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EVALUATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOME ROASTED FOOD DELICACIES IN PORT HARCOURT, RIVERS STATE.

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ABSTRACT

The concentrations of sixteen priority polycyclic aromatic hydrocarbons were determined in some roasted food delicacies (plantain and fish) consumed within Port Harcourt metropolis using Gas chromatography fitted with flame ionization detection. Appreciable amount of Acenaphthene (1.450 mgkg^{-1}); Flourene (0.456 – 2.021 mgkg^{-1}); Anthracene (1.445 mgkg^{-1}); Pyrene (1.235 - 1.346 mgkg^{-1}); Chrysene (0.448- 2.122 mgkg^{-1}); Benzo (k) fluoranthrene (0.036 – 2.531 mgkg^{-1}); Benzo (b) flouranthrene (0.285 mgkg^{-1}) were detected in roasted plantain samples. In the roasted fish samples the detected PAHs were Flourene (2.642 – 3.554 mgkg^{-1}); Anthracene (2.604 – 4.526 mgkg^{-1}); Pyrene (1.371 - 1402 mgkg^{-1}); Chrysene (2.106 – 3.348 mgkg^{-1}); Benzo (b) flouranthrene (0.285mgkg^{-1}). None of the priority PAHs was detected in the raw plantain sample. However substantial amount of Benzo (k) fluoranthrene (0.020 mgkg^{-1}) was detected in the fresh fish. Continual consumption of these foods may consequently put the several consumers at potential health risk.

Key Words: Polycyclic aromatic hydrocarbons; Health risk; roasted, carcinogenic and genotoxic

INTRODUCTION

Polycyclic aromatic hydrocarbons or polynuclear aromatic hydrocarbons (PAHs) are a large class of organic compounds, which is made of two or more fused aromatic rings formed by incomplete combustion or pyrolysis of organic matter. Industrial and anthropogenic activities such as forest fires, volcanic eruptions, tobacco smoke, engine exhausts, coal-derived products, waste incineration among others can contribute to the formation of PAHs (Wang *et al.*, 2001; Dong and Lee., 2009; Mahindrakar *et al.*, 2011; Iwegbue *et al.*,

2013). Humans can get exposed to PAHs through the consumption of smoked, roasted, barbecued, or grilled meat, fish and foods, which are usually contaminated from, industrial food processing and from certain home cooking practices especially roasting of food which is one of the processes that contribute greatly due to incomplete combustion or thermal decomposition of the organic materials (WHO, 2006).

Several researchers have analyzed roasted food items and have proven the presence of carcinogenic and genotoxic PAHs such as benzo[a]pyrene, anthracene, chrysene, benzo[a]anthracene, indeno[1,2,3-c,d]pyrene in different classes of foodstuff (Ogbadu and Ogbadu, 1989; Akpan *et al.*, 1994; Duke and Albert, 2007; Bababunmi *et al.*, 1981; Alonge, 1988). The presence of PAHs in foodstuff occurs as a consequence of environmental contamination, physiological and ecological features of the product as well as the thermal processes to which the foods are subjected to during processing and manufacture of foods (Iwegbue *et al.*, 2013).

Considering the carcinogenic nature of (PAHs), constant monitoring is required to advert the inherent dangers they pose to human health. Therefore for the wellbeing of the general public, there should be monitoring of the concentration of PAH in roasted food at certain time intervals.

This study tends to evaluate the level of PAHs in roasted food delicacies around Choba and the health risk associated with the short and long-term consumption of roasted food containing polycyclic aromatic hydrocarbons.

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MATERIALS AND METHODS

Description of the Study Area

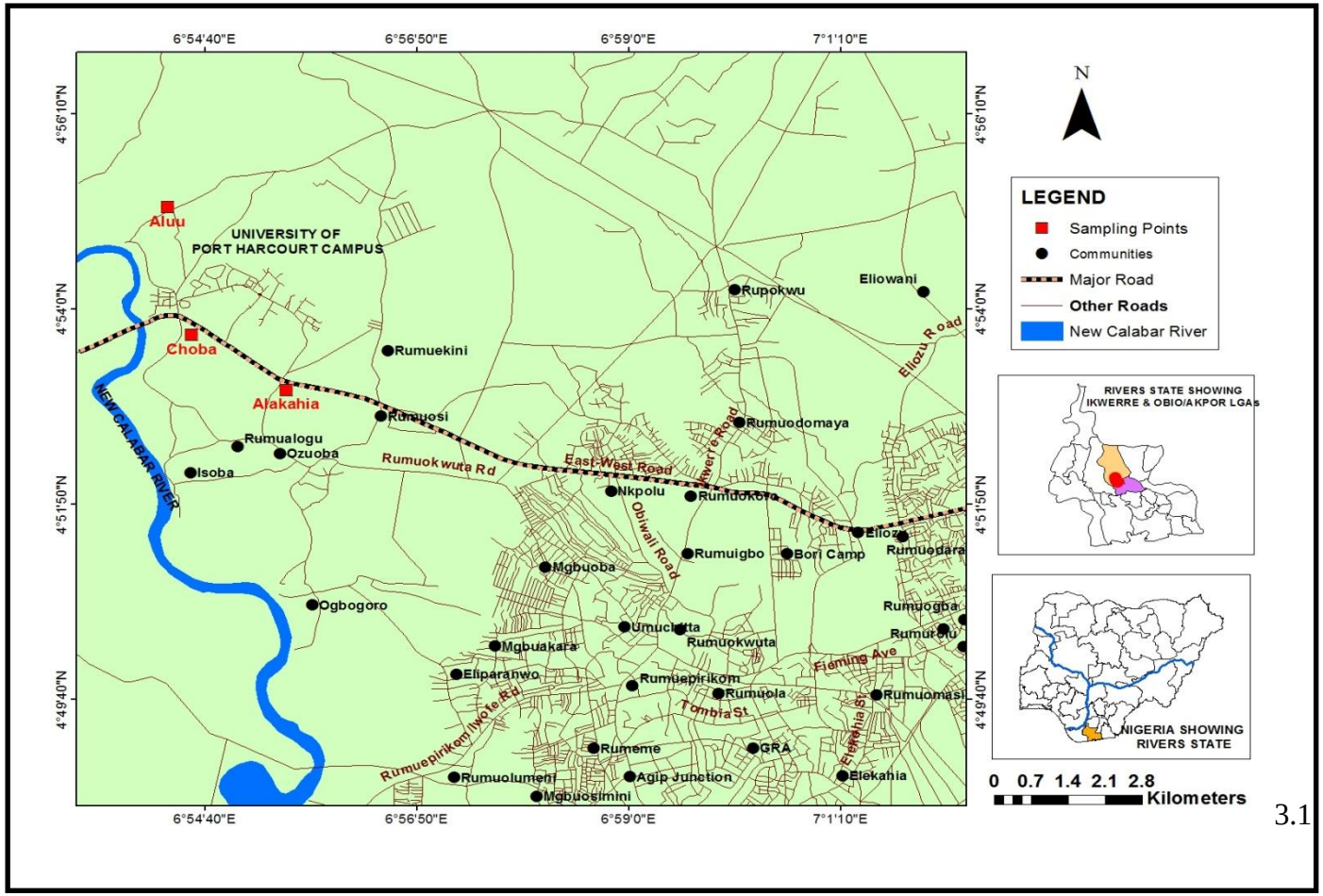

Fig 1: Topographic map of the study area showing sample collection points.

3.1

The study communities lies between latitude 6°54'40"E to 7°1'10"E of and a longitude of 4°49'40"N to 4°56'10"N. Choba, Alakahia and Aluu communities are in Obio-Akpor local government area of Rivers state, Nigeria, and host the University of Port Harcourt with a lot of student and commercial activities going on in the area. The original indigenous occupants of the area are the Ikwerre people. The community's fastest meal is roasted plantain and fish with thousands of students of the University of Port Harcourt partaking also in the roasted delicacies due to the communities' proximity to the school.

Sample collection, preparation and analysis

Six samples of roasted plantain and fish samples were obtained directly from the vendors who prepare them by the roadsides in Choba, Alakahia and Aluu in Port Harcourt, Rivers State. A sample of raw plantain and fresh fish was also obtained from the vendors in Choba and used as the control sample. The heavy influx of students within the area with a high consumption rate of roasted fish and plantain informed the choice of this study.

Extraction of PAHS

Extraction of PAHs was carried out using the method described by Pena *et al.*, (2006), Ten grams (10g) of the roasted plantain and fish were separately homogenized in a mortar and thoroughly mixed with anhydrous sodium tetraoxosulphate (vi) (Na_2SO_4) to dehydrate the sample. 20 ml of di-chloromethane was added to the samples to extract the PAHs. Extracts were then cleaned up using a chromatographic column, moderately packed at the bottom with 1cm glass wool. Ten grams (10g) of activated silica gel (100 mesh size). The silica gel was activated by heating in the oven at 105°C and 1 cm of anhydrous Na_2SO_4 was added to the column while the column was fractionated by elution with 20ml methylene chloride and collected a conical flask. Sample fractions were concentrated to about 2ml under a gentle stream of air in a fume cupboard. The extracts were transferred into 2ml sample vials bottle using a 5ml pipette for GC analysis.

Gas Chromatographic analysis of sample

Gas chromatography (Hewlett-Packard HP-5890) 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 2L 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. Helium was used as a carrier gas with splitless inlet mode. A five point calibration using PAH standards of 10.0 ppm, 20.0 ppm, 40.0 ppm, 80.0 ppm, 160.0 ppm which were prepared from 1000 ppm PAH stock standard (Accustandard) 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 used for the analysis. Compounds were identified by comparing the retention time of standards with that obtained from Standard preparation by serial dilution (Tongo *et al.*, 2017).

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RESULTS AND DISCUSSION

Table 1: Concentration of Polyclic Aromatic Hydrocarbons ($mgkg^{-1}$) in Roasted plantain samples

PAHs	Alakahia	Choba	Aluu	Control
Naphthalene	0.000	0.000	0.000	0.000
2-methylnaphthalene	0.000	0.000	0.000	0.000
Acenaphthene	1.450	0.000	0.000	0.000
Flourene	0.456	0.000	2.021	0.000
Phenathrene	0.000	0.000	0.000	0.000
Anthracene	1.445	0.000	0.000	0.000
Fluoranthene	0.000	0.000	0.000	0.000
Pyrene	1.346	0.000	1.235	0.000
Benzo (a) anthracene	0.000	0.000	0.000	0.000
Chrysene	0.448	2.122	0.000	0.000
Benzo (b) flouranthrene	0.000	0.000	0.522	0.000
Benzo (a) pyrene	0.000	0.000	0.000	0.000
Benzo (k) fluoranthrene	0.703	0.036	2.531	0.000
Indeno (1,2,3) perylene	0.000	2.055	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000
Benzo (g,h,i) perlene	0.000	0.000	0.000	0.000
TOTAL	**5.848**	**4.212**	**6.310**	**0.000**

Table 2: Concentration of Polyclic Aromatic Hydrocarbons ($mgkg^{-1}$) in Roasted Fish samples

PAHs	Alakahia	Choba	Aluu	Control
Naphthalene	0.000	0.000	0.000	0.000
2-methylnaphthalene	0.000	0.000	0.000	0.000
Acenaphthene	0.000	0.000	0.000	0.000
Flourene	2.642	0.000	3.554	0.000
Phenathrene	0.000	0.000	0.000	0.000
Anthracene	0.000	2.604	4.526	0.000
Fluoranthene	0.000	0.000	0.000	0.000
Pyrene	1.402	1.371	0.000	0.000
Benzo (a) anthracene	0.000	0.000	0.000	0.000
Chrysene	2.106	3.189	3.348	0.000
Benzo (b) flouranthrene	0.285	0.000	0.000	0.000
Benzo (a) pyrene	0.000	0.000	0.000	0.000
Benzo (k) fluoranthrene	0.000	0.000	0.000	0.020
Indeno (1,2,3) perylene	0.000	0.000	0.000	0.000
Dibenzo (a,h) anthracene	0.000	0.000	0.000	0.000
Benzo (g,h,i) perlene	0.000	0.000	0.000	0.000
TOTAL	**6.435**	**7.165**	**11.428**	**0.020**

The results of analysis of PAHs from the plantain and fish samples are shown in Tables 1 and 2.

The detected US EPA PAHs in roasted plantain samples are Acenaphthene (1.450 $mgkg^{-1}$); Flourene (0.456 – 2.021 $mgkg^{-1}$); Anthracene (1.445 $mgkg^{-1}$); Pyrene (1.235 - 1.346 $mgkg^{-1}$); Chrysene (0.448- 2.122 $mgkg^{-1}$); Benzo (k) fluoranthrene (0.036 – 2.531 $mgkg^{-1}$); Benzo (b) flouranthrene (0.285 $mgkg^{-1}$) (Table 1 figs 2 & 3). None of the priority PAHs was detected in the plantain sample used as control (Table 1 fig 4). The detected PAHs in the fish samples are Flourene (2.642 – 3.554 $mgkg^{-1}$); Anthracene (2.604 – 4.526 $mgkg^{-1}$); Pyrene (1.371 - 1402 $mgkg^{-1}$); Chrysene (2.106 – 3.348 $mgkg^{-1}$); Benzo (b) flouranthrene (0.285 $mgkg^{-1}$) (Table 2 fig 2). This is in accord with other supposition made by some researchers that raw foods contain no or minimal level of PAHs but they are produced as a result of human processing activities which includes, roasting, baking, smoking or frying (Kayali *et al.,* 1999; Olabemiwo *et al.,* 2011). However substantial amount of Benzo (k) fluoranthrene (0.020 $mgkg^{-1}$) was detected in the fresh fish sample used as control (Table 2 fig 4).

The total distribution of PAHs in roasted fish was found to be higher than roasted plantain samples as shown in (Table 1 & 2). This could be attributed to the high-fat content of the fish compared to that plantain. Strong relationships exist between fish lipids and PAHs with most of them stored in the fatty tissues of fishes (Akpan *et al.,* 1994). Benzo (k) fluoranthene (2.531 $mgkg^{-1}$), was the PAHs with highest concentrations in roasted plantain while Anthracene (4.526) was the PAH with the highest concentration in roasted fish. The health effects of PAHs, under certain circumstances include tumour formation and carcinogenicity in at least, laboratory animals (WHO, 2006; Ogbuagu *et al.,* 2011).

Among the sixteen priority PAHs only one was found in the fresh fish sample thus proving that roasting is responsible for the increment in PAH concentrations of the samples.

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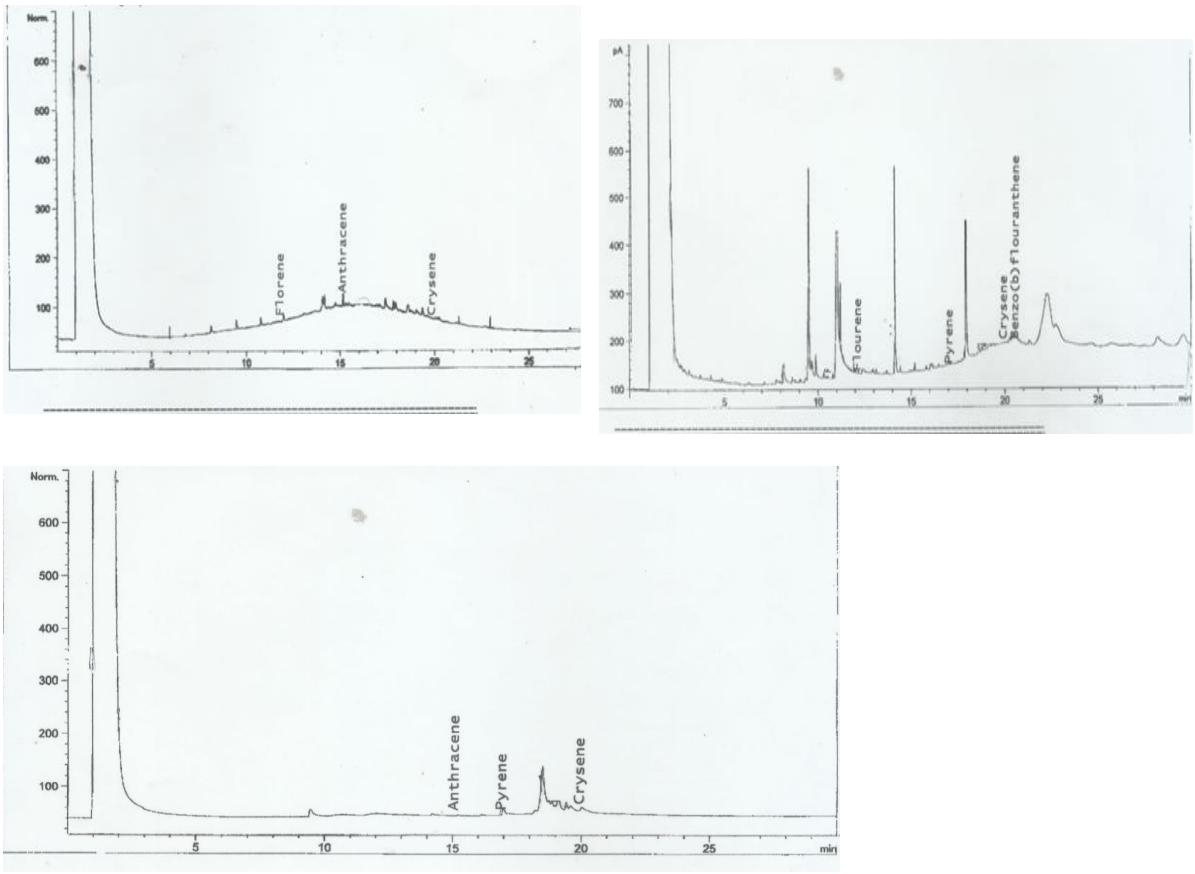

Figure 2. Representative chromatograms for roasted fish from Aluu, Alakahia and Choba communities

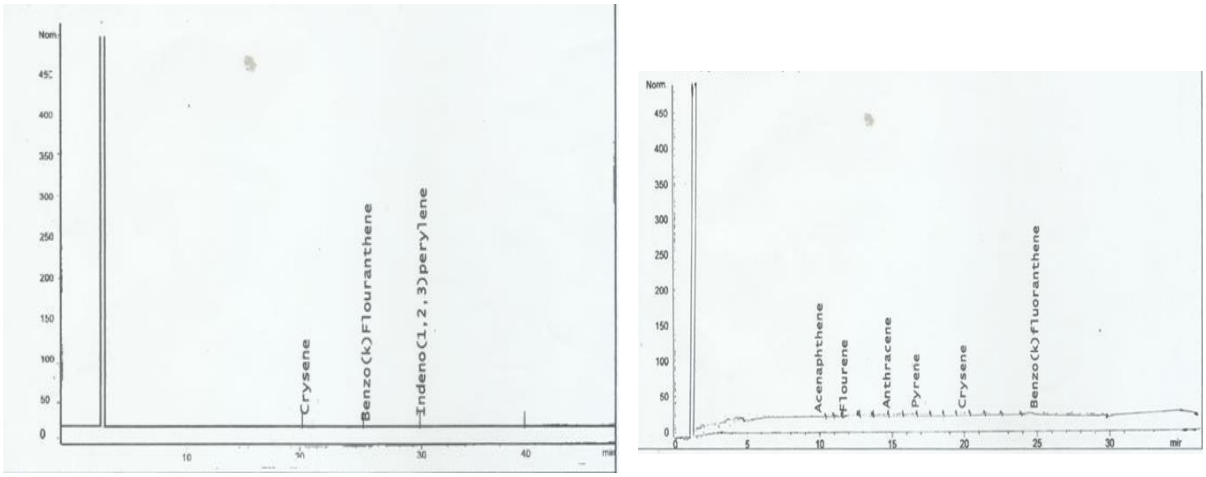

Figure 3. Representative chromatograms for roasted plantain samples from Alakahia and Choba communities

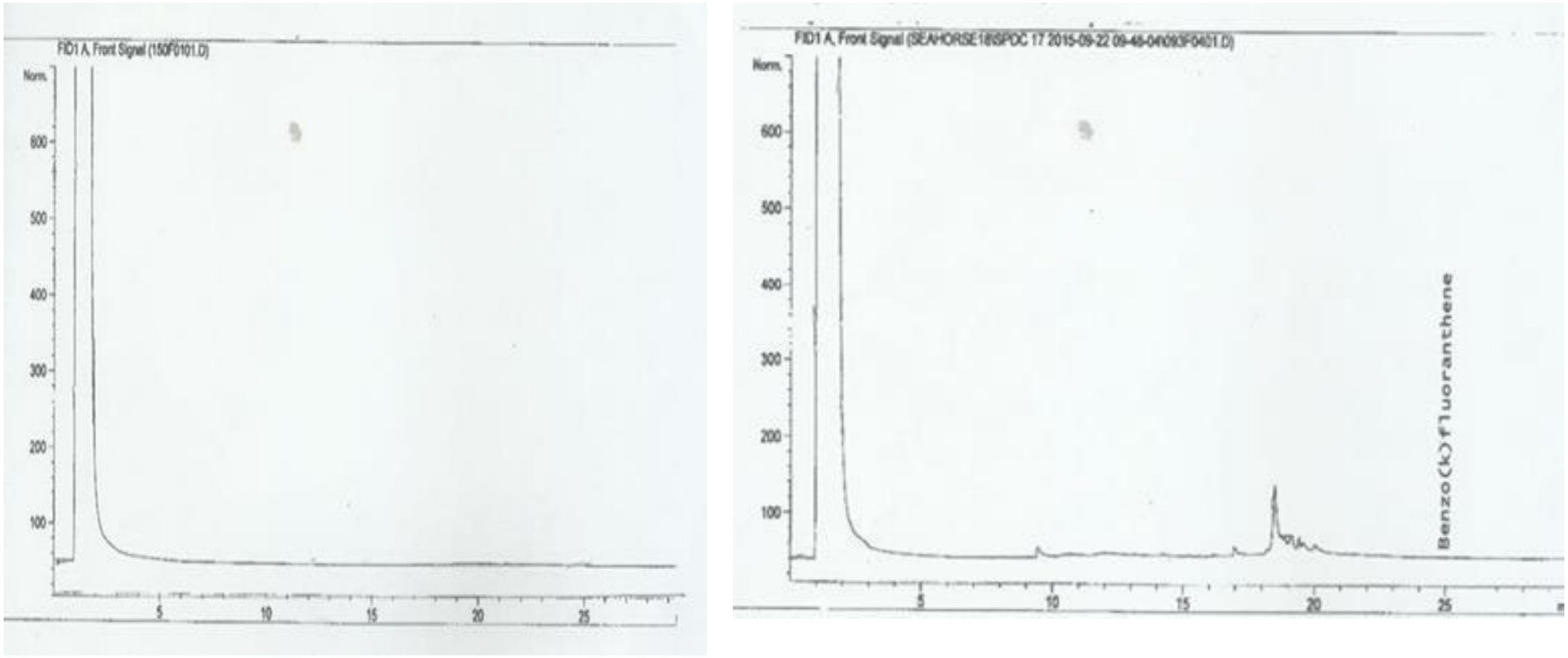

Figure 4. Chromatograms of fresh fish and plantain samples used as control from Choba and Alakahia communities

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PAH Distribution

In the fish samples, 9 PAH compounds were detected in both the roasted fish and roasted plantain while one PAH compound was detected in the raw fish sample used as control and none detected in the control plantain sample.

PAH Composition Analysis showed that Naphthalene and 2- methylnaphthalene were not present in both the roasted fish and roasted plantain samples. Fluorene, Anthracene and Acenaphthene were present in most samples with a total concentration of 7.453 $mgkg^{-1}$, 8.595 $mgkg^{-1}$and 1.450 $mgkg^{-1}$ respectively. They are low molecular weight PAHs (LMW PAHs), and they are relatively unstable. In a previous research work carried out at Amassoma, Nigeria, 15 PAHs were detected in reasonable quantity in the roasted fish and only 3 were found in roasted plantain; but none was detected in the raw food items (Amos-Tatua *et al.*, 2013).This is in agreement with other research work that raw foods do not normally contain high levels of PAHs but they are formed during processing, roasting, baking, smoking or frying (Kayali *et al.*, 1999). As shown in Table 4.1, the sum of all PAHs concentration present in the roasted plantain and fish ranged from 5.848 $mgkg^{-1}$, 4.212 $mgkg^{-1}$and 6.310 $mgkg^{-1}$ for roasted plantain and 5.236 $mgkg^{-1}$, 7.165 $mgkg^{-1}$ and 5.236 $mgkg^{-1}$for roasted fish respectively. The concentration of the various PAH compounds is widely distributed among the high molecular weight PAH and the low molecular weight PAH. In the roasted plantain sample, the highest PAH concentration is 2.531 $mgkg^{-1}$ which is benzo(k)fluoranthene which is a high molecular weight PAH. The roasted fish had its highest PAH concentration as 4.526 $mgkg^{-1}$ which is anthracene a low molecular weight PAH. The raw plantain and raw fish had their highest concentrations at 0.000 $mgkg^{-1}$ and 0.020 $mgkg^{-1}$ respectively the latter being benzo(k)fluoranthene a high molecular weight PAH. Toxicity tends to increase with the number of rings. PAHs containing four fused rings, such as benzo (a)anthracene and chrysene, are weakly carcinogenic. Five- or six-fused ring polycyclic hydrocarbons, such as benzo(b)fluoranthene, benzo(a-) pyrene, and indo(1,2,3-cd) pyrene are very potent carcinogens. However, from these results, low molecular weight PAH compounds recorded highest concentration in both roasted fish and roasted plantain samples. Reports from previous publications have revealed that PAHs with higher molecular weight (HMW) are more carcinogenic and mutagenic than the lower molecular weight (LMW) PAHs (Alonge *et al.*, 1998). The sum of PAH concentration present in the raw fish (0.020 $mgkg^{-1}$) is lower than that of the roasted fish (23.829 $mgkg^{-1}$) samples. The observed higher levels in the roasted fish samples may be attributed to the strong correlation that exists between fish lipids and PAH compounds, and also the close proximity of the fish to the heat source. A study reported that strong correlation exists between fish lipids and PAH compounds; since PAH compounds are stored in fatty fish tissue. The sum average PAHs concentration level present in the roasted plantain, 16.370 $mgkg^{-1}$ is higher than the raw plantain 0.000 $mgkg^{-1}$ as a result of the fat content present in the plantain and its proximity to the heat source. High levels of PAHs have been reported to be associated with the dark colorations in intensively heated products. Several analyses of charcoal roasted/grilled common food items have proven the presence of PAH such as benzo(a)pyrene, anthracene, chrysene,

benzo(a)anthracene, and indeno(1,2,3-c,d)pyrene (Lodovici *et al.,* 1994). In this study, the sum of the amounts of the low molecular weight PAHs (those containing 2 to 4 aromatic rings) such as, acenaphthene, and pyrene, were found lower (9.903 $mgkg^{-1}$) than the high molecular weight PAHs (11.552 $mgkg^{-1}$), those having 4 to 6 aromatic rings such as benzo(a)anthracene, benzo(a)pyrene [BaP], indeno(1,2,3,cd)pyrene in both the roasted fish and roasted plantain samples.

Some Health Effects Associated with Polycyclic Aromatic hydrocarbon

The International Agency for Research on Cancer (IARC) identified some PAHs such as benz(a)anthracene and benzo(a)pyrene, benzo(a)fluoranthene, benzo(k)fluoranthene, and ideno(1,2,3-c,d)pyrene to be carcinogenic to humans (international Agency for Research on Cancer (Tomatis *et al.,* 1978; USEPA, 1985). Polycyclic Aromatic hydrocarbons usually have a low degree of acute toxicity to humans. Studies carried out by some researchers have shown concarcinogenic effects that are based on PAH exposure dose (Gupta *et al.,* 1993). Continual exposure to PAHs may lead to the carcinogenic effects which include pulmonary, gastrointestinal, renal, and dermatologic systems. The metabolites of these PAHs or their derivatives can be potent mutagens. Report from researchers on workers with increased occupational exposure to PAHs shows incidences of lung, skin, and bladder cancers. A few PAHs need metabolism to become more potent carcinogens by forming Diol epoxides—PAH intermediate metabolites which are mutagenic and change normal cell replication. This is capable of causing PAH-induced carcinogenesis can result when a PAH-DNA adduct forms at a site critical to the regulation of cell differentiation or growth. This is one possible genotoxic mechanism of cancer causation (Denissenko *et al.,* 1996)

Many researchers have related the occurrence of several cases of intestinal tract cancer due to frequent intake of roasted food, Long term or chronic exposure to PAHs may include cataracts, kidney and liver damage and jaundice (Bababunmi *et al.,* 1981; Emerole, 1980; Agbozu, 2014).

This research identified some carcinogenic PAHs in some roasted food delicacies (plantain musa paradrica) and fish commonly consumed within the university community. These pose a potential health risk to consumers. The above finding corresponds with interferences made by other researchers that raw (fresh) foods do not normally contain high level of Our findings also proved that roasting is responsible of the increased level of PAHs in these foods because it is an incomplete combustion which leads to the formation of PAHs With this it is therefore recommended that the roadside vendors should come up with an alternative process other than roasting so as to reduce the level of contamination of PAHs and the Ministries of Health and Environment should put up strict policies as regards roadside roasting of food.

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