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Research Article

Polycyclic Aromatic Hydrocarbons (PAHs) Contamination Profile of Barbequed Meats in Some Selected Selling Points within Osogbo, Nigeria

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Abstract

Levels of Polycyclic aromatic hydrocarbon in fresh and barbequed meat (asun and suya) samples sold and consumed from six different locations within osogbo metropolis in Osun state, Nigeria were determined to ascertain their potential toxicity. Levels of the PAHs were determined using gas chromatography - flame ionization detector (GC-FID) after extracting with methylene chloride by soxhlet extraction and cleaned up using column chromatographic packed with anhydrous sodium sulphate and silica gel with a mixture of pentane and methylene chloride as the eluting solvents. All the investigated sites show significant variation in the concentration of PAHs. The mean sum of the 16 PAHs concentration detected in the fresh meat which serves as control ranged between 0.0001 and 0.0004 μg g^{-1}. The total concentration of PAHs in processed samples investigated ranged from 0.001 to 26.82 μg g^{-1} and 0.002 to 9.17 μg g^{-1} for asun and suya meat respectively indicating a significantly contamination according to 0.1 μg g^{-1} stated by ATSDR. The result of this study indicates that the barbequed (asun and suya) meat after preparation has high level of pyrene, fluoranthene, anthracene, and phenanthrene than other constituents due to the continuous exposure to fire while grilling. Asun meat exhibited relatively higher BaPeq than suya meat implying a high carcinogenic burden from anthropogenic sources.

Keywords: PAHs; GC-FID; Barbequed Meat; Carcinogenic Potency; PAHs Contaminations

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are important and known pollutants that had been identified in diverse environmental matrices world-wide (Adebiyi., *et al.* 2008). PAHs are a group of environmental contaminants that emanate from incomplete combustion of fuel or high temperature pyrolysis of fats and oils.

They are widely distributed, environmentally persistent due to their relative chemical stability and resistant to biodegradation and are found in almost every type of environmental sample matrix conceivable (Cohen., *et al*. 2004). Some PAH are semi volatile but most of them tend to adsorb on organic particulate matter [1].

The amount of PAHs generated during thermal food processing depends on some parameters such as temperature, duration of the treatment, distance from the source of heating, fat content, and type of combustible used [2]. High PAHs in processed foods has been estimated to be due to different food processing involving thermal treatments at high temperature and/or direct contact with combustion gases, such as smoking, toasting, roasting or grilling.

Smoking is a processing technique in which meat is exposed directly to wood smoke which may be generated by a variety of methods [3]. Smoked products have traditionally received special attention because considerable amounts of PAH have been detected [2].

The direct exposure of meat products to smoke brings about formation of higher concentrations of PAHs in meat as compared to indirect methods. However, concentration of PAHs in processed meat decreases with time due to photo-degradation and interactions that take place in the meat [4].

Also, the nature of the food including water and fat content also affect the formation of PAHs on heating. On heating of proteins, many nitrated PAHs are formed, some of which are highly carcinogenic and are believed to act as direct mutagens, while other PAHs may play the role of carcinogens or activators. Reports have shown that exposure of human body to PAHs may induce some fatal diseases such as lung and skin cancers (Chen, 2007). Some researchers has also direct link of some set of PAHs to a lot of major health problems, such as cataracts, kidney and liver damage, as well as jaundice (Dahle., *et al*. 2003).

The aim of this study was to report the concentration levels and distribution of PAHs in fresh, roasted and grilled commonly consumed meat and to estimate the potential health risk associated with its consumption.

Materials and Methods

Sampling

Samples of fresh, suya and asun meat were obtained from twenty four randomly selected sales spots in osogbo metropolis, Nigeria. All fresh, roasted (suya) and grilled (asun) samples were deboned and cut in small pieces. An aliquot of the sample obtained (about 100 g) was lyophilized, milled, packed in aluminum foil wraps and stored in the freezer at -20°C prior to analysis.

Extraction of food samples for PAH determination

Homogenized sample (2 g) each was thoroughly mixed with anhydrous Na_2SO_4 salt to absorb moisture and then extracted with of analytical grade dichloromethane (CH_2Cl_2). The dichloromethane extract was cleaned up by passing through a column packed with anhydrous Na_2SO_4. The resulting extract was concentrated on a rotary evaporator to give an oily residue which was again dissolved in 1ml CH_2Cl_2. 1µL was injected into the GC for analysis.

GC-FID analysis

Determination of PAHs was carried out using Agilent GC 7890A. Chromatographic separation of the sixteen PAHs was accomplished within a DB-1 fused-silica capillary column (30m x 0.32 mm I.D, I µm film thickness). Helium was used as a carrier gas at a flow rate of 0.45 mL/min. Sample injection was in the split less mode with an injection volume of 2 µL. The GC oven temperature program was optimized: initial temperature at 70^{0}C held for 2 min and then ramped at 10°C/min to 220°C. It was maintained at this temperature till the end of the run (33 min). The injection port temperature was set at 250°C and that of the FID detector was set at 300°C. The hydrogen and air flow rates were set at 40 mL/min and 400mL/min respectively.

The identification of PAHs was based on comparison of the retention times of the peaks with those obtained from standard mixture of PAHs (standards supplied by instrument manufacturer). Quantification was based on external calibrations curves prepared from the standard solution of each of the PAHs.

Quality control and assurance

Since a suitable reference material with certified content of the target analytes (to test the accuracy of the method) was not available for the meat, a spiking procedure was used to calculate recoveries. A 10 g aliquot of lyophilized raw meat with a low amount of naturally occurring PAHs, was weighed in a round flask and slurred with 20 mL of *n*-hexane containing a known amount of PAH standard mixture. The mixture was efficiently mixed using a rotary evaporator without using the vacuum supply. The solvent was left to evaporate slowly under continuous stirring for 2 hours. After solvent removal the sample was left for 3 days in the darkness before the recoveries (mean of 3 replicate analyses) were calculated by comparing the difference between spiked and unspiked sample with the known amount of PAHs added. Mean recoveries ranging from 78 to 108% was obtained.

Estimation of carcinogenic potency

Carcinogenic potency associated with exposure to a given PAH compound were evaluated using an index of BaP concentration (BaPeq) [5]. Benzo(a) pyrene is the only PAH for which sufficiency carcinogenic toxicology data exist and as such it is used to determine the carcinogenic potency factor of other PAHs. The total toxicity BaPeq for other PAHs was calculated using the following equation:

$$\text{Total BaPeq} = \sum_i C_i \times TEF_I$$

where C_i is the of concentration of some known individual carcinogenic PAHs (BaA, BaP, BbF, BkF, InP and Chr) and Toxicity Equivalent Factor (TEF_i) is their corresponding toxicity equivalency factor of a PAH [6,7].

Statistical analysis

The data thus obtained was subjected to Statistical analysis using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for descriptive and in-

ferential statistics viz; correlation matrix, cluster analysis, T-test and Anova. The cluster analysis was carried out using the complete linkage groupings of the hierarchical cluster dendogram. This was done to group the analyzed PAHs into families. The data were analyzed using T-test to know whether there were significant difference between the concentrations of each PAH at the different sites and samples. Levels of parameters were considered significant if T-test value was less than 0.05. Using the correlation table, correlation coefficients of 0.712 were considered significant for 16 samples and this was done to ascertain if the PAHs actually originated from similar sources/chemical affinity.

Results and Discussion

The average concentrations of all sixteen analyzed prioritized PAH compounds in the fresh and barbequed meat (asun and suya) of some sites within osogbo metropolis are shown in Table 1. Sixteen criteria PAHs namely; Naphthalene (Nap), Acenapthylene (Acy), Acenapthene (Ace), Fluorene (Flu), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flu), Pyrene (Pyr), Benzo(a)anthracene (BaA), Chrysene (Chr), Benzo(b) fluoranthene(BbF), benzo(k) fluoranthene (BkF), Benzo(a)pyrene (BaP), Indeno(1,2,3-cd)pyrene (Ind), Dibenzo(a,h)anthracene (DahA), Benzo(g,h,i)perylene (BgP).

PAHs	Concentration (µg/L)		
	Asun meat	Suya meat	Fresh meat
Nap	0.006 ± 0.001	0.017 ± 0.010	0.0001 ± 0.00001
Acy	0.002 ± 0.003	0.039 ± 0.010	0.0002 ± 0.00001
Ace	0.039 ± 0.001	0.055 ± 0.010	0.0002 ± 0.00002
Flu	0.129 ± 0.020	0.085 ± 0.002	0.0002 ± 0.00001
Phe	0.878 ± 0.001	1.88 ± 0.020	0.0003 ± 0.00004
Ant	6.070 ± 0.320	9.17 ± 0.500	0.0003 ± 0.00001
Fla	6.820 ± 0.100	8.39 ± 1.100	0.0002 ± 0.00001
Pyr	0.749 ± 0.002	9.11 ± 1.500	0.0001 ± 0.00005
BaA	0.132 ± 0.001	0.142 ± 1.000	0.0003 ± 0.00001
Chr	0.064 ± 0.001	0.087 ± 2.500	0.0002 ± 0.00001
BbF	0.011 ± 0.005	0.014 ± 1.000	0.0002 ± 0.00002
BkF	0.008 ± 0.001	0.009 ± 0.500	0.0002 ± 0.00001
BaP	0.110 ± 0.050	0.152 ± 0.200	0.0002 ± 0.00001
InP	0.001 ± 0.001	0.002 ± 0.050	0.0003 ± 0.00002
DaA	0.005 ± 0.001	0.009 ± 0.100	0.0004 ± 0.00001
BgP	0.010 ± 0.002	0.006 ± 0.020	0.0001 ± 0.00001
Total	15.034 ± 5.050	27.167 ± 7.100	0.0035 ± 0.00003

Table 1: Concentrations of the individual PAHs in the samples (µg/L).

The PAH composition in the processed asun and suya meat samples was dominated by varied molecular weight fraction and fresh meat showed relatively similar PAHs profile. The dominant PAH detected in the fresh meat sample before processing were Nap, Chr, Pyr, Ace, BbF, BkF and Flu. The average concentrations of most abundant individual PAHs found in asun beef were Ant (6.07 µg g^{-1}), Pyr (7.49 µg g^{-1}), BaA (0.13 µg g^{-1}), BaP (0.11 µg g^{-1}) Flu (6.82 µg g^{-1}), Phen (0.88 µg g^{-1}) while that of suya meat were Phen (1.58 µg g^{-1}), Ant (9.17 µg g^{-1}), Flu (8.39 µg g^{-1}} and Pyr (9.11 µg g^{-1}) as shown in table 1. These sets of PAHs observed are as a result of combustion of coal and biomass [8].

According to the Agency for Toxic Substances and Disease Registry [9], levels of the sum of PAHs between 0 and 0.1 µg g^{-1} are considered as slight contamination whereas values that fall within the range of 0.1 to 1 µg g^{-1} is an indication of significant contamination. In this study, the total concentration of PAHs in barbequed samples

investigated ranged from 0.001 to 26.82 µg g^{-1} for asun meat and 0.002 to 9.17 µg g^{-1} for suya meat. These values were comparatively higher than what was recorded for fresh meat samples (0.0001 to 0.0004 µg g^{-1}).

Sources of Polycyclic Aromatic Hydrocarbons (PAHs) detected in samples

Some diagnostic ratios are known and used to provide accurate and reliable estimations of the emission sources of PAHs in environmental samples (Guo., *et al.* 2010). Anth/(Anth + Phen) ratios of 0.9 ± 0.01 and 0.8 ± 0.04 were observed for asun and suya meat respectively. These ratios are all greater than 0.1, thus indicating that the PAHs in the selected sites came from a pyrolytic source [10]. This is further confirmed by their Phen/Anth ratios of 0.1 ± 0.03 and 0.2 ± 0.08, which were also less than 10. Usually a Phen/Anth ratio less than 10 indicates a pyrolytic source suggesting that the PAHs detected from the barbequed samples (asun and suya) originated from the roasting process which is as a result of combustion [5]. Even though the two-to-three-ring PAHs are yet to be classified as to their carcinogenicity to humans, their metabolites or derivatives can be potent mutagens [9].

The concentration of total BaPeq in the fresh, asun and suya meat respectively were 0.00007 µg g^{-1}, 0.0352 µg g^{-1} and 0.0312 µg g^{-1}. Asun meat exhibited relatively higher BaPeq than suya meat, implying a high carcinogenic burden from anthropogenic sources.

Data analysis

Correlation matrixes

The results of the correlation matrixes for the 16 PAHs in the barbequed meat using their concentration as variables are presented in Table 2.

	Nap	Acy	Ace	Flu	Phe	Ant	Fla	Pyr	BaA	Chr	BbF	BkF	BaP	InP	DbA	BgP
Nap	1															
Acy	0.979772	1														
Ace	0.988169	0.998872	1													
Flu	0.999313	0.986514	0.993174	1												
Phe	0.756903	0.872375	0.848178	0.780599	1											
Ant	0.888858	0.962569	0.948612	0.905225	0.972214	1										
Fla	0.821011	0.918654	0.898857	0.841602	0.994532	0.991345	1									
Pyr	0.953538	0.99454	0.988462	0.964047	0.918625	0.985598	0.954866	1								
BaA	0.997894	0.990688	0.996036	0.999612	0.797697	0.916704	0.856312	0.971071	1							
Chr	0.987121	0.93514	0.950909	0.980516	0.642609	0.804114	0.719108	0.893063	0.974667	1						
BbF	0.89083	0.963731	0.949971	0.907053	0.971194	0.999991	0.990768	0.986319	0.918423	0.806676	1					
BkF	0.70969	0.836321	0.809343	0.735308	0.997586	0.95361	0.984879	0.888967	0.753891	0.587847	0.9523	1				
BaP	0.767844	0.880515	0.857012	0.791054	0.999857	0.976033	0.996156	0.925174	0.807779	0.655471	0.975083	0.996269	1			
InP	0.598767	0.746935	0.714518	0.628033	0.976634	0.899188	0.948851	0.812243	0.649455	0.46293	0.897289	0.989201	0.972861	1		
DbA	0.999644	0.974085	0.983727	0.997969	0.739201	0.87632	0.80549	0.945162	0.995809	0.991037	0.878393	0.690644	0.750481	0.577189	1	
BgP	-0.20937	-0.00945	-0.05692	-0.17299	0.480573	0.261931	0.386366	0.094956	-0.1455	-0.3631	0.257758	0.540315	0.465678	0.65781	-0.23538	1

Table 2: Pearson correlation matrix of the Polycyclic Aromatic hydrocarbons analyzed in the barbequed meat (asun and suya) samples

n=6, α=0.05, r=0.3 at 95% confidence interval, positive and significant correlation are presented in bold type.

Strong and positive correlation existed among most of the PAHs, especially those with higher molecular weights, thus suggesting that they originated from a similar source and/or having similar properties The relationship between levels of pyrene/benzo(a)anthracene, fluoranthene/anthracene and pyrene/chysene was shown to be significantly correlated in the barbequed meat indicating that the PAHs in the processed meat had presumably come from similar sources and could be related to incomplete combustion [10,11].

Cluster analysis

The cluster analysis was used to establish different groupings within the given suite of parameters in order to confirm if these PAHs have similar sources and/or chemical affinity.

The PAHs cluster results for the processed meat (asun and suya) indicates four major groups viz; (InP, DbA, BgP, NaP, Acy, BkF, Ace, Pyr, Flu, Phe and Fla), (BaA, BbF and BaP), Ant and Chr respectively. It was observed that even the groups are also fairly well correlated as they are majorly a product of incomplete combustion.

Analysis of variance (Anova)

Anova was used to evaluate the differences in the mean concentration level of individual PAH in the analyzed processed meat. The result implies that there are significant difference between the concentrations of analyzed PAH analyzed in the suya and asun meat as seen in table 3.

T-test analysis

A marked difference in the concentration of the PAHs in the set of the samples (asun and suya meat) was observed. The results of the T – test indicate that there are significance differences between the PAHs mean concentrations of the asun meat and suya meat analyzed at different locations.

Groups	Count	Sum	Average	Variance		
Nap	2	0.023	0.0115	6.05E-05		
Acy	2	0.041	0.0205	0.000685		
Ace	2	0.094	0.047	0.000128		
Flu	2	0.214	0.107	0.000968		
Phe	2	2.758	1.379	0.502002		
Ant	2	15.24	7.62	4.805		
Fla	2	15.21	7.605	1.23245		
Pyr	2	9.859	4.9295	34.95316		
BaA	2	0.274	0.137	5E-05		
Chr	2	0.151	0.0755	0.000264		
BbF	2	0.025	0.0125	4.5E-06		
BkF	2	0.017	0.0085	5E-07		
BaP	2	0.262	0.131	0.000882		
Inp	2	0.003	0.0015	5E-07		
DaA	2	0.014	0.007	0.000008		
BgP	2	0.016	0.008	0.000008		
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	223.2624	15	14.88416	5.739071	0.000613	2.352223
Within Groups	41.49567	16	2.593479			
Total	264.7581	31				

Table 3: ANOVA of PAHs levels in the barbequed (asun and suya) meat

PAH	$t_{calculated}$	Remark
Nap	2.055	SD
Acy	1.108	SD
Ace	5.875	SD
Flu	4.864	SD
Phe	2.752	SD
Ant	4.916	SD
Fla	9.688	SD
Pyr	1.179	SD
BaA	27.400	SD
Chr	6.565	SD
BbF	8.333	SD
BkF	17.000	SD
BaP	6.238	SD
InP	3.000	SD
DaA	3.500	SD
BgP	4.000	SD

Table 4: T-test result of PAHs Concentration in the barbequed meat (asun and suya) at different area.

$T_{critical}$ = 2.13, n = 6 and SD = Significant difference.

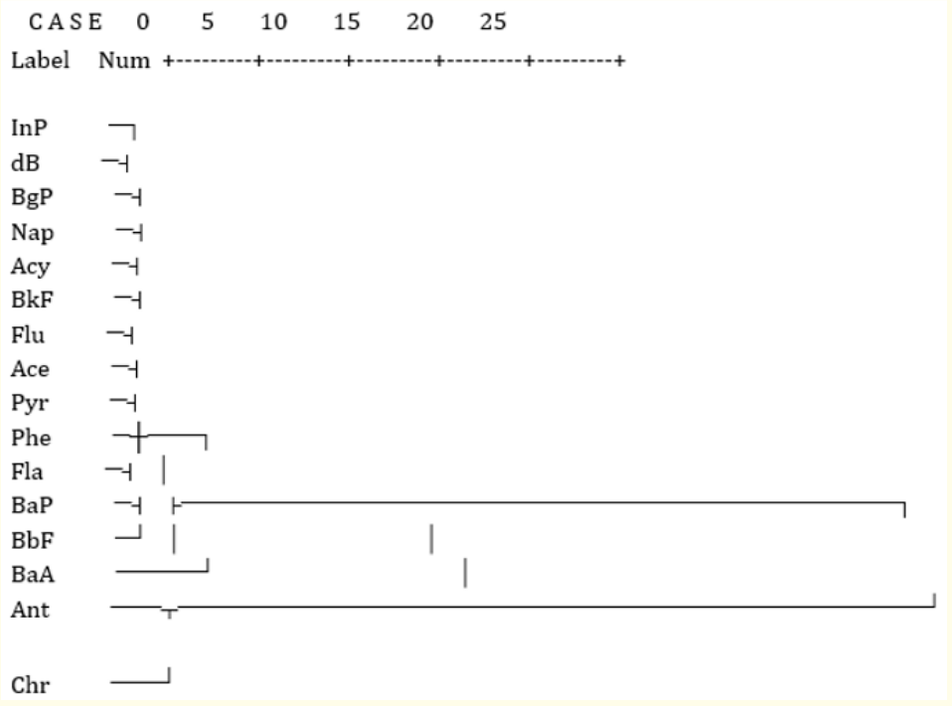

Figure 1: Dendogram showing the hierarchical cluster analysis of the analyzed Polycyclic Aromatic Hydrocarbon in the barbequed meat of the studied area.

PAH	$t_{calculated}$	Remark
Nap	2.055	SD
Acy	1.108	SD
Ace	5.875	SD
Flu	4.864	SD
Phe	2.752	SD
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BaA	27.400	SD
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BbF	8.333	SD
BkF	17.000	SD
BaP	6.238	SD
InP	3.000	SD
DaA	3.500	SD
BgP	4.000	SD

Table 4: T-test result of PAHs Concentration in the barbequed meat (asun and suya) at different area.

$T_{critical}$ = 2.13, n = 6 and SD = Significant difference.

Conclusion

Smoking and/or grilling usually involve direct contact with wood combustion fumes. This is responsible for high contamination levels with carcinogenic polycyclic aromatic hydrocarbons (PAHs). The result of this study indicates that the barbequed (asun and suya) meat after preparation has high level of pyrene, fluoranthene, anthracene, and phenanthrene than other constituents due to the continuous exposure to fire during grilling.

The PAH distribution profile in the asun and suya meat analyzed indicated significant contamination with PAHs. The average total concentrations of PAHs were 0.0035 ± 0.001, 15.034 ± 5.050 and 27.167 ± 7.100 for fresh, asun and suya meat respectively. Asun and suya meat showed values higher than the recommended tolerable limit of 0.1 - 1.0 µg l^{-1} set by the ATSDR for significantly contaminated food. Ratio of Anth/(Anth + Phen) was 0.9 ± 0.01 and 0.8 ± 0.04 for asun and suya meat respectively. These ratios indicates pyrolytic source of contamination since the values are all greater than 0.1 [10]. This assertion is further confirmed by the Phen/Anth ratios of 0.1 ± 0.03 and 0.2 ± 0.08, which were also less than 10.

Asun meat (0.0352 µg g^{-1}) exhibited a relatively higher BaPeq than suya meat (0.0312 µg g^{-1}), thereby implying a high carcinogenic burden from anthropogenic sources.

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