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Determination Of Polycyclic Aromatic Hydrocarbons In Some Selected Fresh And Smoked Food Samples

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***Abstract:** Polycyclic aromatic hydrocarbons (PAHs) are of health and environmental concern because of their associate carcinogenicity. Fresh and smoked/roasted samples of fish, meat and yam were assessed for the levels of Low Molecular Weight PAHs (LPAHs) and High Molecular Weight PAHs (HPAHs). The samples were subjected to ultrasonication extraction using dichloromethane and n-hexane (1:1 v/v) followed by a clean-up with silica gel and PAHs were quantified using GC-FID. A concentration range of 201.74 to 842.41 µg/g for LPAH was observed in smoked beef as against 215.93 to 995.35 µg/g for HPAH. PAHs in the fresh fish samples ranged from 0.78 to 9.418 µg/g. The concentrations were between 0.4918 and 13.53 18 µg/g in smoked fish. The concentrations of PAHs in the roasted yam ranged from 15.24 to 323.40 µg/g for HPAH as against 0.57 to 43.28 µg/g for LPAH in fresh yam. Continuous consumption of these food items could be dangerous over time.*

***Keyword:** PAHs Fish Meat Yam Ultrasonication GC-FID*

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic compounds consisting of large class of organic compounds with two or more aromatic rings. They are generated from partial combustion or pyrolysis of organic material which include fossil fuels such as coal, oil, gas, wood and from burning of organic materials (USEPA, 2008). Natural sources of PAHs include crude oil, forest fires, volcanoes, chlorophyllous plants, fungi, bacteria and shale oil (ATSDR, 1995). They are of environmental concern because of their persistence, ubiquity and toxicity. They are relatively insoluble in water and highly hazardous in nature (Eaton *et al*., 2005). Humans are exposed to PAHs by inhalation, ingestion through food and drinks and dermal absorption (ATSDR, 1995). Generally, low molecular weight PAHs have only 2-3 fused aromatic rings while high molecular weight PAHs have 4 or more aromatic fused rings. They may also be classified as being alterant or non-alterant if their structure is composed of only benzene rings or if 4-6 member non-aromatic rings are included (Wick *et al*., 2011). PAHs are released into the air from burning of residential wood burning, automobiles exhaust, forest fires, volcanoes etc. Industrial plants waste discharge can release them into surface water while their escape from storage containers at hazardous waste sites can contaminate soils and underground water (Ugulu, 2015). They are persistent organic pollutants and degrade slowly in the environment (through the process of photooxidation). The photo-induced oxidation of PAHs in the aqueous phase is brought about by singlet oxygen (most dominant process for the breakdown of PAHs and other organics), ozone, OH-radical and other oxidants (IARC, 2010). PAHs in water originate from surface runoff (erosion of asphalt pavement or from air decomposition of particle) (Yu *et al.*, 2006). Industrial effluent also can contribute to PAHs concentration in surface waters. Their solubility in water is usually very low. Their concentrations in typical drinking water are in the range of 0.02-1.8 ng/L. Estimates of daily PAHs intake from food vary widely, ranging from a few nanogram to a few microgram per person. Smoked cured meats, roasted, baked and fried foods (prepared by high temperature processing and or vegetable grown in contaminated soil) were found to contain PAHs, (JECFA, 2005). Smoking is a traditional way of preserving food. The safety issues related to the possibility of heavy metals and some PAHs are of concern. The subject of PAHs is receiving increasing popularity in food industry due to increasing incidents of contamination in agriculture and seafood sources. Apart from the threat from polluted environment, smoked food is subjected to heavy metals and PAHs contamination during the smoking process. The aim of this research work is to evaluate the level of PAHs in smoked and fresh fish, meat and yam.

II. MATERIALS AND METHODS

SAMPLING

Fresh and smoked samples each of beef, fish and yam were bought at Bariga Market, Lagos, Nigeria in November, 2016. The samples were separately wrapped in aluminium foil, stored in ice-packed coolers and transported into chemistry laboratory, university of Lagos and refrigerated at 4^0C prior to analysis.

SAMPLE EXTRACTION

10 g of each of homogenized samples was mixed with anhydrous Na_2SO_4 to remove moisture and then extracted using a mixture of dichloromethane and n-hexane (1:1, v/v) in an ultrasonic bath. The extract was concentrated using rotary evaporator. The concentrated extract was cleaned up using a column packed with silica gel, eluted with a mixture of DCM and n-hexane and the volume of the eluted fraction was reduced to 2ml, transferred into vials prior to gas chromatography flame ionization detector (GC-FID) analysis of the PAHs.

GC- FID CONDITION

The chromatographic system for PAHs separation was carried out on Agilent Technology 6890N Network System and HP5-Agilent Technology column of internal diameter 0.32 mm and thickness of 0.25 µm. The mobile phase was hydrogen with compressed air. The make up gas was nitrogen. Oven temperature programming was 40^oC to hold for 1 minute, at 15^oC/minute to 210^oC to hold for 1minute, at 50^oC/minute to 310^oC to hold for 8 minutes. The injection mode was split 100:1.

III. RESULTS

The results of the analyses are presented in Tables 1 to 3.

PAHs	Fresh Beef	Smoked beef
LPAH		
Naphthalene	1.21	1.86
Acenaphthylene	1.19	6.58
Acenaphthene	0.32	3.40
Fluorene	BLD	0.79
Phenanthrene	0.19	0.82
Anthracene	1.45	2.81
HPAH		
Fluoranthene	5.18	130.14
Pyrene	1.36	2.54
Benzo[a]anthracene	1.45	2.76
Chrysene	BLD	1.15
Benzo[b]fluoranthene	1.63	1.45
Benzo[k]fluoranthene	BLD	2.82
Benzo[a]pyrene	BLD	2.43
Indeno[1,2,3-cd]pyrene	1.53	1.85
Dibenzo[a,h]anthracene	3.04	3.23
Benzo[g,h,I]perylene	BLD	4.55
Total	17.73	168.63

LPAH= Low Molecular Weight PAH; HPAH= High Mol. Wt. PAH; BLD = Below Detection Limits

Table 1: Concentration (µg/g) of PAHs in Smoked Beef

PAHs	Fresh Fish	Smoked Fish
LPAH		
Naphthalene	BLD	BLD
Acenaphthylene	9.46	13.53
Acenaphthene	1.20	0.51
Fluorene	3.00	3.13
Phenanthrene	0.71	1.24
Anthracene	1.73	2.47
HPAH		
Fluoranthene	0.17	1.59
Pyrene	1.48	2.41
Benzo[a]anthracene	1.44	2.57
Chrysene	1.35	1.45
Benzo[b]fluoranthene	9.07	1.34
Benzo[k]fluoranthene	2.88	3.11
Benzo[a]pyrene	0.78	0.49
Indeno[1,2,3-cd]pyrene	1.76	1.53
Dibenzo[a,h]anthracene	3.00	3.18
Benzo[g,h,I]perylene	1.67	3.49
Total	22.77	53.21

LPAH= Low Molecular Weight PAH; HPAH= High Mol. Wt. PAH; BLD = Below Detection Limits

Table 2: Concentrations (µg/g) of PAHs in smoked fish

PAHs	**Fresh yam**	**Smoked yam**
LPAH		
Naphthalene	BLD	BLD
Acenaphthylene	10.68	66.38
Acenaphthene	BLD	38.04
Fluorene	BLD	68.79
Phenanthene	43.28	BLD

Anthracene	1.32	126.22
HPAH		
Fluoranthene	0.69	BLD
Pyrene	1.43	15.24
Benzo[a]anthracene	1.28	30.57
Chrysene	BLD	323.40
Benzo[b]fluoranthene	1.53	15.39
Benzo[k]fluoranthene	BLD	25.45
Benzo[a]pyrene	0.57	BLD
Indeno[1,2,3-cd]pyrene	BLD	BLD
Dibenzo[a,h]anthracene	BLD	201.31
Benzo[g,h,I]perylene	BLD	BLD
Total	60.78	611.36

LPAH = Low Molecular Weight Polycyclic Aromatic Hydrocarbon; HPAH= High Mol. Wt PAH; BLD = Below Detection Limits

Table 3: Concentrations (μg/g) of PAHs in Fresh and Roasted Yam

IV. DISCUSSION

The concentrations of PAHs in the fresh fish ranged from 0.78 to 9.4 18 μg/g while that of smoked fish ranged from 0.49 to 13.53 μg/g. Acenaphthylene was found to have the highest concentration (9.46 μg/g) of PAHs in the fresh fish analysed while 13.53 μg/g was found in smoked fish. PAHs were consistently high in analysed smoked fish sample when compared with the fresh sample. It was observed that the fresh and smoked food samples were characterized by Low Molecular Weight Polycyclic Aromatic Hydrocarbons (LPAH) which ranged from 23.60 to 21.16 μg/g while HPAHs ranged from 16.17 to 32.34 μg/g. The amount of PAHs formed during the processing of fish depends mostly on the conditions of smoking. In traditional smoking, smoke is generated at the bottom of an oven and the food is placed directly over the smoking wood. These values were higher than the result obtained by Amos-Tautua *et al* (2013) reported for smoked fish. High levels of PAHs pose great health risk such as eye and skin irritation, kidney and liver damage among others. The concentration of PAHs increased during smoking process, and this rendered the food unfit for human consumption.

Toxic Equivalency Factors (TEFs) was identified for a number of individual PAHs to express its potency relative to benzo[a]pyrene (TEF of unity). The concentrations of each of the individual PAHs compounds were multiplied by their corresponding TEF and these are summed to yield benzo[a]pyrene equivalent concentration (Nsikak *et al*., 2016).

PAHs	TEF Values	TEQ (Fresh Fish)	TEQ (Smoked Fish)
Naphthalene	0.001	ND	ND
Anthracene	0.010	1.7e-3	2.5e-3
Fluorene	0.001	3.0e-3	3.1e-3
Phenanthrene	0.001	7.0e-3	1.2e-3
Acenaphthene	0.001	1.2e-3	5.0e-3
Acenaphthylene	0.001	9.5e-3	1.4e-3
Pyrene	0.001	1.5e-2	2.4e-3
Fluoranthene	0.080	1.4e-3	1.3e-2
Benzo[b]flouranthene	0.800	7.3	1.1
Benzo[a]pyrene	1.000	7.8e-2	4.9e-1
∑TEQ		8.2	1.8

ND = No Data

Table 4: Benzo[a]pyrene Equivalent Estimation in Fish

A concentration range of 201.74 to 842.41 μg/g of LPAHs was observed in smoked beef while 215.93 to 995.35 μg/g was obtained for HPAH. Fluoranthene was found to have the highest concentration (130.14 μg/g) of PAHs in the smoked beef while fluorene was found to have the lowest concentration (0.78 μg/g). High Molecular Weight Aromatic Hydrocarbons (HPAHs) concentration was high in the smoked beef and these values were higher than the result reported by Ayejuyo *et al* (2013).” HPAH are more carcinogenic than LPAHs” (Amos- Tautua *et al*., 2013). The HPAHs constitute about 89% of the total PAHs in the smoked beef., fluoranthene concentrations account for 77% of HPAH. Benzo[a]pyrene was also detected in the smoked beef (2.43μg/g). According to Akpambang *et al* (2009), “benzo[a]pyrene is the most studied PAH and it is used as a marker for PAHs contamination in food sample”.

In order to assess the possible human exposure risks associated with carcinogenic or mutagenic PAHs in beef samples, the toxic equivalence factors (TEFs) was calculated relative to a Reference standard, benzo[a]pyrene, B[a]P as reported by (Yusuf et al., 2014). The overall carcinogenicity or mutagenicity of nonvolatile PAHs were estimated based on the weighted sum of individual congener concentrations and equivalence factors (TEFs) relative to the cancer or DNA altering potency to B[a]P. This implies that carcinogenic equivalents (TEQBaP) were calculated as a product of the observed concentrations of the individual PAH congeners with its TEF for cancer potency relative to B[a]P, and MEF for DNA modification capacity relative to B[a]P, respectively.

PAHs	TEF Values	TEQ (Fresh Beef)	TEQ (Roasted Beef)
Naphthalene	0.001	1.21e-3	1.9e-3
Anthracene	0.01	1.5e-3	2.8e-3
Fluorene	0.001	ND	7.9e-4
Phenanathrene	0.001	1.9e-4	8.2e-4
Acenaphthene	0.001	3.2e-4	3.4e-3
Acenaphthylene	0.001	1.2e-3	6.6e-3
Pyrene	0.001	1.4e-3	2.5e-3
Fluoranthene	0.080	4.0e-1	10.4
Benzo[b]fluoranthene	0.800	1.3	1.2
Benzo[a]pyrene	1.000	ND	2.4
∑TEQ		1.7	14.0

ND = No Data

Table 6: Benzo[a]pyrene Equivalent Estimation in Beef

Levels of PAHs in the fresh yam sample were relatively low and PAHs were not detected in some samples. This is in agreement with other inference made by other researchers that fresh foods do not normally contain high levels of PAHs but are formed during processing (Adetunde *et al*., 2012). The concentrations of PAHs in the roasted yam were between

15.24 μg/g and 323.40 μg/g for HPAH while LPAHs ranged from 0.57 to 43.28 μg/g for fresh yam.

PAHs	TEF Values	TEQ (Fresh Yam)	TEQ (Roasted Yam)
Naphthalene	0.001	ND	ND
Anthracene	0.01	1.3e-3	1.3
Fluorene	0.001	ND	6.9e-3
Phenanthrene	0.001	4.3e-3	ND
Acenaphthene	0.001	ND	3.8e-3
Acenaphthylene	0.001	1.1e-3	6.6e-3
Pyrene	0.001	1.4e-3	1.5e-3
Fluoranthene	0.080	5.5e-3	ND
Benzo[b]fluoranthene	0.800	1.2	12.3
Benzo[a]pyrene	1.000	0.6	ND
∑TEQ		1.8	13.6

ND = No Data

Table 7: Benzo[a]pyrene Equivalent Estimation in Yam

Decrease in PAHs concentration of benzo[b]fluoranthene (9.07 to 1.34 μg/g) and acenaphthene (1.20 to 0.51 μg/g) could be due to loss of fat from the fish during the smoking process. However, It has been reported by Mihalcal *et al.*, (2011) that a burning log fire may produce large amounts of PAH and, when used as the source of heat in the smoking of food, very high levels of PAH could be found in the smoked product. When the smoking was carried out over the embers (when flames no longer emerged from the fire) the level of contamination was largely reduced. It seems probable that the use of glowing embers, instead of burning logs, as the source of heat and smoke, could reduce the level of PAH contamination also in traditional smoking.

V. CONCLUSION

This study has revealed that food processing could contribute to the levels of PAHs found in the environment. It is evident from these results that the smoked food contained high levels of PAHs and regular consumption could be harmful. In order to reduce this chemical hazard, it is being suggested that the charred skin of roasted fish, meat or poultry should not be eaten. Methods of smoking should be revisited and readdressed globally.

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