. It has an adult male population of over 90,000 and adult female population of over 80,000. It is politically divided into 10 wards and occupies a land mass of 502 km^2 (Saharareporters.com. Retrieved 2023-01-27)

Figure 1.0 MAP SHOWING EKPOMA GEOGRAPHICAL LOCATION

Materials

Clarias gariepinus commonly called catfish (FRESH and ROASTED)

Chemicals and reagents

All reagents and chemicals used were of analytical grades, standards and include the following:

Acetone (Sigma-Aldrich Corporation, United states)

Dichloromethane (Sigma-Aldrich Corporation, United states)

Cyclohexane (Sigma-Aldrich Corporation, United states)

The three solvents were redistilled before use in order to keep them free from impurity.

Sodium sulphate: granular and anhydrous. The sodium sulphate granules were purified by heating at 400^0C for 4 hours in a shallow tray and cooled in dessicator

Mix 26- the internal standard or surrogate: the mix-26 used as the internal standard comes in 1ml vials and was prepared by diluting the 1ml solution which contains 4000ng/µl in 100ml of dichloromethane. The solution which contains 40ng/100ml will thus have a fluorescent green colour.

Equipment/Apparatus

The equipment/apparatus used include: water bath, , oven, dessicator, blender, ultrasonic bath, SPE cartridges with stand, and gas chromatography instrument, and drier.

Sample Collection

The fish samples, *Clarias gariepinus* also known as catfish (raw and roasted) were obtained from Ambrose Alli University school farm, Ekpoma. The samples were kept in sample bags, labeled properly, kept in coolers with ice and transported to the laboratory where they were stored in refrigerator at 4^0C prior to other treatments.

Methods

The analysis was carried out with a gas Chromatography (GC) – Flame Ionization Detector (FID) equipped with auto sampler.

Sample Preparation:

Extraction and Clean-Up of Samples

The fresh fish samples from sampling sites were washed and re-rinsed with distilled water. The fresh and roasted fish samples were separately grounded using a blender (Mikachi meat grinder) and stored in a refrigerator at 4 ^{0}C prior to extraction and analysis.

50g of each of the fish samples was mixed with 25g of sodium sulphate and 200ml of 50/50 cyclohexane/acetone mixture in a tight fitted covered bottle and10ml of the internal standard was added to each of the bottles. Each bottle containing the sample, solvents mixture, sodium sulphate and the internal standard mix were placed inside an ultrasonic bath (Astrabro ultrasonic cleaner) model 7E for 2hours. The bottles were brought out after every 10mins and shaken. 25ml of the extract was collected using a pipette and filler and concentrated using rotary evaporator to 5ml and each 5ml concentrate was concentrated to 1ml using compressed air and clean up was done using solid phase extractor and cyclohexane was employed as the eluting solvent. The cleaned up sample was concentrated to 1ml using compressed air and stored in 1ml vials and subjected to GC analysis using FID detector.

GAS CHROMATOGRAPHY OPERATING PROCEDURE

Instrument type: Gas chromatography system 6890 series

Product: HP

Detector type: FID

The basic chromatography parameters for the analysis of polycyclic aromatic hydrocarbons are as follows:

Initial Temperature: 100^0C

Rate 1:4^0C/mins

Final temperature: 330^0C

Detector temperature: 300^0C

1ml extract of both the *Clarias gariepinus(fresh and roasted)* kept in vials were analyzed using GC-MS model QP2010SE, Shimazu, Japan.

RESULTS AND DISCUSSION

TABLE 1. Distribution of Polycyclic Aromatic Hydrocarbon (PAHs) in Fresh and Roasted Fish Samples (mg/kg)

COMPONENT	FRFH1	FRFH2	FRFH3	RTFH1	RTFH2	RTFH3
Naphthalene	0.000	0.000	0.000	0.000	0.000	0.000
Acenaphthalene	0.000	0.000	0.000	0.000	0.000	0.000
Acenaphthene	0.000	0.000	0.000	0.000	0.000	0.000
Florene	0.046	0.067	0.136	0.086	0.046	0.062
Phenathrene	0.029	0.072	0.000	0.054	0.024	0.046
Anthracene	0.038	0.063	0.100	0.047	0.000	0.000
Fluoranthene	0.251	0.428	0.195	0.278	0.291	0.248
Pyrene	0.000	0.000	0.000	0.000	2.539	2.115
Benzo(a)anthracene	0.000	0.000	0.038	0.000	0.000	0.000
Crysene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(b)fluoranthrene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(a)pyrene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(k)fluoranthrene	0.000	0.000	0.000	0.000	0.000	0.000
Indeno1,2,3)perylene	0.000	0.000	0.000	0.000	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i)perylene	0.000	0.000	0.000	0.000	0.000	0.000
Total PAH(mg/kg)	0.364	0.630	0.469	0.466	2.899	2.471

KEY:

RTFH – ROASTED FISH
FRFH – FRESH FISH
BDL - BELOW DETECTABLE LIMIT

The summary of the concentration of various Polynuclear Aromatic Compounds (PAHs) present in both fresh and roasted fish samples are shown in Table 1. Table 2 shows the comparison of the result of fresh fish and roasted fish. This research was carried out to evaluate the presence and concentration of 16 PAHs present in fish (fresh and roasted) in Ekpoma, Edo state. The result of the research showed that not all 16 targeted PAHs were present in the fresh and roasted fish

samples. A total of 6 PAHs were discovered in the samples. These are Pyrene, Fluoranthene, Florene, Phenanthrene, Anthracene and Benzo(a)anthracene. The concentration of PAHs in the samples is as shown in Table 2 and 3.

FRESH AND ROASTED FISH SAMPLES:

TABLE 2. Concentrations (mg/kg) levels of PAHs in fresh fish and roasted fish

PAHs	FRFH 1	FRFH 2	FRFH 3	AVERAGE	RTFH 1	RTFH 2	RTFH 3	AVERAGE
Pyrene	0.000	0.000	0.000	0.000	0.000	2.539	2.115	1.551
Fluoranthene	0.251	0.428	0.195	0.291	0.278	0.291	0.248	0.272
Florene	0.046	0.067	0.136	0.083	0.086	0.046	0.062	0.065
Phenanthrene	0.029	0.072	0.000	0.051	0.054	0.024	0.046	0.041
Anthracene	0.038	0.063	0.100	0.067	0.047	0.000	0.000	0.016
Benzo(a)anthracene	0.000	0.000	0.038	0.013	0.000	0.000	0.000	0.000
TOATAL	0.364	0.63	0.469	0.505	0.465	2.9	2.471	1.945

The roasted fish samples had the average highest PAHs concentration. Average value for fresh fish samples was 0.505mg/kg, while that of roasted fish was 1.945mg/kg. This is justified with the fact that when samples are roasted, smoked or grilled, the amount of PAHs increases. Roasted fish 2 had the highest concentration of PAH (Pyrene) with a concentration of 2.539mg/kg. The PAHs concentration in our samples (fresh and roasted) ranged between 0.000mg/kg to 2.539mg/kg.

The lowest value for the concentration of PAHs in our sample was found in fresh fish 3 (Benzo(a)anthracene with a concentration of 0.013mg/kg). Surprisingly, there was no detection of Benzo(a)anthracene in our smoked samples. This clearly shows that though fresh PAHs can be introduced during roasting, however there may be PAHs inherent in fresh samples. This can be attributed to several factors like feed type, materials used in lining ponds, and possibly nature of soil around fishing environment, as well as activities in or around water bodies where these samples were obtained.

The concentration of Fluoranthene in the fish sample; both fresh and roasted were relatively the same. This is an indication that heating was not a factor as to its presence. Its presence could be as a result of feed, antirust used in lining the pond in situations where iron tanks are used or any other factor inherent in the environment.

Pyrene was not detected in any of the fresh fish sample, but was present in relatively high amount in RTFH 2 and RTFH 3. This can be linked to pyrolytic factor which is a function of fat content and heating temperature. Pyrolysis of the fats in the fish generates PAHs that become deposited on the fish. PAHs production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the fish and the proximity of the food to the heat source. Interestingly again, Benzo(a)anthracene was present in fresh fish sample but wasn't detected in any of the roasted samples. What must have been the reason?

In order to determine the source of PAHs in sample, whether its cause is pyrolytic, combustive, petrogenic or from petroleum hydrocarbon, Amos Tauta et. al., 2013 stated thus: 'In order to determine the source of PAHs in detected samples the ratio of Fluoranthene (fla) to Pyrene (pyr) and Phenanthrene (ph) to Anthracene (An) are often used. Ratio of Fluoranthene to Pyrene greater than one (Fla/Pyr >1) is attributed to pyrolytic source and Fla/Pyr <1 is attributed to petroleum hydrocarbon source. Similarly, ratio of phenanthrene to anthracene less than ten (Ph/An<10) indicates combustion source and Ph/An>10 is attributed to petrogenic source.' From the foregoing, the PAHs of the roasted fish can be attributed to combustion source since the ratio is <10.

PAHs CONCENTRATIONS:

Figure 4.1: total mean concentration of PAHs in FRESH and ROASTED sample.

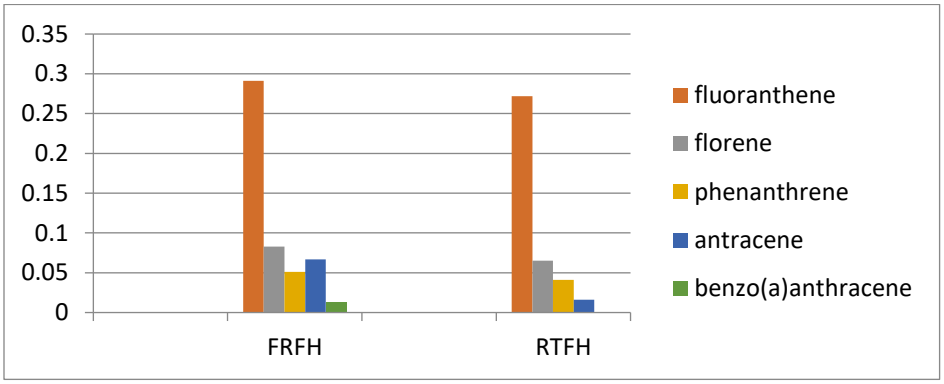
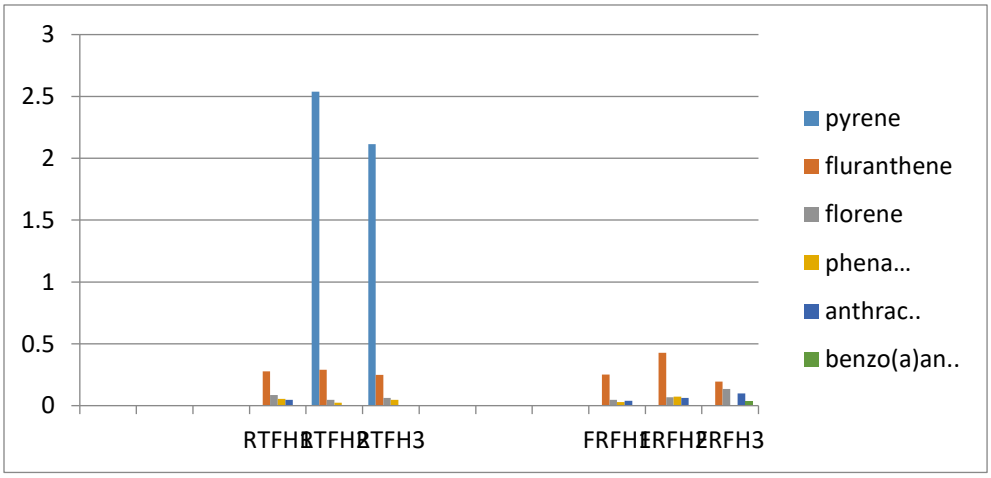


FIGURE 4.2: Polynuclear Hydrocarbon Content (mg/kg)



The highest concentration of total PAHs was detected in RTFH 2, with concentration of 2.9mg/kg. Report by Amos-Tautua B.M.W et. al., 2013 revealed that the sum of the average amount of the low molecular weight (LMW) PAHs (2 to 4 aromatic rings) such as naphthalene, acenapthene and pyrene, were found higher (42.48ug/g) than the higher molecular weight (HMW) PAHs

(20.95ug/g), those having 4 to 6 aromatic ring. However this does not allign with the average concentration of HMW PAHs like Fluoranthene ,benzo(a)anthracene and Pyrene which had a concentration of 0.792mg/kg in our study. Particularly, Pyrene had the highest concentration in samples where they are present as shown in tables 1 – 2. This result can be attributed to temperature of heating and also particle of PAHs sorbed into organic matter (Choi et. al., 2010).

Also, two-four ringed PAHs volatize sufficiently to appear in the atmosphere in gaseous form. Physical state of four ring however can depend on temperature (Walker, T. R., MacAskill, D., Rushton, T., Thalheimer, A., and Weaver, P., 2013). On the other hand LMW PAHs like florene, Phenanthrene and anthracene had relative low sum of avaerage concentration of 0.060mg/kg. This could be as a result of temperature. High temperature generate HMW PAHs and low temperature generate LMW PAHs. This could also be responsible for the high concentration of pyrene.

CONCLUSION

The result of the 6 samples for this study showed a total of 6 PAHs compounds. Both HMW PAHs and LMW PAHs were detected; not in all samples though. Total PAHs concentrations for roasted samples (fish) were higher than those of fresh (fish). This is because when food particularly fish is smoked, roasted , barbecued, or grilled; PAHs are formed as a result of incomplete combustion or thermal decomposition of the organic material (WHO, 2006). From the result of this research the concentration of PAHs is above recommended level; 5.0ug/kg or 0.005ug/g BaP in fishery product and 2ug/kg and 5ug/kg fixed by European standard (Akpoghelie O.J). Benzo(a)anthracene was only detected in FRFH 3. From the foregoing analysis, caution therefore must be taken because of the adverse effect posed by exposure to PAHs through food viz: genotoxicity, mutagenicity, and carcinogenicity. (Elliyana Nadia Hamidi, Parvanch Hajab, Jinap Selemat, and Ahmad Faizal Abdull Razis 2016).

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