Effects of Barbecuing on the Levels of Polycyclic Aromatic Hydrocarbons in Fish (Pseudotolitus Elongatus and Clarias Gariepinus)

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Abstract
The PAHs in fish samples Pseudotolitus elongatus (Kroka fish) and Clarias gariepinus (Catfish) processed by charcoal was investigated in this study with the aim of determining the levels of the potently carcinogenic PAHs in the fish samples. 24 samples of barbecued fish products were purchased from six different barbecued spots, processed and analyzed. The PAHs in the samples were extracted using solvents by ultrasonication and were analyzed for the 16 US EPA polycyclic aromatic hydrocarbons using HPLC with a UV DAD detector. Naphthalene, Acenaphthylene, Acenaphthene, 1, 2-Benzothracene weren’t detected in the entire sampled barbecued fishes across the study locations while Benzo(a)pyrene which is considered one of the most toxic and dangerous PAHs was detected highest in sample of Pseudotolitus elongatus gotten from Ekewan (206.69µg/kg). PAHs with maximum concentrations detected in sampled barbecued fish was Benzo(k)fluoranthene (647.58 µg/g) in Pseudotolitus elongatus from S and T Ugbowo. Total PAHs concentration range from (1797.72 µg/g – 3031.44 µg/kg). The high values recorded in this study should considered relevant in sending out warning signs on the frequent consumption of barbecued fish due to their associated health risk.

Keywords: Barbecued fish, PAHs, Charcoal, Health Risk.

Introduction
Except smokers and occupationally vulnerable populations, most individuals are exposed to PAHs predominantly from dietary sources (Bordajandi et al., 2008). Although for most people, fish and sea food represents only a small part of the total diet, the contribution of this food group to the daily intake of PAHs in some individuals may be comparatively important (Domingo et al., 2007). Traces of PAHs have been detected in many foods (Bartoszek, 2002; EFSA, 2007) including vegetable oils, fruits, sea food (Gianguzza and Santino 2006), grilled and roasted meat, smoked fish, tea and coffee (Simko, P., 2002). In particular, benzo[a]pyrene has been found in these samples at concentration levels between 0.1 and 100mg/kg and hence pose a health risk to consumers (Rey-Salguiero et al., 2008). Contamination of PAHs by intense thermal processing occur due to generation by direct pyrolysis of food nutrients and also due to direct deposition of PAHs from smoke produced through incomplete combustion of different thermal agents. Polycyclic aromatic hydrocarbons have been determined and quantified in some seafood consumed around lakes in Egypt, such as Oreochromis aureus (Nile tilapia), Portuns pelagicus (crabs), Venerupis decussate (bivalves), Strombus tricornis (clams) and Munes spp (gastropods) (Barakat, 2004). Considering the rapid growing spots and love for barbecued fish, together with limited data on the levels of PAH in our commonly barbecued fish in Nigeria, this study was therefore imperative to establish the unknown facts and provide essential data.

Methodology
Sample collection: A total of twenty-four (24) samples of two different barbecued fish species commonly consumed within Benin City namely Pseudotolitus elongatus and Clarias gariepinus were purchased from three different centres from local vendors in Benin City. Each of the samples were purchased from different centres namely; Tips bar located Ekewan Road, S & T Barrack Ugbowo and Megor-Ishior which represents local hangout for many average Benin. After collection, portion of each of the representative sample so obtained (5g) was milled, packed in aluminium foil wraps and stored in the freezer at -20°C before analysis.

EXTRACTION
PAH extraction was carried out by applying the method by (Garcia-Falcon et al., 1996), about 5g milled sample
was weighed into amber glass bottles and extracted sequentially by ultrasonication (Ultrasonic bath 1510 Branson) using 25ml of n-hexane for 1 hour. After ultrasonication the supernatant were decanted into a vial and 15ml of fresh solvent for 1 hour, the combined extracts (50ml) were centrifuged at 2500 rpm for 10 minutes and the supernatant was decanted.

Clean Up
A solid phase extraction (SPE) cartridge was effective as it was used in the clean up. The sorbent of the SPE cartridges were first conditioned with dichloromethane, after which the filtered extracts were loaded on to the cartridges, the analytes were eluted with dichloromethane. The volume of the dichloromethane was blown down to dryness and extract was reconstituted in 2ml after the solvent extraction of the PAHs from the fish samples by ultrasonication, high performance liquid chromatography (HPLC) was used for their separation and analysis. Agilent 6890 Series Gas Chromatography, HPLC system with a quaternary pump, vacuum degasser, a temperature controlled column oven and UV diode-array detector was used to perform the quantification of PAHs. PAHs separation was performed on a monomeric type octadecyl silica column, Supelcosil LC PAH 2 cm x 4.6 mm i.d containing 5µm particles at ambient temperature (25± 1°C) at a flow rate 1.0ml/min. gradient elution using acetonitrile and water was employed (60:40 to 0:100). Peak detection and integration of data was carried out using chemstation software series, external calibration was carried out using mixed PAH standards from the chromatogram, the retention times of the standards were used for the identification and quantization of the individual PAHs. a standard mixture of the USEPA 16priority PAHs and 2 PAHs derivatives (2000 µg/ml, dichloromethane: benzene): naphthalene,acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indo[1,2,3-c,d]pyrene was obtained from SUPELCO, Bellefonte, PA, USA. Appropriate working dilutions of the standard solution with HPLC grade acetonitrile were made and all other solvents used were of high purity analytical grade.

Statistical Analysis
All data were subjected to one-way analysis of variance (ANOVA) using XL Stat program, and the results were presented as mean ±standard Error (95% confident interval)

Results and Discussion
In this study, twenty-four (24) samples of different barbecued fishes were analyzed and the concentrations of PAHs were determined. The data collected after the analysis of the fish were processed and presented in graphs to explain the results of the experiments.

| TABLE 1: PAH determination in Pseudotsolitus elongatus |
|---------------------------------|----------------|----------------|----------------|
| PARAMETERS                      | Ekewan Road    | Sakponba Road  | S & T Ugbowo   |
| Naphthalene                     | ND             | ND             | ND             |
| Acenaphthylene                  | ND             | ND             | ND             |
| Acenaphthene                    | ND             | ND             | ND             |
| Fluorene                        | 99.54±99.54    | 97.78±98.78    | 252.13±181.21  |
| Phenanthrene                    | 165.03±44.93   | 166.50±44.01   | 98.34±98.34    |
| Anthracene                      | 178.74±72.37   | 179.31±74.32   | 64.19±64.19    |
| Fluoranthene                    | 71.89±71.89    | 72.88±72.88    | 43.45±43.45    |
| Pyrene                          | 120.20±120.20  | 115.21±115.21  | 144.19±144.19  |
| 1,2-Benzothracene               | ND             | ND             | ND             |
| Chrysene                        | 84.75±84.75    | 82.73±82.73    | 389.76±317.59  |
| Benzo(b)Fluoranthene            | ND             | ND             | 113.14±113.14  |
| Benzo(k)Fluoranthene            | 615.31±214.85  | 620.85±200.69  | 647.58±419.98  |
| Benzo(a)pyrene                  | 206.69±91.09   | 205.36±94.98   | 128.37±128.37  |
| Dibenzo(a,h)anthracene          | 505.92±249.69  | 512.62±248.22  | 504.10±335.21  |
| Benzo(g,h,i) perylene           | 221.11±88.50   | 224.85±89.34   | 265.28±163.86  |
| Indeno(1,2,3-cd)pyrene          | 295.76±82.67   | 299.16±81.64   | 267.34±171.13  |
| ND/Not detected                 |                |                |                |
A summary of the concentrations of various PAHs present in sampled fish is represented and the mean values are shown in table 1–2. All the 15 targeted PAHs were detected in different quantities in the barbecued fish. Appreciable amounts of PAHs were observed in this study. The concentration of PAH in the barbecued fish ranges between 0 and 647.58µg/g. The distribution of average PAH contents in fish are represented above, as can be seen from the table1 having an average total PAH level of (2564.94 µg/g). This could be ascribed to the high fat content of the fish. (Akpan et al., 1994) reported that strong correlation exists between fish lipids and PAH compounds; since PAH compounds are stored in fatty fish tissue. PAHs with maximum concentrations detected in sampled barbecued fish was Benzo(k)fluoranthene (647.58 µg/g) as represented in table 1. From S and T ugbowo, while the minimum PAH concentration were Naphthalene, Acenaphthylene, Acenaphthene, 1,2-Benzothracene which were not detected in all the sampled barbecue fishes across the study locations. Benzo (a) pyrene which is considered one of the most toxic and dangerous PAHs was detected highest in sample of Pseudotolitus elongatus gotten from Ekewan (206.69µg/kg). Pyrene and benzo[a]pyrene are two of the best characterised PAHs and may be bio transformed in humans and animals to numerous phase 1 metabolites including 1-OH pyrene (1-OH-Pyr) and 3-OH benzo[a]pyrene (3-OH-B[a]P) (Rey-Salgueiro et al., 2009). 3,4-benzopyrene, found in smoked products, serves as an indicator of the possible presence of other polycyclic aromatic hydrocarbons (PAH) and has been used repeatedly as a quantitative index of chemical carcinogens in foods. The level of B (a) P found in the barbecue fish sample across all locations was however higher than the European regulatory maximum level for smoked meat and fishes. Some authors determined the effects of various processing methods, steaming, roasting, smoking, charcoal grilling, etc. on foods (Chen and Chen, 2001; Duedahl-Olesen et al., 2006; Rey-Salgueiro et al., 2009). All mentioned authors attribute the highest PAH generation during grilling or barbecue through pyrolysis during charbroiling of fish products and either deposition and penetration of smoke components into foods and they found a link between fat foods and PAH levels. The hypothesis is that melted fat from the heated fish drips onto the hot coals and is pyrolyzed, giving rise to PAHs generation, which are then deposited on the fish surface as the smoke rises.

The result in table 2 of Clarias gariepinus (Catfish) sample across the two locations was observed to have concentrations of the low molecular weight PAHs such as naphthalene, pyrene, fluoranthene, and anthracene were found to be lower except for chrysene with mean value of 51.48 and 52.53µg/g, phenanthrene 48.06 and 46.56µg/g, fluorine 52.93 and 53.83µg/g whose values were however higher than the European regulatory maximum level. Similarly, significant values were recorded among the higher molecular weight PAHs such as benzo(k)fluoranthene with mean 460.83 and 458.19µg/g, benzo(b)fluoranthene 29.03 and 31.50µg/g. Dibenzo(a,h)anthracene 283.39 and 145.25µg/g and benzo(a)pyrene 145.25 and 152.59µg/g, meanwhile PAHs with higher molecular weight are more carcinogenic than the lower molecular weight PAHs (Moret and Conte 2000). The carcinogenic PAH levels in smoked fish largely depend on the smoke generated by combustion of wood and vary with the fat level of fish as explained earlier. Wood smoke contains appreciable amounts of carcinogenic PAHs, which are the main cause of concern regarding its toxicity.

Benzo(b)fluoranthene was not detected in sample of Pseudotolitus elongatus from Ekewan and Sakponba Road respectively but was recorded in higher concentration from sample gotten from S & T Ugbowo with mean value 113.14 µg/g. Similarly, samples of Pseudotolitus elongatus from Sapele Road recorded mean value of 113.15 µg/g. Results obtained in this study are highly variable and not in agreement with the results obtained by (Simko, 2005; Reinik et al., 2007; Akpan et al., 1994). The discrepancies may be attributed to heat
temperature, time, and the concentration of these compounds in the wood smoke, oxygen accessibility (17).

CONCLUSION
All the polycyclic aromatic hydrocarbons (PAHs) found in this study were among the prioritized PAHs considered as carcinogenic. Most PAHs get into the human system through either direct or indirect ingestion. PAHs at certain significant concentrations can be very dangerous to the health of humans. Thus, Phenanthrene which was present in all the samples inhibits and affects the fluid balance of the body and promotes the abnormal functioning of the body nerves and muscles. The levels of PAHs across the species were higher than the safety limits considering that this study revealed minimum levels of Naphthalene, Acenaphthylene, Acenaphthene, 1, 2-Benzothracene as below detection. A maximum value of 166.50µg/kg for Phenanthrene was found in Pseudotolitus elongatus from Sakponba Road and have been observed to be higher than E.U safety limit.

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References