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Levels of PAHs in Commonly Consumed Barbecued Chicken and Grilled Meat (Suya) in Benin Metropolis

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Abstract
Reports from most health institutions across the country has increased public awareness on the danger of consumption of grilled meat products. The use of charcoals in the preparation of grilled chicken and cow meat (suya) for consumption leaves many residues and contaminants of which polycyclic aromatic hydrocarbons (PAHs) is of major concern. This study investigated the concentrations of PAHs in grilled Chicken and Cow meat (Suya) obtained from five local producers within Benin Metropolis. The PAHs value in the grilled and barbecued meat samples were examined in the laboratory using a GC/HPLC. The result revealed that eight of the most carcinogenic congeners were present. The concentrations of Indeno(1,2,3-cd)pyrene, Dibenzo(a,h)anthracene, Fluoranthene and Benzo(g,h,i)perylene were: 529.92, 1710.8, 562.27, and 763.33µg/kg respectively. PHAs present in the samples were all greater than 5.0µg/kg on the average of the total contamination profile, with dibenzo(a,h)anthracene and benzo(g,h,i)perylene occurring with the highest concentrations (mean contents of 1710.8 and 763.33µg/kg respectively). Irrespective of the sample type analyzed, grilled chicken was potentially more risky, since total PAHs contents for chicken was generally higher as compared to grilled cow meat samples. Significant to the findings of this research is that PAHs levels were far above the European Union recommended level of 5µg/kg in smoked/ grilled meat. It is recommended that the consumption of grilled chicken should be minimized.

Keywords: Grilled meat, Barbecued chicken, PAHs, Benin metropolis

Introduction
Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental contaminants that emanate from incomplete combustion of fuel or high temperature pyrolysis of fats and oils. It is well known that PAHs occur in curing smoke [1] and that they accumulate on meat products being smoked [2] Many studies have demonstrated that carcinogenic PAHs are present in food, being formed through the grilling and smoking of foods [3, 4, 5];.

PAHs are produced in the processed and cooked food as a consequence of organic matter combustion during cooking and smoking process and also direct deposition of PAHs from produced smoke through incomplete combustion of different thermal agents [7, 8]. consumption of contaminated food has been identified as a principal route of human exposure to PAHs as it contributes about 88 to 98% of such contamination (9, 10, 11]. Based on Al-Rashdan et al. [12], exposure of PAHs to human does not present individually, but in complex mixtures of PAHs. , several studies have been carried out evaluating PAHs in processed and cooked food, especially in grilled and smoked food [13, 14, 15, 7, 8].. Recently, Omwukeme et al. [11] reported that PAHs concentration was detected as high as 200 µg/kg of individual PAH in smoked fish and meat. Jahurul et al. [16] has also measured the sum of three PAHs (fluoranthene, benzo(b)fluoranthene, benzo(a) pyrene) and reported that the level of PAHs in beef satay (66.28 ng/g), chicken satay (42.31 ng/g), charcoal grilled fish (40.69 ng/g), mutton satay (30.76 ng/g) and chicken barbecued (17.86 ng/g) [17]

Reink et al., [13] studied the PAHs in meat products and estimated PAH intake by children and the general population in Estonia PAH concentrations detected were 16 µg kg-1 in smoked meat and ham, 19 µg kg-1 in smoked sausage and 6.5 µg kg-1 in smoked chicken samples. In Nigeria, smoking and grilling are prevalent meat cooking methods .The aim of this research was to investigate the levels of pahs in commonly consumed barbecued chicken and grilled meat (suya) in Benin metropolis.

Materials and Methods
Sampling
Grilled meats and barbecued chicken commonly consumed in Benin city, were purchased from local vendors (Sakponba road, Megon-Isihor, Ekewan Road, University of Benin) in Benin city, Edo state Nigeria. Six (6) pieces each of grilled meats and barbecued chicken making a total of 12 pieces from different vendors were pooled together to obtain representative samples for each types of meat and chicken analysed. A small portion

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of each of the representative sample so obtained (10g) was milled, packed in aluminium foil wraps and stored in the freezer at -20ºC before analysis.

Extraction

Extraction of PAHs was carried out based on the method described by Pena *et al.,* [18]10 g of the homogenized chicken and suyua meat samples was thoroughly mixed with anhydrous Na_2SO_4 to dehydrate the sample. 20 ml of the extraction solvent (di-chloromethane) was added to the sample. Samples were covered with aluminium foil to prevent evaporation and sonicated to separate supernatants of extracts. Extracts were concentrated using an evaporator. Extracts were then cleaned up using a chromatographic column, moderately packed at the bottom with 1 cm glass wool. 2 g of silica gel and 1 cm of anhydrous Na_2SO_4 was added to the column while the column was pre-eluted with 20 ml dichloromethane. Extracts were then concentrated and collected in 2 ml vials.

Chromatographic analysis

Chromatographic analysis was carried out based on the method described by [19] .The cleaned up extracts were analysed for naphthalene, acenaphthylene, benzo[b]fluoranthene, phenanthrene, dibenzo[a,h]anthracene, chrysene, benzo[a]pyrene, acenaphthene, benzo[k]fluoranthene, fluorene, pyrene, benzo[a]anthracene, anthracene, fluoranthene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i]perylene. Corresponding results were obtained using Gas chromatography (GC, Hewlett-Packard HP-5890 Series II with flame ionization detection (GC-FID)). The GC was programmed as follows: initial temperature of 60 °C for 2 min and ramped at 25 °C/min to 300 °C for 5 min and allowed to stay for 15 min giving a total of run time of 22 mins. A 2 µL volume splitless injection mode was used and the injection port temperature was set at 250 °C, while 300 °C was maintained for the injection port of the FID detector. A standard mixture of 17 priority PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno(1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene) was obtained and used for the analysis. Compounds were identified by comparing the retention time of standards with that obtained from the extracts and individual analysis of PAHs were used for quantitation.

Results

In this study, six (6) samples of grilled chicken, and six (6) samples of cow meat (suya) were analyzed and the concentrations of PAHs were determined. The PAHs level of the six samples each of grilled (barbecued) chicken and cow meat from Benin City, Edo State of Nigeria were far above the European Commission Standard. Samples of grilled chicken obtained from technical school road market contained indeno(1,2,3-cd)pyrene in variable concentrations. On the basis of the results it was found that grilled (barbecued) chicken contained Indeno(1,2,3-cd)pyrene in concentrations substantially higher (529.92 ug/kg) than other PAHs found. Grilled Cow Liver had the least (115.99 ug/kg) Indeno (1,2,3-cd)pyrene concentration.

Concentration of Indeno(1,2,3-cd)pyrene in grilled chicken

Clearly analyzed samples contained Indeno(1,2,3-cd)pyrene concentration above the European Commission permitted maximum limit of 5µk/kg. The highest content of Indeno(1,2,3-cd)pyrene was detected in grilled chicken (789.39ug/kg), the lowest, in grilled cow liver (115.99ug/kg.

Concentration of Dibenzo(a,h)anthracene in various samples.

The concentrations of dibenzo(a,h)anthracene in grilled chicken ranges between 1710.8 µg/kg to 363.01 µg/kg. On the basis of obtained results it was found that grilled chicken from Sakponba road market contained dibenzo(a,h)anthracene in concentrations substantially higher (1710.8 µg/kg) in comparison with the other samples. The mean concentrations in grilled chicken from technical school road, grilled chicken (Sakponba Road), grilled cow liver (Megon-Isihor), Cow kidney (Uniben), grilled cow meat (Megon-Isihor), and grilled cow meat (Ekehwan Road) were 363.01µg/kg, 1710.8µg/kg, 86.625µg/kg, 504.26µg/kg, 796.39µg/kg, and 412.4µg/kg respectively. The highest content of dibenzo(a,h)anthracene was detected in grilled Chicken (Sakponba Road) 1710.8µg/kg, the lowest; in grilled Cow liver (86.625ug/kg). Napthalene, Acenaphthylene, Acenophthene, Anthracene, Pyrene, 1,2-Benzothracene and Chysene was not found+.

Concentration of Fluoranthene in cow liver-megon-isihor.

The statistical analysis of the Fluoranthene content indicated that there was significant difference across the various PAHs contaminants. The fluoranthene content ranged from 0.00 µg/kg in naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene and Benzo(b)fluoranthene to (562.27 µg/kg) in grilles cow liver Megon-Isihor. However, Fluoranthene had the highest concentration in grilled cow liver from Megon-Isihor.

Concentration of Phenanthrene in various samples

The phenanthrene content presented in table 4 below varied significantly among grilled samples of Cow kidney (Uniben). The phenanthrene content ranged from a minimum of 59.647 µg/kg in grilled Cow kidney and maximum value of 216.51 µg/kg in chicken from Sakponba market. However, phenanthrene was not found in grilled Cow liver from Magon- Isihor. Chicken (Sakponba), Cow meat (Ekehwan), and Suya (Megon) recorded the highest phenanthrene levels in sequence**.**

Concentration of pyrene in various samples

Pyrene content was slightly higher in the samples of cow meat as compared to the samples of Chicken. Table 5 below shows that the result was significantly different (389.16 µg/kg) from the other samples but not cow meat from Ekehwan Road (Table 6). The pyrene content ranged from the least 49.59 ug/kg in Cow kidney and maximum of 389.16 µg/kg in Cow meat from Megon-Isihor. However, Pyrene was absent in Chicken technical school road. Analysis based on comparative examination with the EU standard showed that all the recorded values of pyrene in various samples were within safety limit**.**

Concentration of Benzo(g,h,i)perylene in grilled cow meat from Ekehwan.

The statistical analysis of Benzo(g,h,i)perylene content indicated that there was significant difference across the various PAHs contents in cow meat from Megon-Isihor. Analyzed sample contain Benzo(g,h,i)perylene concentrations that were above the EU permitted maximum limit (Table 6). It ranged from a minimum of 95.39.µg/kg in cow liver-Megon Isihor to maximum of 763.33µg/kg in Cow meat Ekehwan. However, Benzo(g,h,i)perylene was also found to be the highest concentration of PAHs in sample 2 chicken from Sakponba road. Naphthalene and Benzo(b)fluoranthene was not found.

The PAHs content of grilled Chicken and cow meat samples in Benin city are not heartwarming, Indeno(1,2,3cd)pyrene, Benzo(a)pyrene, Fluoranthene, Dibenzo(a,h)anthracene, and Benzo(k)fluoranthene were all higher above 5ug/kg except Benzo(k)fluoranthene. These are all precarious chemicals for the destruction of human life: they are carcinogenic. In addition o the lower PAHs levels across the various samples, the levels were a bit below the safety limits. Most PAHs get into the human system through either direct or indirect ingestion. These PAHs at certain significant concentrations can be very risky by the consumption of man. For instance Indeno(1,2,3-cd)pyrene, Benzo(g,h,i)perylene and Dibenzo(a,h)anthracene which are present in grilled chicken and cow meat samples inhibits and promotes the abnormal functioning of the body nerves and muscles

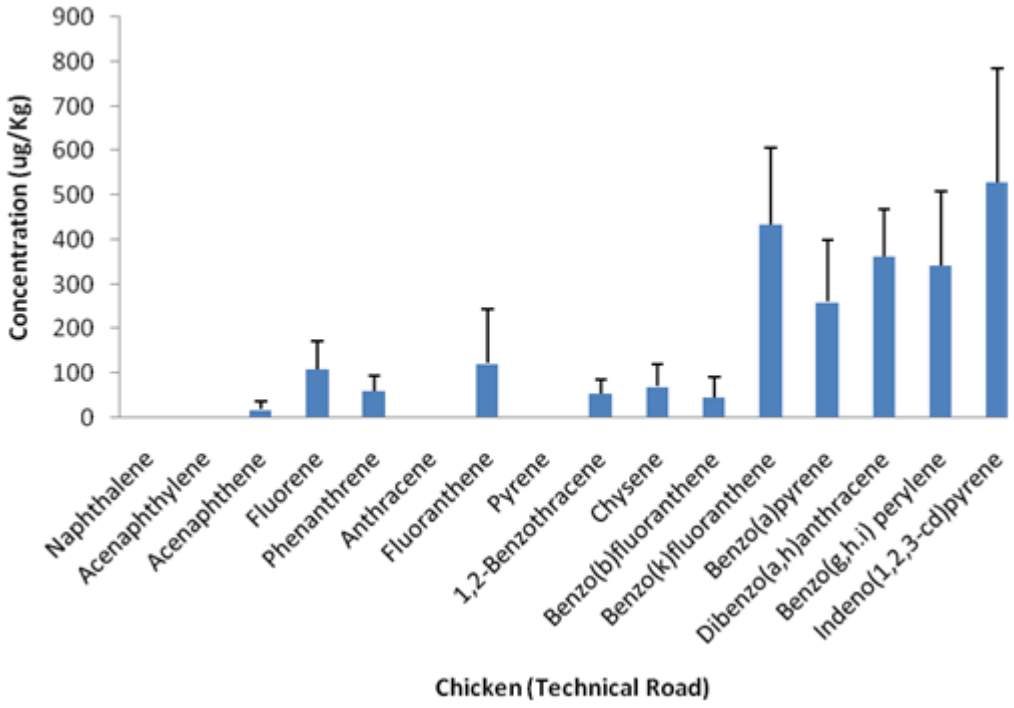

Figure 1: Various PAHs contaminants

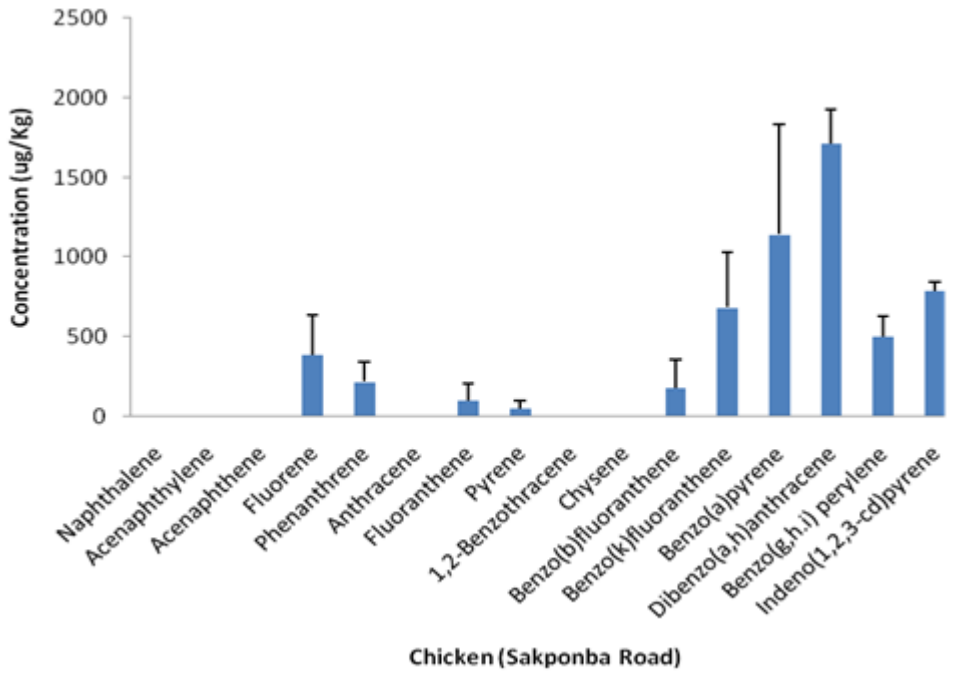

Figure 2: Levels of PAHs in grilled Chicken.

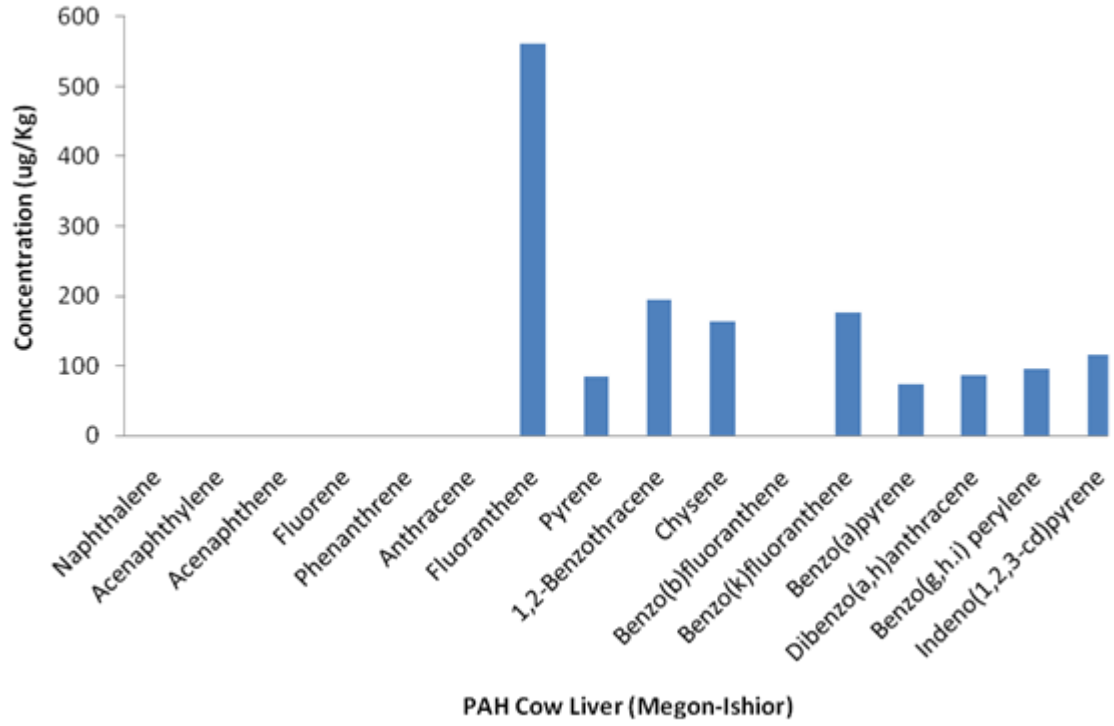

Figure 3: Levels of PAHs in grilled cow liver**.**

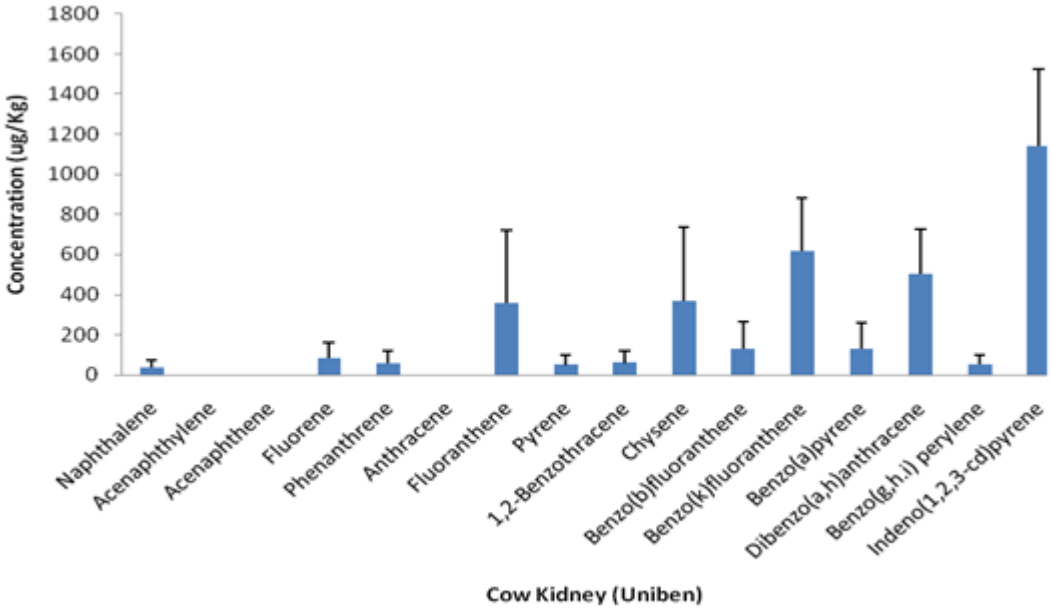

Figure 4: Levels of PAHs in grilled Cow kidney.

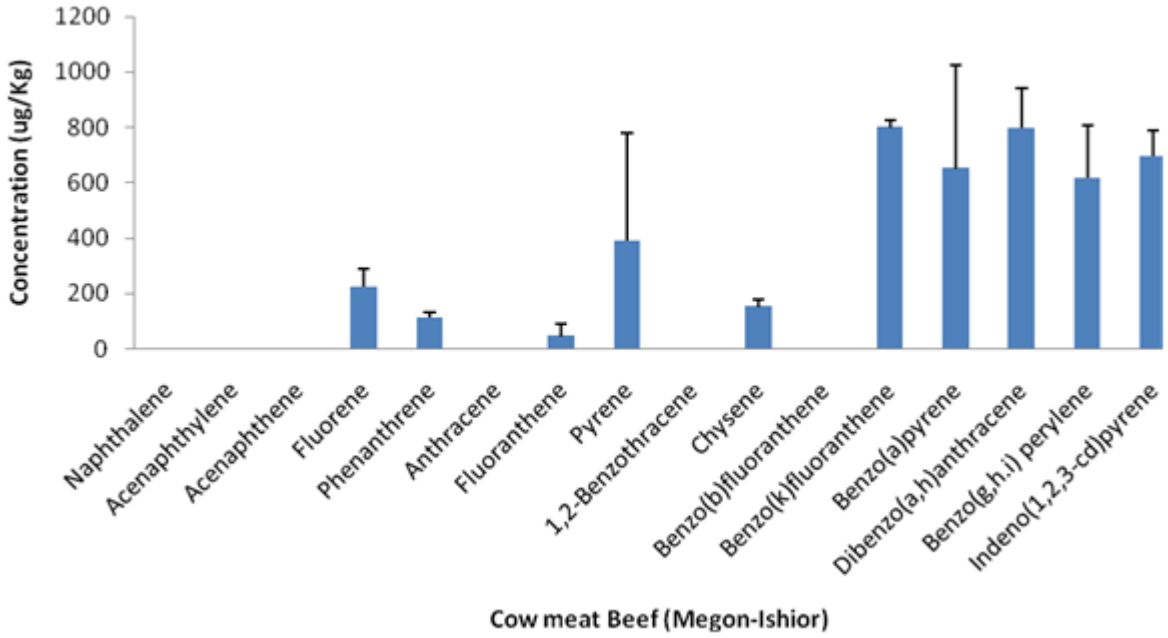

Fig 5: Various concentrations of PAHs in Cow meat (suya) Megon-Isihor

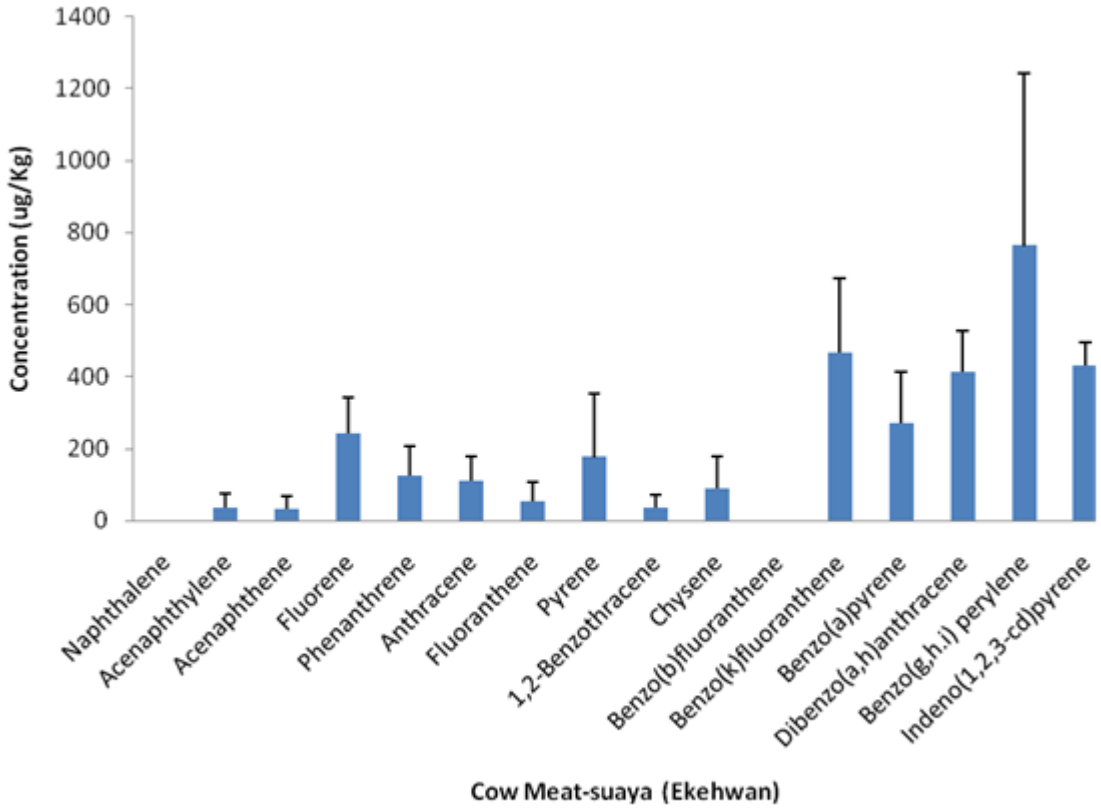

Fig 6: Various concentrations of PAHs in Cow meat (Ekehwan Road).

Concentration of Indeno(1,2,3-cd)pyrene in grilled chicken

Clearly analyzed samples contained Indeno(1,2,3-cd)pyrene concentration above the European Commission permitted maximum limit of 5µk/kg. The highest content of Indeno(1,2,3-cd)pyrene (Table 2) was detected in grilled chicken (789.39ug/kg), the lowest, in grilled cow liver (115.99ug/kg).

This result clearly indicates that the production of grilled chicken (barbecued) with Indeno(1,2,3-cd)pyrene level less than ---µg/kg is possible in non-intensely grilled chicken. Taking into account properties of Indeno(1,2,3-cd)pyrene, the EU recommended that the Indeno(1,2,3,-cd)pyrene content in grilled chicken should be as low as reasonably achievable (ALARA).

Table 2: Sample of grilled chicken obtained from technical school road showing Indeno(1,2,3-cd)pyrene level.

PARAMETERS	MEAN±SE	MIN	MAX	RANGE
Naphthalene	0±0	ND	ND	ND
Acenaphthylene	0±0	ND	ND	ND
Acenaphthene	18.284±18.284	ND	73.136	73.136
Fluonere	108.25±62.94	ND	234.72	243.72
Phenanthrene	59.149±35.044	ND	137.56	137.56
Anthracene	ND	ND	ND	ND
Fluoranthene	122.1±122.1	ND	488.39	488.39
Pyrene	ND	ND	ND	ND
1,2-Benzothracene	54.966±31.735	ND	110.19	110.19
Chysene	70.077±49.077	ND	208.2	208.2
Benzo(b)fluoranthene	46.187±46.187	ND	184.75	184.75
Benzo(k)fluoranthene	433.82±172.43	79.226	88799	808.76
Benzo(a)pyrene	259.82±139.19	67.558	661.64	594.08
Dibenzo(a,h)anthracene	363.01±104.02	103.76	606.26	502.5
Benzo(g,h,i)perylene	340.96±166.37	91.577	818.55	726.98
Indeno(1,2,3-cd)pyrene	529.92±253.63	175.24	1268.5	1093.2

Concentration of Dibenzo(a,h)anthracene in various samples.

The various concentrations of Dibenzo(a,h)anthracene in grilled chicken ranges between 1710.8 µg/kg to 363.01 µg/kg (Table 3). On the basis of obtained results it was found that grilled chicken from sakponba road market contained Dibenzo(a,h)anthracene in concentrations substantially higher (1710.8 µg/kg) in comparison with the other samples. The main concentrations for grilled chicken from technical school road, grilled chicken sakponba road, grilled cow liver megon-isihor, cow kidney uniben, grilled cow meat megon-isihor, and grilled cow meat ekehwan were (363.01µg/kg), (1710.8µg/kg), (86.625µg/kg), (504.26µg/kg), (796.39µg/kg), and (412.4µg/kg) respectively.

The highest content of Dibenzo(a,h)anthracene was detected in grilled chicken-sakponba road (1710.8µg/kg), the lowest; in grilled cow liver (86.625ug/kg) (Table 3). Napthalene, Acenaphthylene, Acenophthene, Anthracene, Pyrene, 1,2-Benzothracene and Chysene was not found.

Table 3: Sample of grilled chicken obtained from Sakponba road.

PARAMETERS	MEAN±SE	MIN	MAX	RANGE
Naphthalene	ND	ND	ND	ND
Acenaphthylene	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND
Fluonere	385.85±251	134.84	636.82	501.99
Phenanthrene	216.51±125.55	90.95	342.06	251.11
Anthracene	ND	ND	ND	ND
Fluoranthene	101.25±101.25	ND	202.5	202.5
Pyrene	49.232±49.232	ND	98.463	98.463
1,2-Benzothracene	ND	ND	ND	ND
Chysene	ND	ND	ND	ND
Benzo(b)fluoranthene	176.24±176.24	ND	352.48	352.48
Benzo(k)fluoranthene	682.24±349.23	333.01	448.32	698.46
Benzo(a)pyrene	1141.6±693.28	448.32	1834.9	1386.6
Dibenzo(a,h)anthracene	1710.8±216.7	1494.1	1927.5	433.41
Benzo(g,h,i)perylene	500.31±128.2	372.11	628.51	256.4
Indeno(1,2,3-cd)pyrene	789.39±52.504	736.88	841.89	105.01

Concentration of Fluoranthene in cow liver-megon-isihor.

The statistical analysis of the Fluoranthene content indicated that there was significant difference across the various PAHs contaminants. The fluoranthene content ranged from 0.00 µg/kg in naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene and Benzo(b)fluoranthene to (562.27 µg/kg) in grilles cow liver Megon-Isihor. However, Fluoranthene had the highest concentration in grilled cow liver from Megon-Isihor.

Table 4: PAHs in grilled Cow liver obtained from Megon –Isihor.

PARAMETERS	MEAN±SE	MIN	MAX	RANGE
Naphthalene	ND	ND	ND	ND
Acenaphthylene	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND
Fluorene	ND	ND	ND	ND
Phenanthrene	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND
Fluoranthene	561.265±1.00	562.27	526.27	ND
Pyrene	83.802±0.6035	84.405	84.405	ND
1,2-Benzothracene	194.976±0.1554	194.82	194.82	ND
Chysene	163.719±0.424	164.14	164.14	ND
Benzo(b)fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	176.6796±0.18685	175.81	175.81	ND

Benzo(a)pyrene	72.787±1.0315	73.819	73.819	ND
Dibenzo(a,h)anthracene	85.755±0.8696	86.625	86.625	ND
Benzo(g,h,i)perylene	96.2425±0,8526	95.39	95.39	ND
Indeno(1,2,3-cd)pyrene	114.585±1.4083	115.99	115.99	ND

Concentration of Phenanthrene in various samples

The phenanthrene content presented in table 4 below varied significantly among grilled samples of Cow kidney (Uniben). The phenanthrene content ranged from a minimum of 59.647 µg/kg in grilled Cow kidney and maximum value of 216.51 µg/kg in chicken from Sakponba market. However, phenanthrene was not found in grilled Cow liver from Magon- Isihor. Chicken (Sakponba), Cow meat (Ekehwan), and Suya (Megon) recorded the highest phenanthrene levels in sequence**.**

Table 5: PAHs in grilled Cow kidney obtained from Uniben

PARAMETERS	MEAN±SE	MIN	MAX	RANGE
Naphthalene	37.137±37.137	ND	74.274	74.274
Acenaphthylene	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND
Fluonere	81.417±81.417	ND	162.83	162.83
Phenanthrene	59.647±59.647	ND	119.29	119.29
Anthracene	ND	ND	ND	ND
Fluoranthene	359.36±359.36	ND	718.73	718.73
Pyrene	49.59±49.59	ND	99.18	99.18
1,2-Benzothracene	60.023±60.023	ND	120.05	120.05
Chysene	369.21±369.21	ND	738.42	738.42
Benzo(b)fluoranthene	131.3±131.3	ND	262.6	262.6
Benzo(k)fluoranthene	618.1±264.48	353.61	882.58	528.97
Benzo(a)pyrene	130.11±130.11	NA	260.21	260.21
Dibenzo(a,h)anthracene	504.26±223.68	280.58	727.95	447.36
Benzo(g,h,i)perylene	50.598±50.598	ND	101.2	101.2
Indeno(1,2,3-cd)pyrene	1143±381.33	761.7	1524.4	762.66

Concentration of pyrene in various samples

Pyrene content was slightly higher in the two samples of cow meat as compared to the two different samples of Chicken. Table 5 below shows that the result was significantly different (389.16 µg/kg) from the other samples but not cow meat from Ekehwan Road (Table 6). The pyrene content ranged from the least 49.59 ug/kg in Cow kidney and maximum of 389.16 µg/kg in Cow meat from Megon-Isihor. However, Pyrene was absent in Chicken technical school road. Analysis based on comparative examination with the EU standard showed that all the recorded values of pyrene in various samples were within safety limit**.**

Concentration of Benzo(g,h,i)perylene in grilled cow meat from Ekehwan.

The statistical analysis of Benzo(g,h,i)perylene content indicated that there was significant difference across the various PAHs contents in cow meat from Megon-Isihor. According to table 6, analyzed sample contain Benzo(g,h,i)perylene concentrations that were above the EU permitted maximum limit. It ranged from a minimum of 95.39.µg/kg in cow liver-Megon Isihor to maximum of 763.33µg/kg in Cow meat Ekehwan. However, Benzo(g,h,i)perylene was also found to be the highest concentration of PAHs in sample 2 chicken from Sakponba road. Naphthalene and Benzo(b)fluoranthene was not found.

Discussion

Studies have shown that eating a charcoal broiled food may expose one to the same quantity of PAHs as one would received from smoking 600 cigarette [20]. The result of this study showed the various PAHs associated with grilled chicken(barbecued) and grilled cow meat (suya) which include: Fluoranthene, Indeno(1,2,3-cd)pyrene, Benzo(g,h,i)perylene, and Chysene, Benzo(k)fluoranthene, 1,2-Benzothrecene, Fluoranthene, Pyrene, Benzo(a)pyrene Indeno(1,2,3-cd)pyrene, Dibenzo(a,h,i)anthracene, Fluorenthene and Benzo(a,h,i)perylene were found in very high concentration ranged from 529.92µg/kg, 789.39µg/kg, 562.27µg/kg, and 763.33µg/kg respectively which is far above the EU standard of 5.0µg/kg. Benzo(a)pyrene was detected in all the samples in the range of 73.819-1141.6µg/kg. The minimum concentration of Benzo(a)pyrene (73.819µg/kg) was detected in grilled liver and maximum concentration of 1141.5µg/kg was detected in grilled chicken.

The high concentration of PAHs detected in theses samples could be as a result of its lipophytic factor (fat content) which penetrate into the tissue wall of the meat during grilling/smoking[21]. It can also be probably due to the fact that food were directly in contact with the flame [22]

In a study by Rey-Salgueiro *et al* [23]direct toasting (flame toasting), (coal grilling or gas oven toasting), and direct toasting (electric oven toasting) of bread samples were investigated to evaluate the level of PAHs. No sample obtained by electric oven and toaster were polluted, otherwise the samples toasted by charcoal and flame grilling presented very high levels and 350mg/g of total PAHs were detected in toasting samples by wood flame. In the study by Janoszka *et al.,* [24] Fluoranthene and benzo(a)pyren were found at concentration of 0.57ng/g and 0,15ng/g respectively, in grilled chicken breast on charcoal which were far less than what was obtained in this study. In addition Kazerouni *et al.,* [25]found that benzo(a)pyrene was present in 200 different meal dishes, of which very well grilled or babecued meat had the highest concentration of PAHs ranged from 0,04 to 1.15ng/g when compared to our result with the studies, chicken grilled on charcoal had higher concentration of PAHs.

Conclusion

Smoking and grilling are prevalent meat preparation methods in Nigeria, the impact of meat products is assumed to be significant in the overall PAHs intake. The PAHs intake values obtained in this study is much higher than the estimates by other studies.

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