LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN FISH SAMPLES FROM DIFFERENT PROCESSING METHODS

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Abstract

Fish is easily contaminated from water prone to water pollution. In this study, the levels of polycyclic aromatic hydrocarbons (PAHs) in fish samples processed, using three methods and marketed in Makurdi, Benue state, were determined. The samples were coded as SF (Smoked Fish), SD (Sundried) and FD (fried) fish samples. PAHs in the samples were quantified, using gas chromatography – mass spectrometer (GC-MS). The Σ_{16} PAH concentrations were in the range of 0.2-10.0 mg/kg. Both LPAHs/HPAHs and the binary diagnostic ratios of PAHs indicated pyrogenic and petrogenic sources Σ_{16} PAH and their dominant PAH ring-types were separately computed for each sample. The six-membered ring PAHs in smoked fish was 32.07% (with Bezo(ghi)perylene as most dominant). High levels of six member ring PAHs (Dibenz(a,h) anthracene) were also found in Sundried fish and fried fish contain Dibenz(a,h) anthracene. Generally, the PAHs concentrations in the samples, pointed more at pyrogenic than the petrogenic source. With exception of the five and six membered rings, estimated PAH levels were within the NAFDAC permissible limits.

Keywords: PAHs, Fish, Pollutants, Petrogenic, Pyrogenic, Diagnostic ratio

Introduction

Harvested fish requires preservation and storage to increase the shelf life. Major methods of fish processing and preservation have been reported [1,2,3]. The traditional methods of processing are often inefficient and unhygienic due to contamination [4]. The presence of PAHs in food is usually a

consequence of the nature of these compounds in the environment at their formation during cooking processes or as a result of the manufacturing processes [5]. Despite being environmental contaminants, PAHs are formed in food processing, such as drying, grilling, roasting, and smoking. PAHs tend to accumulate in water sediments over time [6].

Figure 1: Chemical Structures of Some Persistent Organic Pollutants (POPs) and Methyl Mercury Frequently Detected in the Dorsal Muscle Tissue (red) of Fish [7].

The origin of PAHs in the environments include pyrogenic, petrogenic, and biological sources [8]. Low molecular weight PAHs

(LPAHs) include two to three member rings while the high molecular weight PAHs (HPAHs) contain four to six rings and are

highly carcinogenic. PAH diagnosis, with LPAHs/HPAHs > 1 indicates petrogenic while more than one indicates pyrogenic origin. Several authors used the ratios of individual PAHs levels for source identification [9,18,20].

Table 1 is the priority PAHs list. The 16 PAHs are divided into five groups according to the number of the aromatic rings; 2- rings, 3-rings, 4-rings, 5-rings and 6-rings PAHs [10].

The rising incidence of health issues in which experts have implicated fish consumption as one of the entrant route for the toxicants, creates the need to evaluate the presence and impacts of contaminants in fish, particularly the organic types. This study explores the level of PAHs in fish samples that are processed and sold in Makurdi, Benue state, Nigeria. The limitation on “PAHs contributory role by the fish species used in this study and possibly, their habitat” is acknowledged. However, the study is an expository study of the fish samples processed in Markurdi, with their common and respective processing methods.

Materials and Methods

All reagents were of analytical grade. Dichloromethane (DCM >99 %), HPLC grade acetone and HPLC grade methanol and Commercial PAH standard containing 16 USEPA priority PAHs were supplied by Bristol Chemicals for Sigma Aldrich. The determination of PAHs in the sampled fish was carried out using the ultrasonication

extraction followed by Gas Chromatography-Mass Spectrophotometer (GC-MS) with Agilent Technologies GC-MS made in Germany.

Table 1: The 16 USEPA Priority PAHs

PAHs compounds	Molecular Formula	Carcinogenic activity
Naphthalene	C ₁₀ H ₈	+
Phenanthrene	C ₁₄ H ₁₀	-
Anthracene	C ₁₄ H ₁₀	±
Fluoranthene	C ₁₆ H ₁₀	-
Pyrene	C ₁₆ H ₁₀	-
Chrysene	C ₁₈ H ₁₂	-
Benzo(a)anthracene	C ₁₈ H ₁₂	±
Benzo(b)fluoranthene	C ₁₈ H ₁₂	++
Benzo(k)fluoranthene	C ₂₀ H ₁₂	+
Benzo(e)pyrene	C ₂₀ H ₁₂	±
Benzo(a)pyrene	C ₂₀ H ₁₂	+++
Perylene	C ₂₀ H ₁₂	±
Benzo(ghi)perylene	C ₂₀ H ₁₂	±
Dibenzo(ah)anthracenes	C ₂₂ H ₁₄	+++
Indeno(cd)pyrene	C ₂₂ H ₁₂	+
Coronene	C ₂₄ H ₁₂	±

(+++)- carcinogenic to experimental animals; ± - data are inadequate to permit an evaluation of the carcinogenicity of substance to experimental animals. - no evidence that substance is carcinogenic to experimental animals [10].

Table 1 shows some common PAHs, their molecular formula and carcinogenic activity

Sampling: As presented in Table 2, (i) smoked (ii) Fried and (iii) sundried fish were randomly selected from dealers in Wurukum and Northbank markets, Makurdi. Samples were packaged in perforated polythene bags and were refrigerated at 4°C before analysis [11].

Table 2: Sample description and Codes

S/N	Fish species	Processing Method	Sample Code
1	<i>Tilapia zilli</i>	Smoking	SF
2	<i>Ethmalosa fimbriata</i>	Sun-drying	SD
3	<i>Micropogon undulatus</i>	Frying	FD

Extraction and clean-up experiment

A documented sample pre-treatment method [12] was adopted. The ground samples (10 g) was weighed into conical flask. 20 mL of the mixture of analytical grade Dichloromethane (DCM) and Acetone was added to the samples (1:1 v/v). The samples were sonicated for 45 min. The clear portion was decanted into another beaker. The extraction procedure was repeated by adding 20 mL of the mixture of DCM and acetone and then allowed to

undergo ultrasonication. The extract was combined and later cleaned up [13,14]. For the clean-up experiment, an SPE column was packed with cotton wool containing anhydrous sodium sulphate and silica gel. Method TG51991 was carefully followed. Briefly, the packed SPE cartridge was conditioned with 3 mL hexane. The sample extract was applied to the cartridge and eluted with 5 mL hexane into an evaporator glass tube. The solvent was evaporated to near dryness at 40 °C, using a

blow-down apparatus under gentle stream of nitrogen. Sample was reconstituted in 180 μ L of cyclohexane with 20 μ L of injection standard [15].

GC/MS operating condition: An Agilent Technologies GC-MS comprises of 7890A gas chromatography and MS 5975C mass spectrometer detector was used in this study. The instrument comprises a column thickness of 0.25 μ m, internal diameter of 0.32 mm and Helium as carrier gas at the rate of 1mL/min. The column temperature was initially held at 70 °C for 3mins, increased to 160 °C at a rate of 20 °C/min, then to 210 °C at a rate of 3 °C/min and to a final temperature of 310°C at a rate of 5 °C/min and held for 10mins and transfer line of 320 °C. The mass spectrometer (MS) condition was electron impact positive ion mode. The PAHs identification was based on retention time [13,14].

Analytical Quality Controls

To assure recovery efficiency, triplicate analyses were conducted on PAH standard reference materials provided by national institute of standards and technology (NIST). Recovery studies showed that the efficiency is greater than 90%, with a coefficient of variation (CV) of 6–10 % for all 16 PAHs. The quality assurance was also monitored by analyzing blanks sample spiked with known quantity of each PAHs standard. The values of PAHs in the blanks samples were below the detection of the instrument (0.01 μ g/mL).

Results and Discussion

PAHs Concentrations in Samples:

Figs. 2 is a typical chromatograms obtained in this study. Tables 3-5 showed the result of PAHs concentration in fish samples. The result of Diagnostic ratio is presented (Table 6)

PAHs in smoked fish: From Table 3, the two member rings PAHs detected were Naphthalene, Naphthalene, 2 methyl, 000090-12-0 Naphthalene, Biphenylene, and Acenaphthene with 0.0029 mg/kg, 0.0057 mg/kg, 0.0063 mg/kg, 0.00124 mg/kg, and 0.0222 mg/kg respectively. The elevation of Biphenylene could be as a result of incomplete combustion of materials used during smoking process [16]. Three-member ring PAHs compound detected have the following concentration; 0.0018 mg/kg and 0.0026 mg/kg for fluorine and Phenanthrene respectively. The four member rings estimated have the following concentration; 0.0021 mg/kg, 0.0021 mg/kg and 0.0015 mg/kg for fluoranthene, pyrene and Tryphenylene respectively. The five member rings (Benz (a)anthracene, Benzo(b) Fluoranthene, and Benzo(a)pyrene) has concentrations of 0.0012 mg/kg, 0.0018 mg/kg and 0.0025 mg/kg respectively while the six member rings including Indeno(1,2,3-cd) Pyrene, Dibenz(a,h) anthracene and Benzo(ghi) perylene were available at 0.0051 mg/kg, 0.0076 mg/kg, and 0.0127 mg/kg concentrations. The total concentration of PAHs components and their respective percentage abundance were recorded according to their member rings; 2 rings (0.0385 mg/kg and 9.673 %), 3 rings (0.9044 mg/kg and 9.673 %), 4 rings (0.0057 mg/kg and 7.199 %), 5 ring (0.0054 mg/kg and 6.889 %) and 6 rings (0.050 mg/kg and 32.07 %) respectively. The level of PAHs in smoked fish sample depends on the heat source, temperature, flame intensity, flame combustion. [17].

Figure 2: Chromatogram of PAHs in Smoked Fish sample

Table 3: PAH Concentrations (mg/kg) in Smoked Fish Sample

S/N	PAH	Abbrev.	Conc. mg/kg	in	Abundance %	rings	Σ_{16} PAH in mg/kg / (%)
Two Member Ring PAHs							
1	Naphthalene	NAP	0.0029		3.674	2	
2	Naphthalene, 2 methyl-	2MNPH	0.0057		7.214	2	
3	000090-12-0 Naphthalene	12NAP	0.0063		7.964	2	
4	Biphenylene	BP	0.0012		1.566	2	
5	Acenaphthene	ACE	0.0221		27.945	2	0.0383(9.673)
Three Member Ring PAHs							
6	Fluorene	FLR	0.0018		2.234	3	
7	Phenanthrene	PHE	0.0026		3.250	3	0.9044 (3.250)
Four Member Ring PAHs							
8	Fluorathene	FLT	0.0021		2.689	4	
9	Pyrene	PYR	0.0021		2.603	4	
10	Triphenylene	TRP	0.0015		1.907	4	0.0057(7.199)
Five Member Ring PAHs							
11	Benz (a)anthracene	BaA	0.0012		1.495	5	
12	Benzo(b)Fluoranthene	B(b)FA	0.0018		2.208	5	
13	Benzo(a)pyrene	B(a)P	0.0025		3.186	5	0.0054(6.889)
Six Member Ring PAHs							
14	Indeno(1,2,3-cd)Pyrene	IP	0.0051		6.399	6	
15	Dibenz(a,h) anthracene	D(a,h)A	0.0076		9.630	6	
16	Bezo(ghi) perylene	B(ghi)P	0.0127		16.041	6	0.0509(32.07)
tPAHs			0.0793				0.0793(59.081)

Table 4: PAH Concentrations (mg/kg) in Sundried Fish Sample

S/N	PAH	Abbre.	Conc.(mg/kg)	Abundan ce %	Number of rings	Σ ₁₆ PAH; /(%)	mg/kg
Two Member Ring PAHs							
1	Naphthalene	NAP	0.0022	4.296	2	0.0041(8.225)	
2	Naphthalene,2 methyl-	2MNAP	-	-	2		
3	000090-12-0 Napthalene	12NAP	-	-	2		
4	Biphenylene	BP	-	-	2		
5	Acenaphthene	Ace	0.001964	3.929	2		
Three Member Ring PAHs							
6	Fluorene	FLR	0.0018	3.663	3	0.0041 (8.247)	
7	Phenanthrene	Phen	0.0023	4.584	3		
Four Member Ring PAHs							
8	Fluorathene	FLT	0.0020	4.087	4	0.0045(8.957)	
9	Pyrene	Pyr	0.0013	2.523	4		
10	Triphenylene	Trp	0.0012	2.347	4		
Five Member Ring PAHs							
11	Benzo (a)anthracene	B(a)A	0.0015	3.073	5	0.0051 (10.247)	
12	Benzo(b)Fluoranthene	B(a)F	0.0021	4.153	5		
13	Benzo(a)pyrene	B(a)P	0.0015	3.021	5		
Six Member Ring PAHs							
14	Indeno(1,2,3- cd)Pyrene	IP	0.0075	14.951	6	0.0322 (64.324) 0.05(100)	
15	Dibenz(a,h) anthracene	DB(ah)A	0.0191	38.191	6		
16	Bezo(ghi) perylene	B(ghi)P	0.0056	11.182	6		
tPAHs			0.0500				

PAHs in sundried fish: The results in Table 4 showed that the two-member ring detected were Naphthalene (0.0022 mg/kg) and Acenaphthene (0.0019 mg/kg) with the percentage abundance of 8.225 %. The total concentration (0.0041 mg/kg) for the two member rings are within the permissible limit (0.002 mg/kg and 0.005 mg/kg respectively). The presence of naphthalene in sundried fish could be due to the route of exposure such as air. These confirmed that lower molecular weights PAHs are air borne and certain PAHs are present in both particulate and gaseous phase depending on their volatility. The three member rings detected are fluorene (0.0018 mg/kg) and Phenanthrene (0.0023 mg/kg) with a total concentration of 0.00412 mg/kg and percentage abundance of 8.24 %. The lower

molecular weight PAHs compounds presence are naphthalene, acenaphthalene, fluorine and phenanthrene and this could be as a result of lower or moderate temperature involved during drying. The four member rings detected are Fluoranthene (0.0020 mg/kg), Pyrene (0.0013 mg/kg) and Triphenylene (0.0012 mg/kg). The total concentration of four member rings and the percentage abundance were 0.0048 mg/kg and 8.957 %. All the five member rings were detected with the concentration of 0.0015 mg/kg, 0.0021 mg/kg and 0.0015 mg/kg for Benz(a)anthracene, Benzo(b)fluoranthene and Benzo(a)pyrene respectively. The five membered rings have a total concentration of 0.0051 mg/kg and 10.247 % (percentage abundance). The results obtained showed that the six-member ring

were detected with the concentration of 0.0075 mg/kg, 0.0191 mg/kg, and 0.0056 mg/kg for Benzo (1,2,3), pyrene and Benzo (ghi) perphrene respectively. Total PAHs components detected were 0.0321 mg/kg for concentration and percentage abundance of 64.324 %. Among the six member rings, the Diazo(a) anthracene (0.2 mg/kg) has the highest PAHs component compared to that of smoke dried samples (0.008 mg/kg). The total concentration of PAHs components in sundried fish sample (0.0499 mg/kg) were higher than the permissible limit in fish as recommend by EU (0.002 mg/kg). These result agrees with similar report [18]. This is an indication that high concentration of PAHs component could be due the level of organic matter present in the environment.

PAHs compounds in fried fish: Table 5, showed the PAHs concentrations in fried fish samples. The study showed that only Biphenylene (0.0021 mg/kg) and Acenaphthalene (0.0015 mg/kg) were detected in two members aromatic PAHs rings. They are within the acceptable limit as recommended by WHO and EU (0.002 mg/kg and 0.005 mg/kg). The total concentration and percentage abundance for the two member rings are 0.0036 mg/kg and 8.382 %. Phenanthrene was the only three member rings detected with the concentration of 0.0012 mg/kg with percentage abundance of 2.80463%. The lower concentration of phenanthrene observed in oil fried fish sample could be due to diffusion of phenanthrene from fish fraction which could reduce the level in fish during frying [11]. The four member rings and five member rings were not detected. The six member rings. PAHs concentration detected were Indeno (1,2,3) pyrene (0.0058 mg/kg) Dibenz (a,h)anthracene (0.0274 mg/kg) and Benzo(ghi) perylene (0.0050 mg/kg) respectively. The result showed that Dibenz(a,h)anthracene are higher than the permissible limit (0.002 mg/kg and 0.005 mg/kg). The total concentration and percentage abundance of six-member ring was recorded to be 0.03822 mg/kg and 88.814 %. In this study, $\sum_{16}$ PAHs compounds in fried fish sample was recorded 0.0430 mg/kg.

PAHs sources based on diagnostic ratio

The PAHs formation can either be natural or anthropogenic [19]. Pyrogenic sources are those including combustion of hydrocarbons or indeed any organic materials (engines exhaust, fire, and aluminium smelting resulting in atmospheric deposition and waste water effluents) while Petro-genic sources originated from oil, petroleum, related activities as well as natural sources. Pyrolytic mixture of PAHs enters the aquatic environments through the deposition of atmospheric emission directly into the water or soil followed by soil erosion. PAHs are found in the ambient air in gas-phase and as sorbet to aerosols [20,21]. The PAHs components from two to three member rings and some alkyl substitution points at petrogenic contaminants whereas four to six rings PAHs are more toxic and thermodynamically stable than those from petrogenic source. They were identified with pyrogenic origin. The molecular weight ratios are mostly used to differentiate PAHs from Pyrogenic and petrogenic origins [21]. The result obtained in this study showed the ratio of $\sum \text{LPAH}/\text{HPAH}$ in sundried, smoked fish and fried fish sample are less than 1, which indicated that the source or origin is pyrogenic. This showed that the source of most these fish samples could be derived from the incomplete combustion of organic matter and fossil fuels from wood and fuel respectively. The ratio of $(\text{IPy}/\text{IPy}+\text{Bgghi}) > 0.5$ gives a pyrogenic origin in all the processed samples, but the reverse is the case in smoke fish sample with $\text{IPY}/\text{IPY}+\text{Bgghi} < 0.5$ which indicated petrogenic origin. BaA (BaA +Try) ratio > 0.350 indicated pyrogenic while < 0.2 is attributed to petrogenic source. The study therefore revealed that most of the sample's source was pyrogenic which could be as a result of antropogenic activities and organic waste present in the environment of origin. Statistical test of significance ($p < 0.05$) indicated that the differences in PAH levels between the processed fish samples were not significant. With exception of the six member rings, PAH levels investigated in this study are within threshold limits suggested by existing legislations.

Table 5: PAH Concentrations (mg/kg) in Fried Fish Sample

S/N	PAH	Abbrev.	Conc. (mg/kg)	Abundance %	No of rings	Σ_{16} PAH; mg/kg (%)
Two Member Ring PAHs						
1	Naphthalene	NAP	-	-	2	0.0036(8.382)
2	Naphthalene, 2 methyl	2MNPH	-	-	2	
3	000090-12-0 Napthalene	12WAP	-	-	2	
4	Biphenylene	BP	0.0015	3.474	2	
5	Acenaphthene	Ace	0.0021	4.908	2	
Three Member Ring PAHs						
6	Fluorene	FLR	-	-	3	0.0012 (2.805)
7	Phenanthrene	PHE	0.0012	2.805	3	
Four Member Ring PAHs						
8	Fluorathene	Flt	-	-	4	
9	Pyrene	Pyr	-	-	4	
	Triphenylene	Trp	-	-	4	
Five Member Ring PAHs						
10	Benz (α)anthracene	B(a)A	-	-	5	
12	Benzo(β)Fluoranthene	B(a)F	-	-	5	
13	Benzo(a)pyrene	B(a)P	-	-	5	
Six Member Ring PAHs						
14	Indeno(1,2,3-cd)Pyrene	IP	0.0058	13.505	6	0.0382(88.814)
15	Dibenz(a,h) anthracene	DB(ab)A	0.0274	63.665	6	
16	Benzo(ghi) perylene	B(ghi)P	0.0050	11.644	6	
tPAHs			0.04304			
						0.0430(100.00)

Table 6: Source of PAHs based on Characteristic Diagnostic Ratios

PAHs Ratio	value	Origin (source)	SM	FD	SD
PHen/ANth	<10	Pyrogenic	NA	NA	NA
PHen/ANth	>10	pyrogenic	NA	NA	NA
Pyr<Flth	-	Pyrogenic	Pyrogen	pyrogenic	pyrogenic
FLth>PYr	-	Pyrogenic	Pyrogenic	pyrogenic	pyrogenic
PYR/FLth	>1	Petrogenic	Pyrogenic	pyrogenic	pyrogenic
IPy/(IPy +Bghi)	>0.5	pyrogenic	petrogenic	pyrogenic	pyrogenic
IPy/(IPy +Bghi)	<0.2	Petrogenic	Pyrogenic	pyrogenic	pyrogenic

ΣLPAH/ ΣHPAH < 1 Pyrogenic Pyrogenic pyrogenic pyrogenic

NA-Not Applicable.

Conclusion

PAHs levels in fish samples, processed using different methods were investigated. Results from this studies revealed that the high molecular weight PAHs, majorly the six membered rings were present than the lower member ring PAHs. Generally, PAHs in fish samples underscores the method of preservation since the differences in the PAH values reported were non-significant. Overall, estimated PAHs levels in the fishes, processed using different methods were within acceptable concentrations prescribed by existing legislation. Exceptions to this generalization were the levels of five member ring (Benzo(a)pyrene) and six member ring (Dibenzo(ah)anthracenes) PAHs, known for high carcinogenicity.

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