Evaluation of Sources of Polycyclic Aromatic Hydrocarbons in Thermally Treated Meat and Fish: A Food Pollutant Study

Gina Ifeyinwa Iwu[1]*, Labunmi Lajide[2], Paschal Chima Madu[1], Ibrahim Attai Isah[3]

[1]Department of Chemistry, Nasarawa State University, Keffi, Nigeria

[2]Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria

[3]Pesticide Residues Laboratory, NAFDAC Central Laboratory Complex, Oshodi, Lagos State, Nigeria

***Corresponding Author**

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ABSTRACT

This study assessed the sources of PAH in cooked meat and fish. Gas Chromatography-Mass Spectrometry (GC-MS) performed the sample analysis. The total PAH concentrations (mg/kg) ranged from 0.911 to 2.763. Levels of low-molecular-weight (LMW) PAHs varied from 0.353 mg/kg to 1.786 mg/kg, while high-molecular-weight (HMW) PAHs ranged from 0.516 to 0.977 mg/kg. The study detected 2 to 6-ring PAHs, the 3-ring PAHs were the most prominent. PAHs diagnostic ratios, their plots and principal component analysis (PCA) were used to assess the origin of PAHs in the samples. Values of diagnostic ratios, such as Phenanthrene/Anthracene (1.84–3.58), Anthracene/(Anthracene + Phenanthrene) (0.21–0.35), Fluoranthene/Pyrene (0.00-1.79), Fluoranthene /(Fluoranthene + Pyrene) (0.00-0.64), Indeno[123-cd-]pyrene/(Indeno[123-cd-]pyrene + Benzo[ghi]perylene) (0.00–43), LMW-PAH/HMW-PAH (0.59–1.83) and Benzo[a]pyrene/(Benzo[a]pyrene + Chrysene) (0.00–0.50) indicated that the PAHs in the samples were from mixed sources, with greater contributions from pyrogenic sources. They show that the barbecued samples were contaminated primarily by pyrogenic PAHs (biomass (wood/charcoal) burning, liquid fuel combustion, or automobile exhaust emissions). The grilled and smoked samples were mostly contaminated from different combustion sources (traffic and non-traffic, biomass burning, automobile exhaust and liquid fuel), with little input from petrogenic sources. The combination plots of the ratios also show that PAH was from mixed sources (petrogenic and pyrogenic), but mostly from a pyrogenic source (wood/charcoal burning, liquid fuel combustion, and automobile exhaust emissions) with minor inputs from petrogenic origin, as shown by the value of LMW-PAH/HMW-PAH ratios > 1 in a few samples. PCA equally showed that four factors accounted for 82.47% of the total variability and separated the PAHs into identifiable source groups, comprising mixed sources, combustion of liquid fossil fuel, automobile/diesel engine exhaust, gasoline and oil combustion and biomass burning. The study showed that contamination from the natural habitats of the animals contributed to the PAHs in them in addition to the cooking process. This therefore underscored the impacts of anthropogenic pyrogenic activities on PAHs formation and levels in meat and fish, hence adequate control/reduction of these activities will promote a healthier and safer food environment for all.

Keywords: polycyclic aromatic hydrocarbon, pyrogenic, petrogenic, diagnostic ratios, sources, meat, fish

INTRODUCTION

In recent years, concerns over the presence of polycyclic aromatic hydrocarbons (PAHs) in food items have intensified, given their potential adverse effects on human health (Mekuleyi *et al.*, 2018). Nigeria is no exception to this global challenge. With a burgeoning population and increasing urbanization, the demand for meat and fish has risen substantially, raising questions about the origin and composition of PAHs within these staple food sources.

PAHs are long-range transport organic pollutants comprising two or more aromatic rings (Rengarajan *et al.*, 2015). They are regarded as persistent organic pollutants (POPs) because of their resistant to degradation (Kronenberg *et al.*, 2017), and can bio accumulate in animal and human tissues (Singh & Agarwal, 2018). "Low-molecular-weight'' (LMW)-PAHs have two or three fused benzene rings, while "high-molecular-weight'' (HMW)-PAHs have four or more fused benzene rings (Gupte *et al.,* 2016). HMW-PAHs are carcinogens because they are more stable, more persistent, and more toxic than LMW-PAHs (Adeniji *et al.*, 2017; IARC 2015).

PAHs found in the environment are classified based on their origin (formation process) or on their emission sources (i.e., their sources into the environment). Their formation process is mainly pyrogenic or petrogenic (Hąc-Wydro *et al.,* 2019; Mojiri *et al.,* 2019), whereas their emissions can come from natural or anthropogenic (human activities) sources. Pyrogenic PAHs are formed through rapid, high-temperature (ranging from 350°C to 1200°C) pyrolysis or incomplete combustion of organic matter such as fossil fuels (coal, petroleum, wood) and biomass (forest, grassland, or agricultural) (Balmer *et al.,* 2019; Mauro & Roush 2008; Patel *et al.,* 2020). They occur in high concentrations in urban areas, from where they spread into the atmosphere (Lima *et al.*, 2005 as cited in Balmer *et al.*, 2019; Yang *et al.*, 2021). Petrogenic PAHs come from petroleum-related activities via biogenic processes that are slow but have long-term moderate to low temperatures (100°C to 150°C) such as in the formation of fossil fuels and petroleum products (Lawal & Fantke, 2017). Their distribution in the environment is mainly anthropogenic such as the outcome of extensive transportation, storage, and use of crude oil and its products, petroleum products (Crawford & Quinn, 2017), oceanic and freshwater oil spills (Sen & Field, 2013), storage tank leaks, seepage of petroleum products, and erosion of sedimentary rocks containing petroleum products and related substances (Abdel-Shafy & Monsour, 2016), leakages and spillages during exploration, production (Gupte *et al.,* 2016).

PAHs can pollute the environment (air, water, and biota) and subsequently find their way into the food chain. Their level in food is therefore a pointer to the degree of anthropogenic activities in the environment. Understanding the specific sources of PAHs in food is crucial for implementing effective mitigation strategies and ensuring the safety of the food supply. This is achieved using diagnostic ratios and other methods (Evans *et al.*, 2016; Marques dos Santos *et al., 2017). Despite their flaws, diagnostic ratios help to identify th*e source of PAHs in the environment. PAH isomers from the same source/origin have identical physical and chemical properties and thus transform and degrade at the same rate; this helps to generate diagnostic ratios (Clement *et al.*, 2015). HMW PAHs make up most pyrogenic sources and are associated with low thermodynamic stability, while LMW PAHs make up most petrogenic sources and have high thermodynamic stability (Marris *et al.*, 2020). Many studies have reported using PAH diagnostic ratios for source apportionment purposes, either alone (Yunker *et al.*, 2002; Yusuf, 2015; Agbozu *et al.*, 2020) or with other source apportionment strategies (Tay *et al.*, 2022; Dosunmu *et al.*, 2012; Al-Nasir *et al.*, 2022). PAHs of molecular masses 178, 202, 228, and 276 are frequently used to distinguish between combustion and petroleum sources (Agbozu *et al.*, 2017, 2020). Each PAH ratio has associated threshold values, used in differentiating between source types (Table 2) (Yunker *et al.*, 2002). Applying diagnostic ratios to discern the sources of PAHs in meat and fish is crucial, given the implications of environmental exposure and food safety.

The aim of this paper, therefore, was to investigate and identify the potential sources of PAH in the cooked meat and fish, and to shed light on the potential impact of environmental and cooking practices on the levels of PAHs in these staple foods. A part of the data set was used to evaluate the health risks because of regular consumption of thermally treated meat/fish (Iwu *et al.*, 2023 & 2024). The outcomes of this study will hence contribute to the scientific understanding of PAHs in food, provide actionable insights for policymakers, food safety regulators, risk assessors, and the public to design targeted interventions for reducing PAH exposure in food, as well as promote a safer and healthier food environment for all.

MATERIALS AND METHODS

A. Chemicals and Standards

A United States Environmental Protection Agency (US EPA) priority mix PAHs standard solution comprising of Naphthalene (Naph), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phen), Fluoranthene (Flt), Anthracene (Anth), Pyrene (Pyr), Chrysene (Chr), Benzo[a]anthracene (B[a]A), Benzo[b]fluoranthene (B[b]F), Benzo[K]fluoranthene (B[k]F), Benzo[a]pyrene (B[a]P), Benzo[ghi]perylene (B[ghi]P), Indeno[123-cd]pyrene (I[cd]P), Dibenzo[ah]anthracene (Db[ah]A) in acetonitrile was procured from Sigma-Aldrich Bellefonte, PA, United States of America (USA).

Acetone (HPLC grade) (99.99 %) purity was acquired from Fischer Scientific, United Kingdom. Acetonitrile (HPLC grade) (purity > 95%) was from Merck, Japan. n-hexane (98.0 %), magnesium sulfate (MgSO4) (fine) (>98%) for clean-up, and sodium chloride (NaCl) (99.5%) were obtained from Merck, Germany. MgSO4 anhydrous grit (coarse) was obtained from Sigma-Aldrich, USA. While disodium hydrogen citrate sesquihydrate (C6H6Na2O7.1.5H2O) was purchased from Sigma-Aldrich, Germany. Trisodium citrate dehydrates (Na3C6H5O7.2H2O) were from Sigma-Aldrich, Japan. Bondesil Primary Secondary Amine (PSA) sorbents were purchased from Agilent Technologies, USA, while C-18 sorbents were acquired from Supelco, USA. Others are distilled water (HPLC grade), and 5 % Formic acid solution in acetonitrile.

B. Sample Collection and Preparation

Commercially cooked meat and fish samples including beef barbecued with firewood (BBF), beef barbecued with charcoal (BBC), lean chicken barbecued with firewood (LCBF), lean chicken barbecued with charcoal (LCBC), fattened chicken barbecued with charcoal (FCBC), fattened chicken barbecued with firewood (FCBF), fattened chicken barbecued with charcoal without basting (FCBCN), fattened chicken grilled with electric oven (FCGE), fattened chicken grilled with charcoal (FCGC), lean fish grilled with charcoal (LFGC), catfish grilled with charcoal (CFGC), smoked bush meat (SBM), smoked mackerel fish (SMF), smoked catfish (SCF) were randomly purchased from different outlets and vendors in different parts of the Federal Capital Territory, Abuja. Fifty-eight meat and fish samples were purchased from the vendors and supermarkets selling these products.

The samples were pooled together to get representative samples for each type. They were shredded and further blended using a Waring 800 EG food blender to obtain a homogenized sample, packed in bottles, and frozen at 4 °C (Duedahl-Olesen *et al.,* 2015).

C. Extraction and Clean-up

Determination of PAH was according to the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method (EN, 2008); which is based on the extraction of analyte using acetonitrile, subsequent clean-up, and then concentration of analyte (Iwu *et al.,* 2023).

D. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of PAHs

Identification and quantification of PAHs was performed with Shimadzu Gas Chromatography GC-2010

Plus coupled to Mass Spectrometry (MS) QP2010 Ultra (Shimadzu) with a VF5 MS cross-linked capillary column of 30 m × 0.25 µm x 0.25 mm ID. Compounds were identified by comparing the retention times of the PAHs in the calibration standards with those obtained from the sample extracts.

While quantitation was based on external standard calibration involving a comparison of the instrument (peak) responses from the sample to the target compound responses in the calibration standard using the six-point calibration curve.

E. Quality Control

Reagent blanks were analyzed with each batch of samples for quality assurance and quality control to show the lack of carryover from run to run. Method blanks and fortified method blanks (prepared with 5 mL of distilled water in place of the sample composite and extracted using similar procedures as in the sample) were also analyzed with each batch of samples to monitor for contamination from laboratory sources. Six calibration concentrations (0.05 to 2.0 µg/mL) were used.

Chromatograms of PAH analytes detected in some of the samples are shown in supplementary figures S2, S3, S, S5 respectively. The detection limit ranged from 0.024 to 0.060 mg/kg, the R2 values exceeded 0.995 for all analytes, recoveries ranged from 83 to 100% for all analytes (except I[cd]P), and percent relative standard deviations (%RSDs) were within 5.67.1%. The recovery values were within the recommended range of 70–130% (US EPA, 2018). Al-Thaiban *et al.* (2018) reported similar values (74–117%).

F. Data Analysis

The computer-based GC software provided by Shimadzu Corporation processed the data, while MS Excel version 15.0 and SPSS version 25.0 did statistical analysis, and presented the results as total PAH, total LMW-PAH, total HMW-PAH, sum of (2-ring-PAH, 3-ring-PAH, 4-ring-PAH, 5-ring-PAH, and 6-ring-PAH), and PAH ratios including Phen/Anth, Anth/(Anth + Phen), ΣLMW-PAH/ΣHMW-PAH, B[a]P/(B[a]P + Chr), Chr/Baa, Baa/(Chr + Baa), Flt/Pyr, Flt/(Flt + Pyr), I[cd]P/(I[cd]P + B[ghi]P) and Bap/B[ghi]P, ΣCombPAH/ΣPAH16. ΣCombPAH is the sum of the HMWPAHs (Flt, Pyr, B[a]A, Chr, B[K]F, B[b]F, B[a]P, I[cd]P, D[ahA] and B[ghi]P).

RESULTS AND DISCUSSION

Figure 1 shows the concentrations (mg/kg) of total PAH16, total LMW-PAHs, and total HMW-PAHs. While Figure 2 depicts the sum of levels of Σ2-ring PAHs, Σ3-ring PAHs, etc., in the cooked sample. Table 2 shows the correlation coefficients between various PAH levels in the cooked samples. Tables 3 and 4 show the evaluated PAH diagnostic ratios, while Figures 3 to 6 showed the plots of diagnostic ratios. Table 5 displayed the Rotated Component Matrix of Principal Component Analysis.

A. Concentrations of PAHs in the Cooked Samples

The levels (mg/kg) of PAH vary across different meats and fish, and this is because of different factors such as cooking methods (grilling or smoking), type of meat/fish, fuel type, origin of PAHs, as well as environmental factors. For instance, the firewood-smoked bush meat (SBM) has higher concentrations of PAHs compared to other samples. Both the HMW PAHs and LMW PAHs showed prominent presence, indicating that the PAHs detected might have petrogenic and pyrogenic origins (Zelinkova & Wenzel, 2015). The data also show that the concentrations differ based on ring structures. The 3-ring-PAHs have the highest total concentrations in every sample compared to the other ring structures.

The levels (mg/kg) of total PAH16 ranged from 0.757 in electric-grilled chicken (FCGE) to 2.765 in firewood-smoked game meat (SBM). High total PAHs discovered in the smoked samples is likely because of smoke deposits coming from fuel (wood, grass, or charcoal) combustion and equally from the transformation of meat's fatty acids during smoking (Aydin & Şahan, 2018) as well as the technique of the smoking process (Sahin *et al.,* 2020). The ΣLMW-PAH levels (mg/kg) varied between 0.298 in FCGE and 1.786 in SBM, while the ΣHMW-PAH concentrations (mg/kg) varied from 0.393 in FCBCN to 0.979 in SBM. The pattern of occurrence of ΣLMW-PAH and ΣHMW-PAH in SBM is suggesting that PAH in it may be from mixed source.

TABLE 1 SOME PAH DIAGNOSTIC RATIOS AND THE THRESHOLD VALUES.

PAH Ratios	Value	Source
Phen/Anth	< 15	Pyrogenic
	> 15	Petrogenic
Chr/B[a]A	< 0.4	Petrogenic
	> 0.9	Pyrogenic
Anth/(Anth + Phen)	< 0.1	Petrogenic
	> 0.1	Pyrogenic
Flt/Pyr	< 1	Petrogenic
	> 1	Pyrogenic
Flt/(Flt + Pyr)	< 0.4	Petrogenic
	0.4 – 0.5	Liquid fossil fuel combustion
	> 0.5	Wood, grass, or coal combustion
B[a]A/B[a]A + Chr	< 0.2	Petrogenic
	0.2 – 0.35	Pyrogenic (diesel exhaust emissions)
	> 0.35	Wood, grass, or coal burning
I[cd]P/(I[cd]P + B[ghi]P	< 0.2	Petrogenic
	0.2 – 0.5	Vehicle and crude oil combustion
	> 0.5	Wood, grass, or coal combustion
ΣLMW-PAH/ΣHMW-PAH	< 1	Pyrogenic
	> 1	Petrogenic
	< 0.5	combustion Liquid fossil fuel, automobile exhaust emission etc.
	> 0.5	Combustion of solid fuels like wood
B[a]P/B[ghi]P	< 0.6	Non-traffic sources
	> 0.6	Traffic source
B[a]P/(B[a]P + Chr)	< 0.2	Petrogenic
	0.2 – 0.35	Pyrogenic (diesel exhaust emissions)
	> 0.35	Wood, grass, or coal combustion
ΣComboPAH/ΣPAH16	approx. 1	Combustion

^{a} (Source: Yunker *et al.*, 2002; Adeniji *et al.*, 2019; Onojake *et al.*, 2016, 2021; Al-Nasir *et al.*, 2022, Evans *et al.*, 2016, Jiao *et al.*, 2017; Tsymbalyuk *et al.*, 2011; Tobiszewski & Namiesnik, 2012).

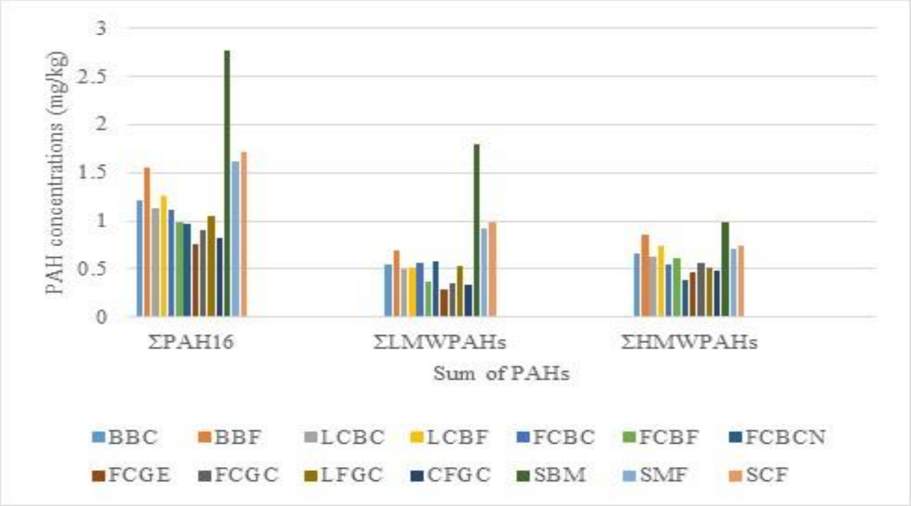

Fig. 1 Levels (mg/kg) of ΣPAH16, ΣLMWPAHs, and ΣHMWPAHs in cooked Samples.

[b] Data are given only for the purpose of information in SBM. Further research is required.

B. Concentrations of Indicators of PAH Occurrence and Toxicity (B[a]P and PAH4) in samples

The Panel on Contaminants in the Food Chain (the CONTAM panel) adopted PAH4 and PAH8 as better indicators of PAH occurrence and toxicity than only B[a]P (EFSA, 2008). Hence, maximum permitted levels of 0.002 and 0.012 mg/kg were respectively set for B[a]P and the sum of PAH4 [Σ (B[a]A, Chr, B[a]P, and B[b]F)] in traditionally smoked meat and meat products, as well as traditionally smoked fish and fishery products. While levels of 0.005 and 0.030 mg/kg were in turn given for B[a]P and ΣPAH4 in heat-treated meat or fish and their products (including barbecued and grilled meat or fish) (European Union, 2014).The mean B[a]P and the PAH4 concentrations in the tested samples were above the EU limits, except in LCBC, FCBCN and FCGE, This implies that chronic oral exposure to high levels of these PAHs in those samples may increase the risk of developing certain types of cancer and other health issues (Zheng *et al.*, 2018).

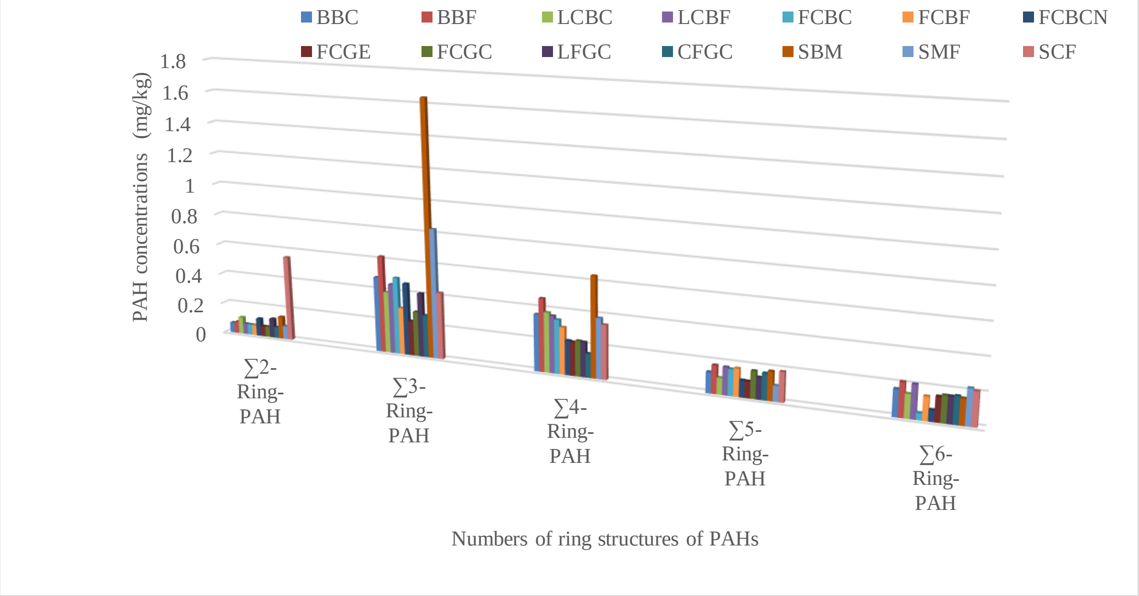

Fig. 2 Levels (mg/kg) of ΣPAHs based on Numbers of ring

[c]Data are given only for the purpose of information in SBM. Further research is required

TABLE 2 PEARSON CORRELATION COEFFICIENTS BETWEEN VARIOUS PAH LEVELS IN THE COOKED SAMPLES

Variable	BBF	BBC	LCBF	LCBC	FCBF	FCBC	FCBCN	LFGC	CFGC	SBM	SMF	SCF	FCGC	FCGE
BBF	1													
BBC	0.8	1												
LCBF	0.93	0.69	1											
LCBC	0.82	0.72	0.83	1										
FCBF	0.82	0.64	0.91	0.8	1									
FCBC	0.8	0.81	0.68	0.62	0.61	1								
FCBCN	0.63	0.61	0.51	0.58	0.37	0.78	1							
LFGC	0.68	0.46	0.8	0.52	0.78	0.58	0.46	1						
CFGC	0.73	0.48	0.79	0.6	0.75	0.56	0.67	0.75	1					
SBM	0.51	0.44	0.57	0.45	0.43	0.56	0.55	0.64	0.47	1				
SMF	0.56	0.43	0.69	0.49	0.56	0.43	0.36	0.74	0.51	0.92	1			
SCF	0.24	0.28	0.3	0.49	0.35	0.26	0.47	0.48	0.41	0.18	0.21	1		
FCGC	0.83	0.61	0.9	0.67	0.89	0.67	0.55	0.86	0.91	0.43	0.53	0.37	1	
FCGE	0.74	0.53	0.85	0.81	0.92	0.45	0.38	0.72	0.75	0.38	0.48	0.4	0.82	1

[d] Data are given only for the purpose of information in SBM. Further research is required.

C. Pearson Correlation

The correlation analysis in the cooked samples show a strong positive correlation ($r \geq 0.70$) between the regular barbecued beef samples (BBF and BBC), the regular barbecued chicken samples (LCBC, LCBF, FCBC, and FCBF) as well as grilled samples FCGE and CFGC (Table 2). All the regular barbecued chicken samples except FCBC, also had a similar relationship, as well as between LCBC and FCBF, LCBC and FCGC. BBC only correlated with BBF, LCBC and FCBC suggesting a similar source of PAH. However, between the non-basted barbecued chicken (FCBCN) and other cooked samples excluding FCBC, there was a moderate or weak positive correlation. FCBC and FCBCN had a strong positive correlation, showing a related source of PAH in them.

SBM and SMF showed strong positive correlation, whereas a weak positive relationship existed between them and SCF, a sign that a separate factor may have contributed to PAHs found in SCF. The smoked samples did not correlate strongly with other samples, a pointer that the PAH in them is from the smoking process.

There was also a strong positive correlation between PAH occurrence in LFGC and CFGC, SMF, FCGC, and FCGE respectively. FCBF equally correlated with the grilled samples (LFGC, CFGC, FCGC and FCGE) respectively. Furthermore, CFGC correlated with FCGC and FCGE respectively. While FCGC related strongly with FCGE. These findings show the existence of common factors influencing the co-occurrence of PAHs in samples that showed strong or moderate positive correlation. All other relationships are moderate or weak. They also show that similar sources, cooking processes or environmental factors may have led to the concurrent formation of those PAHs in these samples. Further investigation was done with the use of other model to explore these factors.

TABLE 3 PAH DIAGNOSTIC RATIOS FOR BARBECUED MEAT AND FISH SAMPLES

PAH Diagnostic Ratios	BBC	BBF	LCBC	LCBF	FCBC	FCBF	FCBCN
Phen/Anth	1.84	2.87	2.42	2.64	2.45	2.31	2.45
Chr/B[a]A	0	3.27	0	4.36	0	0	0
Anth/(Anth+ Phen)	0.35	0.26	0.29	0.27	0.29	0.3	0.29
Flt/Pyr	1.54	0.94	1.79	0.83	1.18	0.77	1.12
Flt/(Flt + Pyr)	0.61	0.49	0.64	0.45	0.54	0.44	0.53
B[a]A/(B[a]A + Chr)	0	0.23	0	0.19	0	0	0
Ind[cd]P/(I[cd]P + B[ghi]P)	0.43	0.32	0	0.31	0	0	0
∑LMW-PAH/∑HMW-PAH	0.84	0.82	0.8	0.7	1.02	0.59	1.47
B[a]P/(B[a]P + Chr)	0.34	0.32	0	0.35	0.34	0.37	0
B[a]P/(B[ghi]P)	0.64	0.46	0	0.47	0	0.33	0
∑CombPAH/∑16PAHs	0.54	0.55	0.56	0.6	0.49	0.63	0.41

TABLE 4 PAH DIAGNOSTIC RATIOS FOR GRILLED AND SMOKED MEAT AND FISH SAMPLES

PAH Diagnostic Ratios	FCGE	FCGC	LFGC	CFGC	SMF	SCF	SBM
Phen/Anth	3	2.33	2.6	3.79	3.02	2.25	3.58
Chr/B[a]A	0	0	0	0	5	0	2.43
Anth/(Anth+ Phen)	0.25	0.3	0.28	0.21	0.25	0.31	0.22
Flt/Pyr	0.53	0.52	0	0.49	0.81	1.58	1.07
Flt/(Flt + Pyr)	0.35	0.34	0	0.33	0.45	0.61	0.52
B[a]A/(B[a]A + Chr)	0	0	0	0	0.17	0	0.29
I[cd]P/(I[cd]P + B[ghi]P)	0	0.42	0.41	0.41	0.3	0.31	0.39
∑LMW-PAH/∑HMW-PAH	0.62	0.63	1.03	0.72	1.3	1.32	1.83
B[a]P/(B[a]P + Chr)	0	0.47	0.36	0	0.45	0.5	0.33
B[a]P/(B[ghi]P)	0	0.7	0.67	0.64	0.63	0.56	0.6
∑CombPAH/∑16PAHs	0.62	0.61	0.49	0.58	0.43	0.43	0.35

[e] Data are given only for the purpose of information in SBM. Further research is required.

D. Source apportionment of PAHs in the samples using diagnostic ratios

The data in Figures 1 and 2, as well as in Table 2, provided little insight into the sources of PAH in the samples. Other approaches were used to examine and gain an understanding of the origin of PAHs in these samples. Determining the origin of PAHs in meat and fish helps mitigate human exposure, and the associated health risks. PAH diagnostic ratios and principal component analysis (Agbozu *et al.,* 2020; Tay et al., 2022) were used to evaluate the origin of PAHs in this study. It is worth mentioning that, in nature, there is rarely a single emission type, and hence, there are cases of co-contamination from more than one source.

In this study, the evaluated PAH molecular ratios comprising Phen/Anth, Anth/(Anth + Phen), ΣLMW-PAH/ΣHMW-PAH, ΣCombPAH/ΣPAH16, B[a]P/(B[a]P + Chr), Chr/Baa, Baa/(Chr + Baa), Flt/Pyr, Flt/(Flt + Pyr), I[cd]P/(I[cd]P + B[ghi]P) and Bap/B[ghi]P assigned PAHs detected in the meat and fish to various sources (Tables 3 and 4). The plots of these ratios also gave more insights into the sources of PAHs in the samples (Figures 3 to 6).

The values of the Phen/Anth ratio were < 15, showing major contributions from combustion/pyrogenic origin. Anth/(Anth + Phen) ratio has values > 0.1 in all the samples, also showing predominantly pyrogenic origin. B[a]P/(B[a]P + Chr) ratios varied from 0.00 to 0.50 in all samples, showing combustion sources (specifically diesel exhaust/automobile emissions, wood, and charcoal).

Chr/B[a]A displayed values ranging from 0.00 to 5.00. In four firewood cooked samples (BBF, LCBF, SBM and SMF), their values show the dominance of combustion source. All samples produced Flt/Pyr ratios ranging from 0.00 to 1.79; six samples (BBC, LCBC, FCBC, FCBCN, SCF and SBM) showed combustion origin; and seven others, including all firewood barbecued samples, three grilled samples and SMF, showed petrogenic source. LFGC had zero.

Similarly, the values of Flt/(Flt + Pyr) ratios ranged from 0.00 to 0.64. All grilled samples showed petrogenic input; BBF, LCBF, FCBF, and SMF showed inputs from liquid fossil fuel combustion; and BBC, LCBC, FCBC, FCBCN, SCF and SBM showed combustion from wood, grass, or charcoal.

BBF, LCBF, SMF and SBM generated B[a]A/(Chr + B[a]A) ratios, varying from 0.00 to 0.29 and their values pointed to mixed sources (petroleum and combustion). LCBF and SMF showed petrogenic source, whereas BBF and SBM showed combustion from diesel exhaust emission.

The values of I[cd]P/(I[cd]P + B[ghi]P) ranged from 0.00 to 0.43. The results showed PAHs to be from vehicle and liquid fuel combustion in most of the samples (Adeniji *et al.*, 2019; Onojake *et al.*, 2016, 2021).

The values of the B[a]P/B[ghi] ratio varied from 0.00 to 0.70 and showed that PAHs were from both traffic sources and non-traffic sources.

The ΣLMW-PAH/ΣHMW-PAH ratios ranged from 0.59 to 1.83, showing pyrogenic inputs for some samples and petrogenic for others. All the smoked samples showed petrogenic inputs, most barbecued and grilled samples showed contributions from solid fuel combustion. The ΣComb/ΣPAH16 varied from 0.35 to 0.63, suggesting mixed source.

From Figure 3, the plot of Flt/Pyr against Phen/Anth ratio showed the values of the firewood-barbecued samples (BBF, FCBF, and LCBF), charcoal-grilled (FCGC, LFGC and CFGC) and electric-grilled FCGE samples and the firewood-smoked SMF lying mainly in the ‘combustion’ region of Phen/Anth and in the petrogenic region of Flt/Pyr. This suggests that the occurrence of these PAHs in them are from mixed sources. Charcoal-barbecued BBC, LCBC, FCBC and FCBCN, as well as firewood-smoked SCF and SBM, are on the combustion regions of the two ratios indicating predominant input from pyrogenic sources.

In Figure 4, the Flt/(Flt + Pyr) versus Anth/(Anth +Phen) plot showed BBF, LCBF, FCBF and SMF to be in the liquid fossil fuel combustion region of Flt/(Flt + Pyr) and also in the combustion region of Anth/(Anth + Phen), signifying greater contributions to be from the combustion source and precisely from liquid fuel combustion. All grilled samples are positioned in petrogenic region of Flt/(Flt + Pyr) and in combustion region of Anth/(Anth + Phen), suggestion that PAH in them are from mixed sources; while BBC, LCBC, FCBC, FCBCN, SCF and SBM are residing in the wood, grass, or charcoal combustion area of Flt/(Flt + Pyr) and in the combustion region of Anth/(Anth +Phen), indicating inputs from wood, grass, or charcoal combustion.

Figure 5 represents the B[a]P/(B[a]P + Chr) versus B[a]P/B[ghi] plot. It shows LCBC, FCBCN and FCGE in the petrogenic region of B[a]P/(B[a]P + Chr) and in the non-traffic combustion region of B[a]P/B[ghi] signifying mixed sources. CFGC is in the petrogenic area of B[a]P/(B[a]P + Chr) and traffic combustion region of B[a]P/B[ghi] also suggesting inputs from mixed sources. While BBF, LCBF, and FCBC are positioned in the diesel exhaust emission region of B[a]P/(B[a]P + Chr) and non-traffic combustion region of B[a]P/B[ghi] also suggesting inputs from mixed sources. BBC and SBM are in the diesel exhaust emission region of B[a]P/(B[a]P + Chr) and traffic combustion region of B[a]P/B[ghi] likewise suggesting inputs from similar but mixed sources. FCBF and SCF are in the region of wood, grass, or charcoal combustion of B[a]P/(B[a]P + Chr) and non-traffic combustion region of B[a]P/B[ghi], while SMF, LFGC and FCGC are positioned in the region of wood, grass, or charcoal combustion of B[a]P/(B[a]P + Chr) and traffic combustion region of B[a]P/B[ghi], indicating mixed sources. This plot shows that B[a]P, Chr and B[ghi] in the samples are from mixed sources.

In Figure 6, the plot of ΣComPAH/Σ16PAHs versus ΣLMW-PAHs/ΣHMW-PAHs showed that FCBC, FCBCN, LFGC, SBM, SMF and SCF are in the petrogenic regions of both indicator ratios, suggesting major inputs from petroleum sources for PAHs in the samples. While BBC, BBF, FCBF, LCBC, LCBF, CFGC, FCGE, and FCGC are in the wood, grass, or charcoal combustion region of both ratios, signifying greater contributions from pyrogenic sources in them.

The PAH diagnostic ratios and the plots showed PAH was from mixed sources (petrogenic and pyrogenic), but mostly from anthropogenic pyrogenic sources (wood/charcoal, liquid fuel combustion, and automobile exhaust emissions) with minor inputs from petrogenic origin, as shown by the value of LMW-PAH/HMW-PAH ratios > 1 in a few samples. The pyrogenic input may have come from the cooking processes and combustion of fuel (Tsymbalyuk *et al.,* 2011; Evans *et al.,* 2016; Jiao *et al.,* 2017), and equally from the environment (input of atmospheric deposits arising from automobile exhaust emissions and wood/grass burning processes, etc.) (Lopez *et al.,* 2019). Whereas, the petrogenic PAHs are likely from anthropogenic activities such as crude oil drilling, oceanic and freshwater oil spills, leakages, and spillages during exploration, production, transport, and storage of petroleum products, etc. (Adeniji *et al*. 2019; Gupte *et al.,* 2018; Lopez et al., 2019) within the natural habitats of the animals. It may also come from ingested food, water, or even inhaled air, possibly contaminated by petroleum and its products (Adeniji *et al.,* 2019; Gupte *et al.,* 2016; Zelinkova & Wenzl, 2015).

E. Principal component analysis

Principal component analysis with varimax rotation was performed on PAH levels to examine the variances in the distribution of PAH and then further provide insight into the sources of PAHs detected in the analysed meat and fish samples. A factor variance must be greater than or equal to 7%. High or signification loading should be 0.70 or above. Four main factors (Table 5) described the origin of PAH in the samples, which accounted for 82.48% of the total variability in the data set and distinguished the PAHs into different source groups.

Factor 1, which accounted for 47.29% of the total variance, has high loadings for anthracene, phenanthrene, pyrene, acenaphthylene, benzo[a]anthracene, acenaphthene, and fluoranthene. This represented a typical mixed source but with greater contributions from petrogenic origins and less from combustion origins.

Factor 2 contained 14.57% of the total variance and contained high loadings for a benzo[a]pyrene, an HMW-PAH associated with incomplete combustion of organic matter indicative of biomass burning and automobile diesel exhaust emissions. The negative loading for benzo[k]fluoranthene may suggest a different origin for this PAH compared to others, possibly showing variations in combustion conditions.

Factor 3 represented 11.53% of the total variance with a high loading for benzo[ghi]perylene, another HMW-PAH associated with incomplete combustion and suggestive of biomass burning.

Factor 4 accounted for 9.08% of the total variance, and the dominant PAH was naphthalene, a LMW-PAH found in emissions from biomass burning, gasoline and oil combustion, etc.

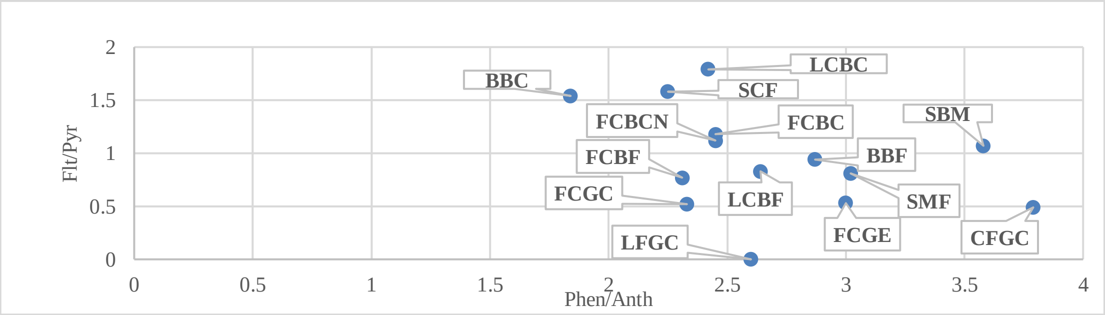

Fig. 3 Plot of Flt/Pyr against Phen/Anth

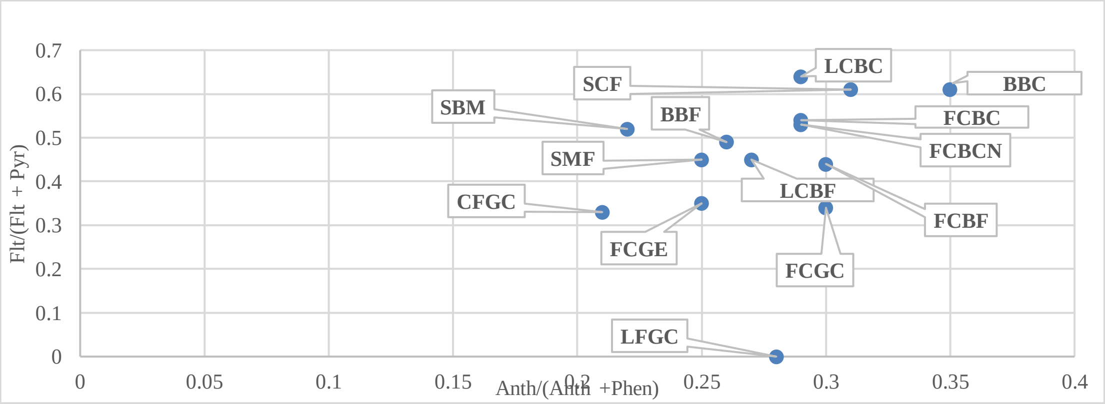

Fig. 4 Plot of Flt/(Flt + Pyr) against Anth/(Anth + Phen)

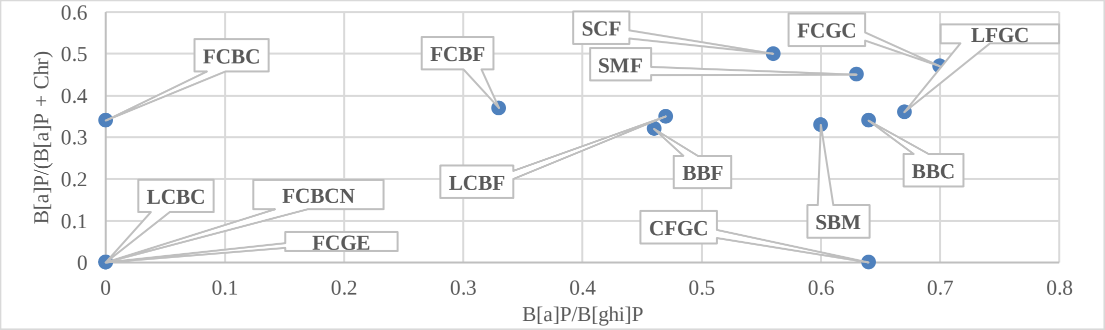

Fig. 5 Plot of B[a]P/(B[a]P + Chr) against B[a]P/B[ghi]P

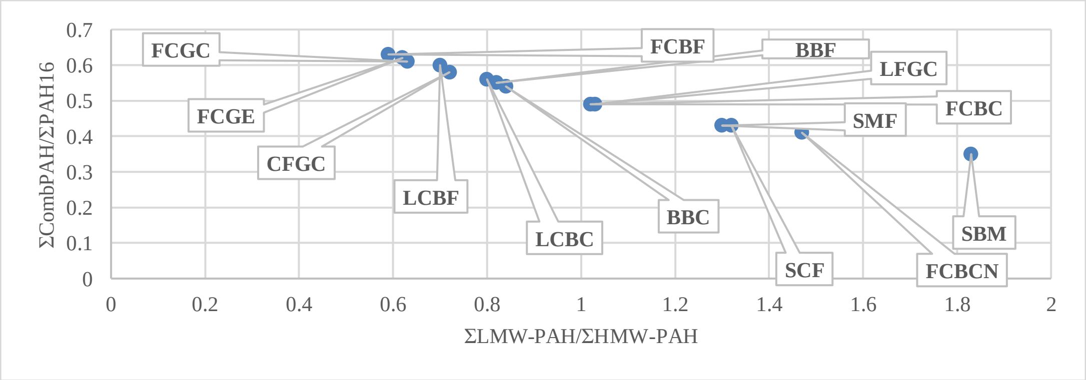

Fig. 6 Plot of Σ ComboPAHs/Σ16PAHs against ΣLMW-PAH/ΣHMW-PAH

TABLE 5 ROTATED COMPONENT MATRIX OF PRINCIPAL COMPONENT ANALYSIS

PAHs	Factor 1	Factor 2	Factor 3	Factor 4
Phenanthrene	.958			
Pyrene	.948			
Anthracene	.941			
Acenaphthylene	.927			
Benzo[a]anthracene	.897			
Acenaphthene	.841			
Fluoranthene	.740	-.304		.455
Fluorene	.596		-.595	
Chrysene	.567		.494	
Benzo[a]pyrene		.828		
Benzo[k]fluoranthene		-.715		
Indeno[123-cd]pyrene		.666	-.574	
Benzo[ghi]perylene			.919	
Naphthalene				.960
Variance %	47.29	14.57	11.53	9.08
Pollution source	Mixed	Biomass burning and Automobile exhaust emission	Biomass burning	Biomass burning, gasoline and oil combustion

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalisation

CONCLUSION

The analysis of polycyclic aromatic hydrocarbons (PAHs) in meat and fish samples using diagnostic ratios and principal component analysis revealed the origins of these PAHs. The diagnostic ratios identified both petrogenic (originating from petroleum and related products) and pyrogenic (resulting from combustion processes) sources. They show that pyrogenic PAHs, which could be from wood burning, liquid fuel combustion, or automobile exhaust emissions, contaminated primarily the barbecued samples. While that in the grilled samples were mostly from different combustion sources (traffic sources, biomass, and liquid fuel), with little input from petrogenic sources. Various combustion sources (traffic and non-traffic, firewood burning, automobile exhaust) majorly contaminated the smoked samples and a little input from petrogenic sources. The PCA analysis accounted for 82.47% of the total variability in the data set, also showing a robust explanation of the sources and distribution of PAHs in the meat and fish samples. Factor 1 (47.29%) was the most significant contributor, highlighting the predominance of mixed sources (petrogenic and combustion origins). Factors 2, 3, and 4 explained smaller yet significant portions of the variance, highlighting specific sources like biomass burning, liquid fossil fuel combustion, and automobile emissions.

Pyrogenic sources, especially from anthropogenic activities including cooking processes and environmental pollution from vehicle exhaust emissions, liquid fossil fuel combustion, and combustion of wood/charcoal, were the predominant contributors. Minor contributions came from petrogenic sources, likely related to petroleum activities and contamination in the natural habitat. Adequate control/reduction of these anthropogenic activities and usage of improved cooking practices will promote a healthier and safer food environment for all.

Future Research should focus on conducting additional studies to identify other potential sources of PAHs and their impacts on different environmental compartments and food items. Exploring the effectiveness of various intervention strategies to reduce PAH levels in the environment and food products, as well as developing more precise diagnostic tools that can help in identifying and mitigating PAH sources effectively.

TABLE 6 SUMMARY OF SOURCES OF CONTAMINATION IN SAMPLES USING DIAGNOSTIC RATIOS

Samples	Pyrogenic	Petrogenic
Barbecue	Primarily from pyrogenic sources (wood burning, liquid fuel combustion, or automobile exhaust emissions).	Little input from petrogenic sources
Grilled	Mostly from different combustion sources (traffic sources, biomass, and liquid fuel).	A little input from petrogenic sources.
Smoked	Various combustion sources (traffic and non-traffic, firewood burning, automobile exhaust) majorly contaminated the smoked samples.	A little input from petrogenic sources.

TABLE 7 SUMMARY OF SOURCES OF CONTAMINATION IN SAMPLES USING PCA

Factor	Pollution sources	% Variance
Factor 1	Mixed sources (petrogenic and combustion origins).	47.29
Factor 2	Combustion (Biomass burning and Automobile exhaust emission).	14.57
Factor 3	Combustion (Biomass burning).	11.53
Factor 4	Combustion (Biomass burning, gasoline and oil combustion).	9.08
Overall/conclusion	Predominantly from anthropogenic pyrogenic activities.	82.47

RECOMMENDATIONS

1. **Improve Cooking Methods**: Implementing cleaner cooking technologies and practices can reduce PAH exposure. Using electric grills or gas instead of wood/charcoal can minimize PAH formation.
2. **Monitor and Regulate Emissions:**Strengthen regulations on automobile and industrial emissions to reduce atmospheric PAH deposition. Implementing stringent emission controls and promoting the use of cleaner fuels can be effective. Advocate for reduced reliance on fossil fuels and increased use of renewable energy sources to decrease overall PAH emissions.
3. **Public Awareness and Education**: Educate the public on the sources and risks of PAH exposure from food and environmental sources to promote better-informed choices. Encourage practices that reduce PAH formation during cooking.
4. **Source Control and Mitigation:**Implement stricter regulations and monitoring of petroleum product handling and spills to minimize petrogenic PAH contamination in the environment. Promote cleaner combustion technologies and improve emission controls on diesel engines to reduce PAHs from incomplete combustion.
5. **Environmental Clean-up:**Enhance efforts to clean up oil spills and reduce petroleum leakages in water bodies. Monitoring and managing industrial discharges can help mitigate PAH contamination.
6. **Environmental and Food Safety:**Regularly monitor PAH levels in meat and fish to ensure they remain below harmful thresholds, thus protecting consumer health.
7. **Public Awareness and Education:**Educate the public on the sources and risks of PAH exposure,

particularly in food items, to promote better-informed choices.

By addressing the identified sources and implementing these recommendations, it is possible to reduce PAH contamination in meat and fish, safeguarding public health and the environment.

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