Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 65-74 *https://dx.doi.org/10.4314/sa.v20i1.6*
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OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN ELECTRIC GRILLED FOODS COMMONLY CONSUMED IN PORT HARCOURT, NIGERIA: DISTRIBUTION AND CONTAMINATION PROFILES

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Received: 06-01-2021
Accepted: 23-02-2021

ABSTRACT

United State Environmental Protection Agency (USEPA) priority polycyclic aromatic hydrocarbons (PAHs) were investigated in electric grilled (EG) foods: plantain (EG-PN), meat (EG-MT), yam (EG-YM) and fish (EG-FH), commonly consumed in Port Harcourt, Rivers State, southern Nigeria. Distribution and concentrations of PAHs in the EG food samples were obtained by gas chromatography (GC) analyses, after extraction using hexane/dichloromethane (1:3 v/v) and clean up by column chromatography. GC analyses identified 13 to 16 PAHs in the food samples with 10 observed to occur in all. Fluoranthene, pyrene (4-ring PAHs) and benzo(a)pyrene (5-ring PAH) were the most abundant, while the 2- and 3-ring PAHs were generally minor constituents or absent. Concentrations of PAHs in the EG foods ranged from 15.73 to 67.13 μg/kg and was observed to decrease in the order EG-PN > EG-MT > EG-YM > EG-FH with increase in grilling time. Ratios used as diagnostic indices of PAH formation processes indicate a combustion source for PAHs in the food samples and revealed electric grilling generated PAHs which contaminated the foods. Benzo(a)pyrene (BaP), used as a marker for PAH contamination in foods, had concentrations of 7.51 μg/kg, 2.68 μg/kg, 2.33 μg/kg and 1.85 μg/kg in EG-PN, EG-MT, EG-YM and EG-FH respectively. These values were above the maximum limit of 2 μg/kg set by the European Union, except for EG-FH which was slightly lower.

Keywords: Food, Polycyclic aromatic hydrocarbons, Electric grill, Concentration, Diagnostic ratio, Nigeria.

INTRODUCTION.

Polycyclic aromatic hydrocarbons (PAHs) are of global concern because they are widespread in the environment and show toxic characteristics, such as carcinogenicity, at low concentrations (Zhang *et al*., 2006). These chemical compounds are made of two or more aromatic rings fused. They are mainly derived from the incomplete combustion or burning of organic substances such as wood, coal, garbage, tobacco, petroleum and its derivatives (Yunker and Macdonald, 1995; Suchanova *et al*., 2008; Tipmanee *et al*., 2012). There are over 100 PAHs which occur in the environment as complex mixtures. However, the United State Environmental Protection Agency (USEPA) has designated 16 PAHs as priority pollutants, based on their occurrence and carcinogenicity (Jiang *et al*., 2018). PAHs are harmful to humans due to their potential to form carcinogenic and mutagenic diols and epoxides that react with DNA, resulting in many health

problems such as lung and skin cancer. Among the US EPA priority PAHs, benzo (a) anthracene, chrysene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene and dibenzo(a,h)anthracene are classified as probable human carcinogens (ATSDR, 1995).

The presence of PAHs in foods is of concern because this is one of the routes of human exposure. WHO (1998) reported that for an adult who does not even smoke, PAHs levels in foodstuff gives above 90% of total exposure. Several studies have reported PAHs in foods such as fruit, vegetable, fats and oils, fish, meat, milk, flour, cereals, seafood and baked ready-to-eat foods (Kazerouni *et al*., 2001; Ogbuagu and Ayoade, 2012; Iwegbue, 2016; Nitonye *et al*., 2019). PAHs occurrence in foods can also arise from thermal methods of preparation like drying, smoking, roasting, baking, or frying. According to Knize *et al*. (1999), these preparation methods raise the level of PAHs in foods, whereas boiling and steaming scarcely bring about PAHs formation. For instance, about 130 µg/kg was observed in barbecued meat while for uncooked foods the average background values are between 0.01 to 1 µg/kg (SCF, 2002). Evaluation of PAHs in meats and fishes sold in Abobo market in Abidjan, Côte d'Ivoire showed smoking produced more PAHs than frying or grilling with benzo(a)pyrene appearing in the majority of the samples in quantities above the European Union fixed limit (Manda *et al*., 2012). Assessment of PAH levels in roasted plantain and plantain chips in Warri, Delta State using gas chromatography (GC) equipped with flame ionization detector (FID) showed the total concentration of 16 PAHs range from 6.9 to 18.3 µg/kg for roasted plantain and 3.8 to 10.5 µg/kg for plantain chips (Ossai *et al*., 2014). Due to the occurrence of harmful PAHs in foods arising from thermal preparation processes, this article examines the levels of PAHs in some electric grilled foods; plantain, yam, fish and meat, commonly consumed in Port Harcourt City, southern Nigeria.

MATERIALS AND METHODS

Sample collection and preparation

Four foods commonly consumed in the city of Port Harcourt, Rivers State, situated in southern Nigeria, were used for this study. The foods were fish, meat, plantain and yam. They were purchased from the popular Mile 3 Market in Port Harcourt, Rivers State. The food samples were shared into five portions each. Four portions of each food sample were grilled separately using an electric Halogen multi-cooker (IGENIX model no. IG1150, made in China). Temperature was set at 200 ^{o}C and each of the 4 plantain samples grilled for 30 minutes, meat samples grilled for 35 minutes, yam samples grilled for 40 minutes and fish samples grilled for 55 minutes, rendering all cooked and ready for consumption. The fifth portion of each of the food samples was not grilled and served as reference. All the food samples were air-dried, homogenized with an electric powered blender and the four portions of each electric grilled food sample were mixed and labelled appropriately.

Sample Extraction and Clean-up

Sample extraction and clean-up were accomplished using the methods reported by Nitonye *et al*. (2019). Five (5) grams of each homogenized and mixed food sample was weighed into a conical flask. 20 ml of hexane/dichloromethane (1:3 v/v) was

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 65-74 *https://dx.doi.org/10.4314/sa.v20i1.6*
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measured out and poured into the conical flask. The mixture was stirred, agitated with a mechanical vibrator for 30 minutes and filtered. The filtrate was concentrated by evaporation under a gentle stream of dry nitrogen in a fume cupboard and transferred onto the top of a glass chromatographic column (25 cm x 1 cm) packed with four grams of activated silica gel (mesh 100-200). 20 ml of hexane was poured into the column to elute the saturated hydrocarbons and 20 ml of dichloromethane poured to elute the aromatic hydrocarbons. The aromatic hydrocarbon fraction was concentrated under a gentle stream of nitrogen in a fume cupboard.

Polycyclic Aromatic Hydrocarbon (PAH) Analysis

Detailed PAH analysis was achieved according to Onyema, *et al*. (2017). The concentrated aromatic fraction of each food sample was dissolved in hexane, transferred into labelled glass vials and with the aid of the automatic liquid sampler (ALS) one microlitre (1µL) was injected into an Agilent 7890B gas chromatography (GC) fitted with an HP-5 silica capillary column (30 m x 320 µm id and 0.25 µm film thickness). The GC was coupled to a flame ionization detector (FID) and operated in a splitless injection mode. Helium was used as the carrier gas and oven temperature for the PAH analysis was programmed from to increase from 35 ^{o}C to 325 ^{o}C at 10 ^{o}C/min with 2 min hold at 35 ^{o}C and 10 mins hold at 325 ^{o}C. Peaks were identified by comparing their retention times with internal standard and quantification was acquired by the Chemstation software OPEN LAB CDS Edition.

RESULTS AND DISCUSSION

Distribution and Concentration of PAHs in the Electric grilled foods

Gas chromatographic (GC) analyses of the food samples showed polycyclic aromatic hydrocarbons (PAHs) were present in the electric grilled (EG) foods and absent in the reference food samples. The GC results indicate that electric grilling generated PAH compounds which were incorporated into the food samples, thereby contaminating them. Total PAH concentrations of the food samples grilled at a temperature of 200 ^{o}C ranged from 15.73 to 67.13 µg/kg (Table 1). Plantain, meat, yam and fish, which were grilled for 30, 35, 40 and 55 minutes, had total PAH concentrations of 67.13 µg/kg, 32.68 µg/kg, 32.31 µg/kg and 15.73 µg/kg respectively. This shows that an increase in grilling time decreases the total concentration of PAHs in the food samples.

A total of 13 PAHs were identified in electric grilled plantain (EG-PN), yam (EG-YM) and fish (EG-FH), while in electric grilled meat (EG-MT) 16 PAHs were identified (Table 1). The PAHs identified in the EG food samples are among the 16 US EPA priority pollutants. Among the PAHs detected, 10 were observed to occur in all the EG food samples (Table 1). They are Anthracene (Ant), Fluoranthene (Fth), Pyrene (Pyr), Benzo (a) anthracene (BaA), Chrysene (Chr), Benzo (k) fluoranthene (BkF), Benzo (a) pyrene (BaP), Indeno (1,2,3-*cd*) pyrene (IP), Dibenzo (*a*,*h*) anthracene (DA) and Benzo (*g*,*h*,*i*) perylene (BP). Other PAHs detected are Naphthalene (Nap) Acenaphthylene (Acy) Acenaphthene (Ace) Fluorene (Fle) Phenanthrene (Phe), Benzo(b)fluoranthene (BbF). The concentrations of PAHs in the four electric

grilled food samples (EG-PN, EG-MT, EG-YM and EG-FH) are presented in Table 1.

Table 1: Concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) in the Electric grilled (EG) food samples (plantain, meat, yam and fish).

	PAHs	Ring Size	Concentration in grilled food samples (μg/kg)			
			EG-PN	EG-MT	EG-YM	EG-FH
1.	Naphthalene (Nap)	2	n.d.	0.04	n.d.	0.03
2.	Acenaphthylene (Acy)	3	n.d.	0.15	0.18	n.d.
3.	Acenaphthene (Ace)	3	0.06	0.05	n.d.	0.03
4.	Fluorene (Fle)	3	n.d.	0.04	n.d.	0.02
5.	Phenanthrene (Phe)	3	0.10	0.14	0.14	n.d.
6.	Anthracene (Ant)	3	1.82	1.64	1.11	0.45
7.	Fluoranthene (Fth)	4	37.00	14.67	18.54	7.89
8.	Pyrene (Pyr)	4	13.53	7.20	7.79	3.04
9.	Benzo(a)anthracene (BaA)	4	1.55	1.31	0.29	0.58
10.	Chrysene (Chr)	4	0.90	1.68	0.33	0.77
11.	Benzo(b)fluoranthene (BbF)	5	0.26	0.28	0.11	n.d.
12.	Benzo(k)fluoranthene (BkF)	5	0.98	0.94	0.46	0.11
13.	Benzo(a)pyrene (BaP)	5	7.51	2.68	2.33	1.85
14.	Indeno(1,2,3-*cd*)pyrene (IP)	6	0.33	0.58	0.24	0.25
15.	Dibenzo(*a,h*)anthracene (DA)	5	0.98	1.15	0.61	0.61
16.	Benzo(*g,h,i*)perylene (BP)	6	2.12	0.13	0.17	0.10
	Σ 2-3 ring LMW PAHs		1.98	2.06	1.43	0.53
	Σ 4-6 ring HMW PAHs		65.15	30.63	30.88	15.21
	Total PAHs		67.13	32.68	32.31	15.73

EG-PN, electric grilled plantain; EG-MT, electric grilled meat; EG-YM, electric grilled yam and EG-FH, electric grilled fish; n.d., not detected; LMW, low molecular weight; HMW, high molecular weight.

Individual PAH concentrations in EG-PN ranged from 0.06 to 37.00 μg/kg, with Fth (37.00 μg/kg), Pyr (13.53 μg/kg) and BaP (7.51 μg/kg) being the most abundant. Ace (0.06 μg/kg), Phe (0.10 μg/kg) and BbF (0.26 μg/kg) were the least abundant, while Nap, Acy and Fle were absent. In EG-MT, PAH concentrations ranged from 0.04 to 14.67 μg/kg. Fth (14.67 μg/kg), Pyr (7.20 μg/kg) and BaP (2.68 μg/kg) were the most abundant while Nap (0.04 μg/kg), Fle (0.04 μg/kg) and Ace (0.05 μg/kg) were the least abundant. Concentration of PAHs in EG-YM ranged between 0.11 and 18.54 μg/kg. Fth (18.54 μg/kg), Pyr (7.79 μg/kg) and BaP (2.33 μg/kg) were the most abundant, while BbF (0.11 μg/kg), Phe (0.14 μg/kg) and BP (0.17 μg/kg) were the least abundant. Nap, Ace and Fle were absent. PAH concentrations in EG-FH ranged from 0.02 to 7.89 μg/kg. The most abundant PAHs were Fth (7.89 μg/kg), Pyr (3.04 μg/kg) and

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 65-74 https://dx.doi.org/10.4314/sa.v20i1.6
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BaP (1.85 µg/kg). Fle (0.02 µg/kg), Nap (0.03 µg/kg) and Ace (0.03 µg/kg) were the least abundant while Acy, Phe and BbF were absent. From the concentration results, Fth, Pyr and BaP, 4- and 5-ring PAHs were the most abundant in the electric grilled foods while the 2- and 3-ring PAHs were generally minor constituents or absent.

PAHs are classified into two groups based on their ring size; namely, low and high molecular weight PAHs. Low molecular weight (LMW) PAHs have two to three rings, while the high molecular weight (HMW) have four to six rings (Palm *et al.*, 2011). Total concentrations (Σ) of the 2-3 ring LMW and 4-6 ring HMW PAHs in the EG food samples range from 0.53 to 2.06 µg/kg and 15.21 to 65.15 µg/kg respectively (Table 1). PAHs from petroleum sources usually show abundance of the LMW, while abundance of the HMW is characteristic of combustion source (Yunker and Macdonald, 1995; Chen *et al.*, 2004). The concentrations of PAHs in the EG food samples showed the 4-6 ring HMW were significantly more than the 2-3 ring LMW with the HMW/LMW ratio range from 14.88 - 32.84. This indicates PAHs in the EG food samples were predominantly combustion derived.

Composition Profile of PAHs in the Electric grilled food samples

The composition profiles of PAH compounds in the four electric grilled food samples is presented in figure 1.

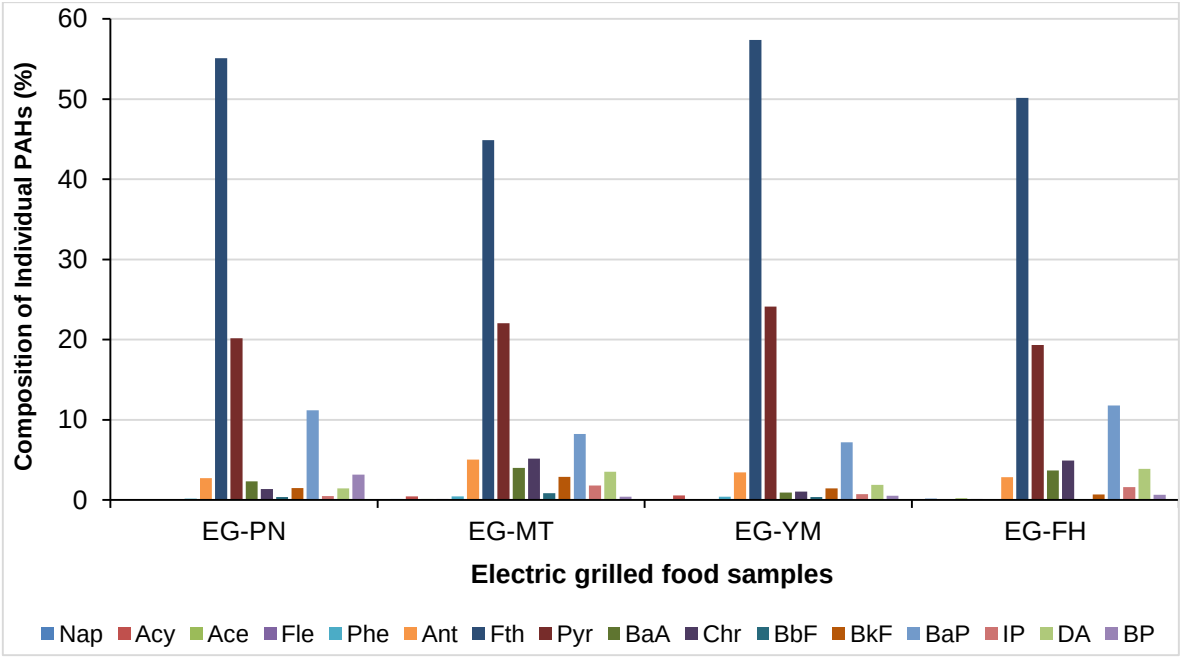

Figure 1: Composition Profile of Polycyclic Aromatic Hydrocarbons (PAHs) in Electric grilled Plantain (EG-PN), Meat (EG-MT), Yam (EG-YM) and Fish (EG-FH)

From figure 1, Fluoranthene (Fth), Pyrene (Pyr) and Benzo(α)pyrene (BaP) were prominent PAHs in all the EG grilled food samples. Fth was predominant with a significantly high composition of 55.11% of total PAH amount in EG-PN, 44.87% in EG-MT, 57.37% in EG-YM and 50.15% EG-FH. Pyr composition range from 19.32

- 24.10% and is dominant over BaP with composition from 7.20 - 11.78% (Fig. 1).

Fluoranthene and pyrene are isomers and their composition is an effective diagnostic index of PAH formation processes. Values of Fth/Fth+Pyr ratios less than (<) 0.40 indicate PAHs were petroleum-derived and ratios greater than (>) 0.40 are attributed to combustion of fossil fuel (Hwang *et al*., 2003; Yunker and Mcdonald, 2003). The calculated Fth/Fth+Pyr ratio for EG-PN is 0.73, EG-MT 0.67, EG-YM 0.70 and EG-FH 0.72 (Table 2). These ratio values (> 0.50) indicate PAHs in the EG food samples were of combustion source. PAHs isomer ratios commonly used as diagnostic index with Fth/Fth+Pyr are Ant/Ant+Phe and BaA/BaA+Chr (Khalili *et al*., 1995; Guo *et al*., 2003). The calculated ratios of Ant/Ant+Phe and BaA/BaA+Chr are presented in Table 2.

Table 2: Diagnostic Index Ratios of PAH Formation in the Electric grilled foods samples

Samples	Ant/(Ant+Phe)	Fth/(Fth+Pyr)	BaA/(BaA+Chr)
EG-FH	1.00	0.72	0.75
EG-MT	0.92	0.67	0.78
EG-PN	0.95	0.73	1.71
EG-YM	0.89	0.70	0.88

From Table 2, the calculated values of Ant/Ant+Phe ratio for EG-PN, EG-MT, EG-YM and EG-FH are 0.95, 0.92, 0.89 and 1.00, while values of BaA/BaA+Chr ratio are 1.71, 0.78, 0.88 and 0.75 respectively. Ant/Ant+Phe ratios < 0.10 suggest petroleum source while ratios > 0.10 indicate combustion source. BaA/BaA+Chr ratios < 0.20 suggest petroleum source, ratios > 0.35 are attributed to combustion, while values between 0.20 and 0.35 suggest input from either petroleum or combustion source (Bhupander *et al*., 2012). The values of Ant/Ant+Phe and BaA/BaA+Chr ratios were considerably higher than 0.10 and 0.35 respectively. This indicates combustion source for PAHs in the EG food samples. Results from the calculated PAH ratios revealed electric grilling of plantain, meat, yam and fish generated PAHs which contaminated the foods.

Contamination Levels of PAHs in Electric grilled food samples

Benzo(a)pyrene (BaP) is a well-known PAH and classified as a human carcinogen. It is widely used as a marker for PAH contamination in food (Alomirah *et al*., 2010). The European Union (EU) has established a maximum limit of 2 μg/kg for BaP in food (EFSA, 2008). BaP concentrations in the EG food samples are 7.51 μg/kg in EG-PN, 2.68 μg/kg in EG-MT, 2.33 μg/kg in EG-YM and 1.85 μg/kg in EG-FH. From the result, concentrations of BaP for EG-PN, EG-MT and EG-YM were above the maximum limit set by the EU while EG-FH was slightly lower than the set limit.

PAHs with 4-6 rings are seen to be more carcinogenic than the 2-3 ring and linear ring configurations tend to be less carcinogenic compared to the highly angular ring configurations (Silva *et al*.,

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 65-74 https://dx.doi.org/10.4314/sa.v20i1.6
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2011). Following this, the European Food Safety Authority (EFSA) has suggested the sum of the concentrations of four PAHs (PAH4): BaA, Chr, BbF and BaP, to better reflect the levels of PAH contamination in foods than BaP alone, due to their carcinogenic and genotoxic activity. PAH4 concentrations for EG-PN, EG-MT, EG-YM and EG-FH were 10.21 µg/kg, 5.95 µg/kg, 3.06 µg/kg and 3.21 µg/kg. These concentrations in the EG food samples were all below the PAH4 maximum limits of 12 µg/kg established by the EU (EFSA, 2008).

CONCLUSION

Gas chromatographic (GC) analysis of electric grilled (EG) plantain (EG-PN), meat (EG-MT), yam (EG-YM) and fish (EG-FH) identified polycyclic aromatic hydrocarbons (PAHs) which were absent in the reference (i.e. raw) food samples. This revealed the electric grilling process incorporated PAHs into the EG food samples. Total concentrations of PAHs in the EG food samples decreased in the order EG-PN > EG-MT > EG-YM > EG-FH with increase in grilling time. 10 PAHs occurred in all the EG food samples with Fth, Pyr and BaP being prominent and most abundant, while the 2-3 ring PAHs were generally minor or absent. Ratios used as diagnostic index of PAH formation processes indicate electric grilling of plantain, meat, yam and fish generated PAHs which contaminated the foods. Benzo(a)pyrene (BaP) concentrations, used as a marker for PAH contamination in foods, were above the maximum limit set by the European Union, except for EG-FH which was slightly lower.

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