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Polynuclear Aromatic Hydrocarbons Concentrations in Char-Broiled Meat Suya

[1]Okoro Duke and [2]Ikolo O. Albert
[1]Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria
[2]Mail-In Oilfield and Laboratory Services Limited, Plot 5, 38th Street, DDPA, Effurun, Delta State, Nigeria

Abstract: Polynuclear aromatic hydrocarbons (PNAs) concentrations in char-broiled meat suya have been determined in samples obtained from four different selling points in Warri Metropolis of Nigeria. The sixteen EPA priority PNAs were detected using Gas Chromatography and Flame Ionization Detector. Concentrations of total PNAs determined in the four sampling points were: EF1 (134.82±8.53 μg kg^{-1}), EF2 (113.83±7.93 μg kg^{-1}), WR3 (115.14±7.77 μg kg^{-1}), WR4 (81.95±6.76 μg kg^{-1}). Benzo(a)pyrene, which is often used as a reference indicator for PNAs carcinogenicity, was determined at levels above 5 μg kg^{-1} recommended as maximum limit by Commission of European Communities for smoked meat and smoked meat products. It was however, observed that the 2-3 rings PNAs including naphthalene, fluorene, acenaphthylene, acenaphthene, phenanthrene and anthracene were more abundant owing to their high percentage composition in the matrix of the charbroiled meat. Although the levels observed for benzo(a)pyrene in the beef suya exceeded standard guidelines of European Commission, it may take the diet to consist of frequent consumption of barbecued meat before a significant contributions of PNAs contaminant to the human system can be thoroughly assessed.

Key words: Benzo(a)pyrene, carcinogens, char-broiled, GC/FID, meat, extraction, PNAs

INTRODUCTION

Polynuclear aromatic hydrocarbons (PNAs) are a group of environmental pollutants with carcinogenic properties which are permanently formed by all sorts of incomplete combustion and hence may be considered to be ubiquitous. They are widely distributed in the environment and human exposure to them is unavoidable. A number of them, such as benzo(a)pyrene, are carcinogenic and mutagenic and they are widely believed to make a substantial contribution to the overall burden of cancer in humans (Phillips, 1999). Smoked food is suspected of containing contaminants, such as polynuclear aromatic hydrocarbons, harmful to human health (Guillén *et al*., 1997). Polynuclear aromatic hydrocarbons are highly soluble in lipids and owing to the relatively high proportions of fat in meat; it might be thought that PNAs are easily retained when smoking process of meat is on. Char-broiled meat popularly and locally known as suya is the most widely consumed barbecue meat in Nigeria. It is mostly prepared in the evenings prior to or around close of work awaiting workers who usually hang out in small and medium pub bars where they take some drink before leaving for their various homes. Some homes do have barbecue stands with charcoal used only during occasional parties for making suya. PNAs are found in substantial quantities in some food, depending on the mode of cooking, preservation and storage and are detected in a wide range of meats, fishes, vegetables and fruits. Study of Dutch market basket dietry components for 18-year-old males involving determination of 17 different PNAs revealed that all these compounds were detected (De Vos *et al*., 1990). Traditional smokehouses are still used fairly widely. Since the generation of wood smoke is an example of incomplete combustion, PNAs are generated (Nordholm *et al*., 1986). The European Commission's Scientific Committee on Food (SCF) in a report in December, 2002, considered some list of contaminants including PNAs as genotoxic carcinogens. A classification of the PNAs by International Agency for Research on Cancer is shown in Table 1.

PNAs are non-polar, chemically inert and hydrophobic, but they undergo metabolic activation in mammalian cells to diol-epoxides that bind covalently to cellular macromolecules, including DNA (Phillip, 1983), thereby causing errors in DNA replication and mutations that initiate the carcinogenic process. When meat is cooked over an open flame, PNAs are formed. Since the meat is in direct contact with the flame, pyrolysis of the fats in the meat generates PNAs that can become deposited on the meat. Nevertheless, fat dripping on to the flame or hot coals generates the compounds which are then carried back onto the meat (Philip, 1999). Similarly,

Corresponding Author: Okoro Duke, Department of Chemistry, Delta State University, Abraka, Delta State, Nigeria
Tel: +234 8033834446, 2348023320809

Table 1: Health effects of PNAs with regards to carcinogenicity

PNAs	Effects
Naph	Possibly carcinogenic to humans
Acep	-
Aceh	-
Flu	Not classifiable as to carcinogenicity to humans
Phe	Not classifiable as to carcinogenicity to humans
Anth	Not classifiable as to carcinogenicity to humans
Fluo	Not classifiable as to carcinogenicity to humans
Pyr	Not classifiable as to carcinogenicity to humans
B[a]A	Probably carcinogenic to humans
Chry	Not classifiable as to carcinogenicity to humans
B[b]F	Possibly carcinogenic to humans
B[k]F	Possibly carcinogenic to humans
B[a]P	Probably carcinogenic to humans
D[a,h]A	Probably carcinogenic to humans
B[g,h,i]P	Not classifiable as to carcinogenicity to humans
I[1,2,3-cd]	Possibly carcinogenic to humans

Adapted from the international agency for research on cancer (IARC, 1987)
The abbreviations are defined in Table 2

PNAs released by cooking over charcoal is a function both of the fat content of the meat and the proximity of the food to the heat source. Generally, it has also been reported that charred food contains PNAs, but normal or frying of food does not produce profuse quantities of PNAs (Howard and Fazio, 1980).

Triger values are set based on accumulation of thousands of data on the levels of an analyte or a contaminant in a particular matrix. Standard guidelines for levels of PNAs in smoked food in Nigeria are not available. Although there is a possible link between high consumption of smoked food and the incidence of stomach cancer among the population of Iceland (Bartsch *et al*., 1989), however, a link has not been established yet between PNAs levels in human and cases of cancer patient in hospitals in Nigeria. Baseline study on the levels of PNAs in smoked or grilled food is therefore necessary. This research was carried out in Warri metropolis to assess levels of PNAs in char-broiled meat (suya) sold at different points in the city. It was done by the extraction of these PNAs from the suya matrix, isolation and separation using gel permeation chromatography. Quantification was done by means of gas chromatography with flame ionization detector (GC/FID). The PNAs studied were the 16 PNAs named as EPA priority.

MATERIALS AND METHODS

Study area: Warri metropolis referred to Warri City and its adjoining town like Effurun. The study area was divided into four sampling points to ensure an effective coverage of the entire area of study and to enable representative sample collection, aimed at providing data that are reflections of the environment. The sampling points were chosen to reflect two selling points in Effurun i.e., EF1 and EF2 and two selling points in Warri i.e., WR3 and WR4. Samples of Char-broiled meat (Suya) were bought at the four different selling points in the metropolitan city of Warri. EF1 represents a suya selling point adjacent to Winas nite club, along Refinery road, Effurun with a geographic coordinate of Latitude 5° 33' N and Longitude 5° 45' E; EF2 represents a suya selling point adjacent to Owode pub bar, adjacent to citi international bank, along Effurun/Sapele road, Efurun with Latitude 5° 32' N and Longitude 5° 46' E; WR3 represents a selling points at Jolly Roger nite club, Ogunu, Warri with Latitude 5° 31' N and Longitude 5° 42' E while WR4 represents the biggest selling outlets in the metropolis of Warri, the Hausa Quarters with Latitude 5° 33' N and Longitude 5° 44' E.

Sampling, handling and treatment: Beef suya were the only meat used for the study. Sampling was done in the month of October 2005. The suya were bought from the selling points at different time interval and pooled together to form a composite sample for each site in a day. The samples were similarly made for another six days to enable sampling for one week. The samples were collected and stored in aluminium foil bags. Quality assurance/quality control formed an integral part of the sampling process. Sample chain of custody forms were used for the registration and tracking of samples from the selling points to the laboratory. The homogenizing bucket was constantly cleaned after each composite sampling. Aluminium foil sheet lining the bucket and the disposable hand- gloves were also constantly changed after each bulk/composite sampling. Suya samples were rapped in aluminum foil paper. In the laboratory, the samples were kept in the freezer prior to treatment and laboratory analyses.

Sample preparation and conditioning: About 10.00 g aliquot of homogenate was weighed into a beaker and saponified with 1.78 M KOH solution in 75% ethanol for 2 h. Fifty milliliter of iso-octane solvent was added to the samples and spiked with 1 mL of the surrogate mix. Sample was sonicated for about 10-15 min at about 70°C. The extract solvent was poured into a round bottom flask. Extraction was repeated once more with an additional 50 mL of solvent, sonicated and the beaker allowed to settle before being decanted into the same round bottom flask. The solvent extract was washed with warm water and extracted with 1, 2, 2-trichlorotrifluoroethane (TCTFE). The TCTFE phase is added with water and back-extracted with cyclohexane. The cyclohexane extract is finally dried with 10.00 g of sodium sulphate and cleaned-up by silica

gel permeation chromatography. The columns were packed with 10.00 g of 100-200-mesh silica gel pre-conditioned (baked) at 105°C over night. The silica was mixed with cyclohexane to form slurry. The solvent extract was thereafter concentrated to 2 mL by rotary evaporation and analysed by GC/FID.

Instrumental analysis

Gas chromatography operating procedure.

Instrument type: Gas chromatography HP6890 series. The basic GC parameters for the analysis of Polynuclear Aromatic Hydrocarbon were as follows: Injector Temp: 250°C; Temperature Program: Initial temp: 100°C; Initial time: 1; Rate 1: 4°C min^{-1}; Final Temp: 310°C; Detector Temp: 300°C; Detector Type: FID; Column type: High Performance Capillary Column (HP-5, Crosslinked PH ME siloxane 19091J-413; Film thickness: 0.25 um, Length: 30 m; Phase Ratio: 320 Column ID: 0.32 mm; Carrier Gas: Helium; Inlet Mode: Splitless; Linear velocity =: 30 cm sec^{-1}; Detector Type: Flame Ionization detector; Hydrogen: 35 mL min^{-1}; Air: 350 mL min^{-1}; Data Acquisition System: Computerized system for collecting, storing and processing detector output.

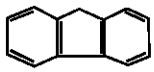
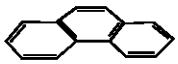
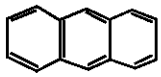
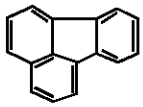
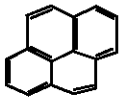
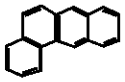
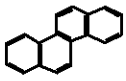
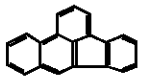
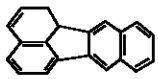
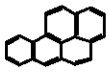
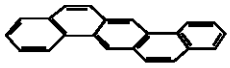
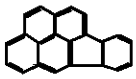
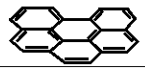
Initial calibration and calculation: Before sample was analyzed, the instrument was always calibrated for the analysis. This was done by injecting a series of PNA standards. The volume injected was 1 μL. A five-point calibration curve was prepared using the PAH standard mixture that was commercially obtained. The range of the curve was 2.0 to 20 μg mL^{-1}. The response factor (RF) was calculated for each component of the PNA mixture standard using the area response and the amount of standard material. The relative standard deviation percentage (%RSD) of the RF was calculated for each component across the calibration curve. The value was never in excess of 30% for the curve to be deemed valid. The recovery efficiencies for each PNAs were always > 70%. The average response factor for the weight ranges were calculated and used for sample quantification. Identification of individual PNA compound was performed by comparison of retention times of the standards with retention times of the identified substances. The contribution from the solvent front and the surrogate compound were excluded from the total area of the sample. Statistical treatment of the data was performed with the aid of kyplot 2.0 statistical software and all calculations were done within a confidence interval of 95%. The one way ANOVA of the data from the four different sampling points were also calculated using the kyplot 2.0.

RESULTS

The 16 polynuclear aromatic hydrocarbons (PNAs) analysed in the suya with their abbreviations, structures and molecular weight are shown in Table 2. The PNAs identified in the suya samples from the various selling points and their concentrations in μg kg^{-1} are given in Table 3. The results shown in the Table 3 are from duplicate analysis of two aliquots per sample. PNAs of low molecular weight such as naphthalene, fluorene, acenaphthelene, acenaphthene, phenanthrene and anthracene were all detected and high molecular weight PNAs including flouranthene, pyrene, the benzofluoranthenes, benzo(a)anthracene, chrysene, benzo(a)anthracene, dibenzo(a,h)anthracene, benzo(g,h,i) perylene and indeno(1,2,3-cd)pyrene were also detected. Concentrations of total PNAs determined in the four sampling points were: EF1 (134.82±8.53 μg kg^{-1}), EF2 (113.83±7.93 μg kg^{-1}), WR3 (115.14±7.77 μg kg^{-1}), WR4 (81.95±6.76 μg kg^{-1}). There was no statistically significant difference in the levels across the four sampling points ($p>0.05$). All the Carcinogenic polynuclear aromatic hydrocarbons were detected in the charbroiled beef meat (suya). The concentrations of the carcinogenic PNAs in the different sampling points which includes benzo(a)pyrene, benzo(b)fluoranthene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene are shown in Table 3. The sum of the Carcinogenic PNAs detected, Σ Carcinogens are 12.80, 9.92, 21.83 and 6.73 μg kg^{-1}, respectively for points EF1, EF2, WR3 and WR4. There were high levels of naphthalene in the matrix of the suya. The values observed for EF1, EF2, WR3 and WR4 are 20.31±1.02, 25.35±0.89, 19.32±0.72 and 26.34±1.11 μg kg^{-1}, respectively. Although levels of PNAs were detected in all four sampling points, naphthalene for instance was highest in WR4, while levels of benzo(a)anthracene was the highest in EF1 with concentration of 19.26±0.88 μg kg^{-1}. However, there was a statistically significant difference in concentrations of Fluorene, benzo(a)anthracene, benzo(a)pyrene and benzo(g,h,i)perylene with a p-value of less than 0.05 at 95% confidence interval.

The compositional distribution of the different ring size PNAs from the four sampling points are given in Fig. 1-5. In sampling points EF1, 2-3 rings PNAs which includes naphthalene, acenaphthelene, acenaphthene, fluorene, phenanthrene and anthracene are the predominant PNAs which constitutes about 67% of the total PNAs. This was followed by the 4-rings PNAs: fluoranthene; pyrene; benzo(a)anthracene; chrysene with a percentage composition of 23%. The

Table 2: The 16 Polynuclear aromatic hydrocarbons (PNA) species analyzed, their abbreviations, structure and molecular weight

PNA	Structure	MWT
Naphthalene (Naph)		128.2
Acenaphthylene (Acep)		152.2
Acenaphthene (Aceh)		154.2
Fluorene (Flu)		166.2
Phenanthrene (Phe)		178.2
Anthracene (Anth)		178.2
Fluoranthene (Fluo)		202.3
Pyrene (Pyr)		202.3
Benzo(a)anthracene (B[a]A)		228.3
Chrysene (Chry)		228.3
Benzo(b)fluoranthene (B[b]F)		252.3
Benzo(k)fluoranthene (B[k]F)		252.3
Benzo(a)pyrene (B[a]P)		252.3
Dibenzo(a,h)anthracene (D[a,h]A)		278.4
Indeno(1,2,3-cd)pyrene (I[1,2,3cd]P)		276.3
Benzo(g,h,i)perylene (B[ghi]P		267.0

5-rings PNAs including the benzofluoranthenes, benzo(a)pyrene and dibenzo(a,h)anthracene constituted about 10% of the Total PNAs while the percentage composition of the 6-rings PNAs such as indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene were relatively infinitesimal (<0.0%). In sampling point EF2, 2-3

Table 3: Mean concentrations of PNAs in char-broiled meat of sampling points in warri metropolis, their individual and total concentrations expressed in (μg kg^{-1})

PNAs	EF1	EF2	WR3	WR4	p-value
Naph	20.31±1.02	25.34±0.89	19.32±0.72	26.34±1.11	>0.05
Acep	10.20±0.19	4.58±0.12	7.59±0.03	5.83±0.21	>0.05
Aceh	7.49±0.33	5.48±0.06	6.50±0.08	4.96±0.32	>0.05
Flu	26.35±1.11	18.38±0.75	9.38±0.07	1.27±0.03	<0.05
Phe	16.49±0.52	19.21±0.74	23.11±1.44	8.94±0.81	>0.05
Anth	10.35±0.51	9.46±0.43	7.31±0.23	11.21±0.10	>0.05
Fluo	3.45±0.05	4.37±0.02	5.73±0.11	2.37±0.210	>0.05
Pyr	6.35±0.64	4.36±0.34	7.48±0.64	4.88±0.33	>0.05
B[a]A	19.26±0.88	11.57±0.75	5.83±0.45	8.59±0.09	>0.05
Chry	1.29±0.01	0.59±0.07	0.92±0.01	0.83±0.22	>0.05
B[b]F	0.42±0.01	0.01±0.01	ND	0.06±0.02	>0.05
B[k]F	0.32±0.03	0.56±0.01	0.09±0.01	ND	>0.05
B[a]P	12.36±7.23	9.46±2.77	21.47±4.77	6.47±1.71	<0.05
D[a,h]A	ND	0.26±0.02	0.32±0.04	0.02±0.01	>0.05
B[g,h,i]P	0.15±0.11	ND	0.05±0.04	ND	<0.05
I[1,2,3-cd]P	0.02±0.11	0.19±0.21	0.04±0.010	0.18±0.22	>0.05
Total PNA	134.82±8.52	113.83±7.93	115.14±7.77	81.95±6.76	>0.06

Concentrations are given as Mean±Standard Deviation; ND: Not Determined

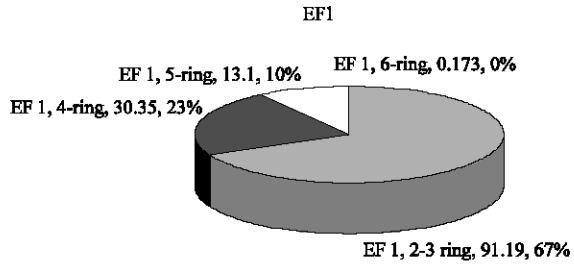


Fig. 1: Compositional distribution of PNAs in suya from EF1

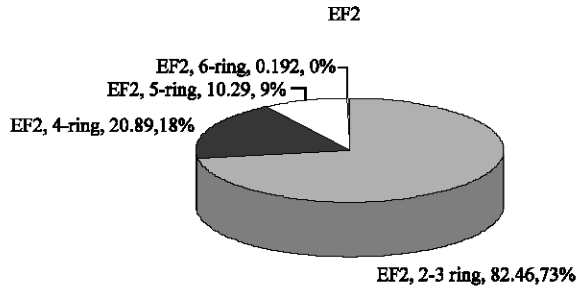


Fig. 2: Compositional distribution of PNAs in suya from EF2

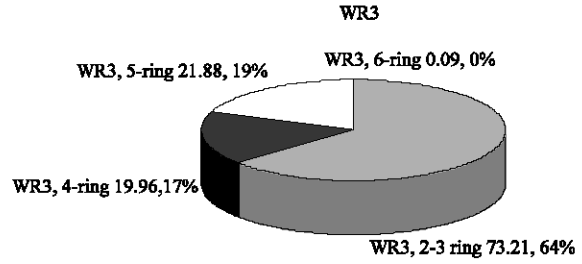


Fig. 3: Compositional distribution of PNAs in suya from WR3

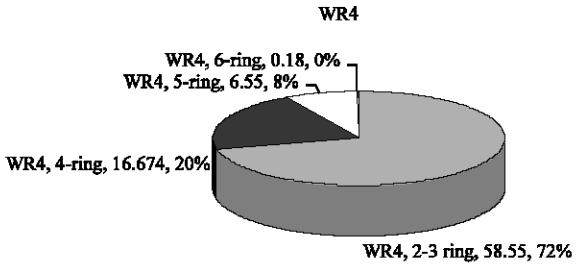


Fig. 4: Compositional distribution of PNAs in suya from WR4

rings PNAs constituted the highest values with a percentage composition of 73%. 4-ring PNAs were 18% of the Total PNAs; 5-rings PNAs were 9% while the lowest values were calculated for the 6 rings PNAs (< 0.0%). The compositional distribution of the different ring size PNAs showed that in sampling point WR3 that the 2-3 rings were also more predominant with a percentage composition of 64%. 4-rings PNAs were 17%, the 5-rings PNAs were higher in composition (19%) compared to the other sampling points. The six rings PNAs were also very low in composition (<0.0%) in the WR3. The last sampling point WR4 also has the 2-3 rings PNAs as the predominant with percentage composition of 72%. The 4-rings PNAs were also fairly large in composition with about 20%. 5-ring PNAs had composition of 8% while the 6-rings PNAs were similarly the lowest in composition (< 0.0%). Benzo(a)pyrene, because it is thought to be one of the most potent carcinogens, is often used as a reference indicator. There was however a statistical significant difference in the four sampling points ($p<0.05$) as given in Table 3. The daily concentrations of benzo(a)pyrene in the suya of the four sampling points are given in Fig. 5. Daily concentrations of the PNAs did not produce any significant difference (p-value>0.05).

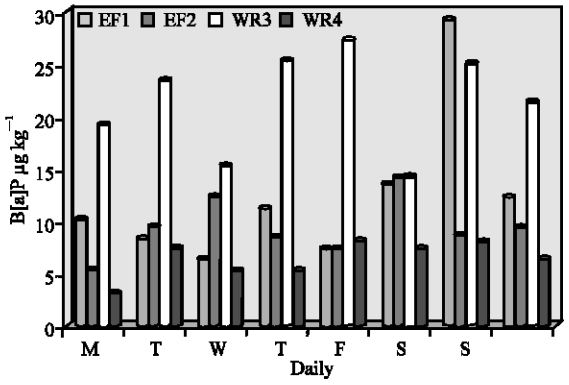


Fig. 5: Concentrations of Benzo(a)pyrene in charbroiled meat of the sampling points

There was a predominance of B[a]P in WR3 sampling points compared to the other points. WR4 can be seen to have the lowest concentration of B[a]P (Fig. 5).

DISCUSSION

The total PNAs concentrations in the suya had a range of 81.95-134.82 µg kg^{-1}. This is well above the levels observed in an earlier study of polycyclic aromatic hydrocarbons in smoked meat (Gomaa *et al*., 1993). This difference could be as a result of uncontrolled process in terms of the wood pyrolysis temperature. In a similar study, PNAs has been detected in barbecued meat samples at level of up to 164 ppb total PNAs in Canada (Panalaks, 1976). The concentrations of the sum of carcinogenic PNAs determined in this study, ΣCarcinogens, were within the range reported for smoked salmon in another study (Gomaa *et al*., 1993). With respect to the concentrations of the PNAs determined, a similar pattern was observed in the four sampling points. The highest concentrations corresponded to the lowest molecular weight PNAs (naphthalene, fluorene, anthracene), while the lowest concentrations corresponded to the heavier PNAs (dibenzo(a,h,) anthracene, benzo(b)fluoranthene, indeno(1,2,3-cd)pyrene). As molecular weight of the PNAs increases, the concentration decreases to very small levels such as 0.01±0.01 µg kg^{-1} of benzo(b)fluoranthene in suya of EF2 and even to levels below detection limit i.e., ND (non-detected). The difference in levels of benzo(a)pyrene and some PNAs observed in the sampling points could be attributed to differences in the nature of wood used for the smoking process (Guillén *et al*., 2000). The levels observed for benzo(a)pyrene exceeded the amount recommended as maximum limit (5 µg kg^{-1}) by the commission of the European Communities for smoked meats and smoked meat products (2005/10/EC). Benzo(a)pyrene for which maximum limit is set, is used as a marker for the occurrence and effect of carcinogenic PNAs. It was also noted that the 2-3 rings PNAs were more abundant in the study with their high percentage composition with the lowest levels coming from the 6-rings PNAs.

CONCLUSIONS

The crunchy taste observed in Char-broiled meat (suya) during eating is as a result of the organoleptic properties induced on it by smoking which is very well appreciated by consumers. Since it has been observed that normal frying of food does not produce profuse amount of PNAs, it is recommended that meat are better fried than smoked owing to the fact that B[a]P was detected beyond recommended limit in this study. Although the levels observed for benzo(a)pyrene in the beef suya exceeded standard guidelines of European Commission, it may take the diet to consist of frequent consumption of barbecued meat before a significant contributions of PNAs contaminant to the human system can be thoroughly assessed. It is also recommended that biomonitoring procedures are developed to assess human exposure to PNAs in other to know the major source of exposure.

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