# THE EFFECTS OF THREE TRADITIONAL SMOKING METHODS ON THE CONCENTRATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SMOKED FISHES.


## Abstract

The effects of three traditional smoking methods on the concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) in smoked fishes were studied to determine the concentration of PAHs in locally available and commonly consumed smoked fish species. Samples of two highly traded species of fish, *Scomber scombrus* and Horse markerel, among the low income people for immediate consumption were purchased from the market and processed using sawdust smoke, firewood smoke and charcoal smoke respectively. Some of the fresh fishes were also analyzed as control. The PAHs content were extracted with standard dichloromethane using solid-liquid extraction, and analyzed using Gas chromatography – Mass spectrophotometer (GC-MS) method. The results showed that fish samples processed with sawdust smoke recorded the highest concentrations of total PAHs, having 1.295mg/kg in Horse markerel and 2.020mg/kg in *Scomber scombrus*, followed by firewood smoked samples with total PAHs content of 0.910mg/kg in Horse markrel and 1.175g/kg in *Scomber scombrus* while charcoal smoked samples recorded the least total PAHs levels of 0.590mg/kg in *Horse markerel* and 0.960mg/kg in *Scomber scombrus.* Benzo(a)pyrene concentrations which is usually used to estimate the carcinogenicity of other PAHs was below detection level in both species of fish. PAH4 was proposed by European food safety authority, recommendation level of 30mg/kg was concluded by the EU regulation. Any PAHs have been associated with intense carcinogenicity in humans, and thus have implication for the quality and safety of these fish products. Therefore, it is imperative that regulatory bodies conduct awareness campaigns to educate the smoked fish processors, traders and consumers on the need to discourage the use of sawdust in smoking fish and adopt safer and improved methods of smoking fishes.

Keywords: PAH, *Scomber scombrus*, *Horse markerel*, sawdust smoke, firewood smoke and charcoal smoke, carcinogenicity

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are an important group of compounds of major environmental concern. There are several possible sources of PAHs in the environment, anthropogenic activities are, however, considered major sources of PAHs in the environment. Among the anthropogenic sources, petrogenic and pyrolytic sources are considered to be the most important. PAHs typically disperse from urban and sub-urbannon-point sources through road run-off, sewage, and atmospheric circulation and subsequent deposition of particulate air pollution (Hylland, 2006). Soil and river sediment near industrial sites such as creosote manufacturing facilities can be highly contaminated with PAHs. Oil spills, creosote, coal mining dust, and other fossil fuel sources can also distribute PAHs in the environment (Achtenand Hofmann 2009).

More than 100 PAHs have been characterized, 16 of which were classified by United States Environmental Protection Agency (USEPA) as priority pollutants because of their toxicity (EPA 1993). They include: 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 (Chen and Chen, 2001; EPA, 1993).

PAHs have been reported to be highly mutagenic and carcinogenic in humans (Simko 2002). Dietary exposures are the major source of human exposure to PAHs. PAHs are found in food as a result of food processing techniques like curing, drying, smoking, roasting, grilling, barbecuing and refining. These food pis smoked fish (Palm et al., 2011). In developing countries, smoked fish is a common source of protein in most diets, smoke not only gives the fish special taste and aroma, it also improves

preservation due to its dehydrating and bactericidal properties (Silva et al., 2011). However, smoke especially wood smoke contains PAHs, many of which are carcinogenic (David 1999). In developing countries, smoking is the most common method employed in preserving fish. In Nigeria, smoked fish products constitute about 61% of the total 194,000 mT of dry fish produced (Silva et al., 2011) Smoked fish products are the most available form of fish product for consumption, which could be attributed to the fact that most of the fishing communities have limited access to electricity to preserve their fish products (Silva et al., 2011). This has however increased the risk of PAHs contamination through consumption. Food Safety is of growing concern globally and PAHs residues if present in smoked fish above recommended levels could pose serious public health concerns (Muyela et al., 2012). Previous studies have shown the presence of PAHs in smoked fishes; however, studies on human health risk associated with consuming smoked fish are rather scanty (Yoon *et al*.,) especially for reported studies from Nigeria. Processing steps are known to generate and increase the level of PAHs in the food (Scientific Committee on Foods - SCF, 2002). One significant food source of PAHs

**Objetives:** The objective of this study is to determine the concentration of PAHs in locally available and commonly consumed smoked fish species (Horse mackerel and *Scomber scombrus*) from markets in South western Nigeria, in order to assess possible human health risks associated with consumption

**MATERIALS AND METHOD**

SAMPLE COLLECTION

The fishes are collected from a cold room at Iyana Iba market, Lagos located at the south western part of Nigeria. The fish's average length and weight were recorded and details are below

*Scomber scombrus* average length is 33cm with average weight is 330g while *Horse markerel* average length is 34cm and average weight is 350g.

COLLECTION AND TRANSPORTATION OF EXPERIMENTAL FISH

The table size fishes of *Scomber Scombus* and *Horse markerel* popularly known as Titus and Kote respectively were obtained from a cold room in Lagos metropolis. The fishes were transported using a cooler with ice(Close transportation system) to the fish hatchery of Department of Fisheries where the smoking was carried out.

Experimental site The Fishes were smoked in the Fish Hatchery of the Department of Fisheries, Lagos State University (LASU) Lagos Nigeria (latitude 0746°28'1.20" N 3°10'58.80" East) (with annual mean temperature at 30°C) and was sent to Nigeria Institute for Oceanography and Marine Research (NIOMR) Lagos, Nigeria for PAHs Analysis.

FISH PREPARATION AND SMOKING

Fish preparation and smoking *Scomber Scombus* and *Horse Markerel* used in this study and were weighed. Their length was taken using a ruler. The fish were properly washed with brine solution degutted and placed on metal meshed trays. Traditional method of smoking was adopted in this study by using smoking klin. Wind whisler, sawdust and charcoal were used for smoking at interval. Fire was obtained by striking matches on the materials to be used as fuel. Time interval of 20 minutes was allowed for the fire to stabilize before the meshed trays were placed on the drums. And then fire was reduced to increase quantity of smoke. The smoking drum was locked to trap the smokes inside and have maximum effect. It took 2 hour 30minutes for the fish to get dried, and then the smoked fishes were stored in a refrigerator at 4°C prior extraction and analysis.

EXTRACTION AND FRACTIONATION OF FISH SAMPLES:

The fish sample was homogenized with a blender. A 2g portion of the homogenate saponified with 200ml methanol/KOH(12% KOH in 95% methanol) solution in an ultrasonic bath at 60 0 C, for 30min. The sample was cooled and filtered through glass cool into separating funnel. The filtrate was extracted twice with 100ml hexane. The extract was washed with methanol/water (4:1) mixture, and then concentrated to 1ml with a rotary evaporator. The concentrate was fractionated through a silica gel column, first eluted with 10ml hexane to collect the aliphatic hydrocarbon fraction, and then with 15ml methylene chloride to collect the aromatic hydrocarbon fraction. Both fractions were concentrated to 1ml and stored capped in GC vials. 3.6 Determination of PAHs in the Solid Samples: 5g of the ample was weighed into an extraction bottle and 20ml of dichloromethane was added and sonicated in an ultrasonic sonicator for 2 hrs. The extract was concentrated to 20cm3 in a rotary evaporator. 20ml of0.5 KOH in 100ml of methanol was added and the mixture was refluxed for 1hr in a water bath at 60oC.20cm3deionized water was added and extracted with hexane (20cm3). The extract was dried over anhydrous sodium sulphate and the extract was concentrated at 60oC in a rotary evaporator to 20cm3. The extract was passed through silica gel column (DB 5 MS (30m x0.25mm x 0.5μm) which had been pre-conditioned with hexane. The extract was eluted with 20cm3 of hexane for aliphatic fractions. To some column, 20cm3 dichloromethane was added for the elution of PAHs and the eluent was concentrated to 1cm3 and solvent exchanged with 1cm3 of actonitrile. 1μL of the extract was injected into a pre-programmed GC vials (HP 6890A). The concentration of the PAHs was calculated from the peak area of the calibration standards. 1μL of each of the fractions was injected into the GC, set-up for the quantification of PAHs and the petroleum hydrocarbons (WHO, 2003; APHA, 2005).

GC Operating Conditions for PAHs:

Initial oven temp-400C; Initial hold time-2 min; Ramp – 12o C/min 40 to 300oC at 12oC/min to

300oC for 10 min; Final oven temp- 300oC; Detector temp- FID 350o C; Injector temp- 350o C; Carrier gas- Hydrogen,4 ml/min; constant flow; Injection volume-1µL, splitless, (hold 2 min).

All the data were log transformed to get normal distribution. One Way Analysis of Variance (ANOVA) was performed to assess the variation among species. Means were compared using the Bonferroni multiple comparison test. All the calculations were done using statistical software, IBM, SPSS version 23.

RESULTS.

Concentrations of Individual PAHs in *Scomber scombrus*

Table 3 shows the mean concentrations of the individual PAHs in *Scomber scombrus* processed by the different smoking methods. The results revealed that most of the individual PAHs were recorded in the samples processed using saw dust smoke. However, the samples processed with sawdust smoke recorded the highest mean level of almost all the PAHs, the level recorded for anthracene (0.260±0.000) and Benzo (a) anthracene (0.395±0.148). This suggests that smoke is the major contributor of the PAHs contamination in the processed fishes.

Concentrations of Individual PAHs in *Horse markerel*

Table 3 shows the concentration of the individual PAHs recorded in *Horse marckerel*. The trend of the PAHs contamination also revealed that most of the PAHs were recorded in the samples processed using saw dust and firewood with the highest PAH found in anthracene (0.175±0.92) and benzo(a)anthracene (0.235±0.092) respectively. Samples smoked using charcoal and firewood has no record of acenaphtylene (0.000±0.0000). The results also showed that benzo(a)pyrene was not detected in any of the processed using all the smoking methods except firewood with mean concentration of 0.005±0.007.

CONCENTRATIONS OF TOTAL PAHS IN THE FISHES

The results in Table show that the concentrations of total PAHs in samples all the samples were 3.315 mg/kg, 2.085mg/kg, and 1.550mg/kg respectively for the saw dust, firewood and charcoal Smoked samples.

In the *Scomber scombrus* samples, the concentrations of total PAHs were: 2.020mg/kg sawdust smoked, 1.175 mg/kg firewood smoked, and 0.960mg/kg charcoal smoked.

The *Horse markerel* recorded total PAHs levels of 1.295mg/kg in the saw dust smoked sample, 0.910mg/kg inthe firewood smoked, and 0.590g/kg in the charcoal smoked samples.

Table 1: Total concentration of PAHs in *Scomber scombrus.*

| Scomber scombrus | Raw | Charcoal smoked | Sawdust smoked | Firewood smoked |
|---|---|---|---|---|
| Total PAHs conc. mg/kg | 0.230±0.183 | 0.960±0.396 | 2.020±0.946 | 1.175±0.445 |

Table 2: Total concentration of PAHs in *Horse markerel*.

| Horse markerel | Raw | Charcoal smoked | Sawdust smoked | Firewood smoked |
|---|---|---|---|---|
| Total PAHs conc. mg/kg | 0.185±0.218 | 0.590±0.337 | 1.295±0.785 | 0.910±0.495 |

Concentration of individual PAH in the smoked fishes

With regards to the various method of smoking, Naphtalene is found to be highest in Scomber scombrus smoked with sawdust 0.155 mg/kg , then 0.045mg/kg found in hourse markerel smoked in firewood and lowest in *Scomber scombrus* smoked with charcoal 0.025mg/kg. (Table 3,).

Acnapthalene was found to be highest in *Scomber scombrus* smoked with sawdust 0.215mg/kg, followed by *Scomber scombrus* smoked with charcoal 0.130mg/kg and lowest in horse markerel as it was fairly below detection limit across all the biofuels used. (Table 3,).

Flourene recorded highest in *Scomber scombrus* 0.230mg/kg smoked with sawdust, 0.155mg/kg was found in *Scomber scombrus* smoked with firewood and the lowest in *Horse markerel*

0.045mg/kg smoked with charcoal. (Table 3,)

Acenapthene was highest in *Horse markerel* smoked with sawdust 0.170mg/kg followed by firewood smoked horse markerel 0.125mg/kg, *Scomber scombrus* was smoked with charcoal have no trace of acenapthene but were found in sawdust 0.140mg/kg and firewood 0.075mg/kg.(table 3)

Phenenthrene recorded highest in sawdust smokes *Scomber scombrus* 0.210mg/kg, then 0.140mg/kg in *scomber scombrus* smoked with charcoal, the least was found in charcoal smoked *Horse markerel* with value of 0.035mg/kg. (Table 3)

Anthracene has highest in sawdust smoked *Scomber scombrus* with value as high as 0.260mg/kg, then closely by firewood smoked *Scomber scombrus*, with 0.245mg/kg and found least in charcoal smoked horse markerel 0.090mg/kg. (Table 3) Anthracene was untraceable in the control. (Table 3)

Flourathene has 0.150mg/kg in sawdust smoked sawdust, 0.140 in sawdust smoked *Horse markerel* and untraceable in *Scomber scombrus* charcoal smoked. (Table 3)

Pyrene found in sawdust smoked scomber scombrus recorded the highest level of pyrene, 0.195mg/kg , followed by 0.170mg/kg in sawdust smoked *Scomber scombrus* and least in charcoal smoked *Scomber scombrus* as shown in table 3)

Benzo (a) anthracene as outlined by table 3, shows 0.395mg/kg in sawdust smoked *Scomber scombrus*, this is also the highest single PAH level for all the parameters considered, 0.235mg/kg was found in sawdust smoked *Horse markerel* and least value was found in (0.065mg/kg) charcoaled smoked *Horse markerel.*

Chyresene, highest singular value was found in charcoal smoked *Scomber scombrus*, 0.105mg/kg, followed by 0.080mg/kg in sawdust smoked *Scomber scombrus*, and below untraceable limit in *Horse markerel* irrespective of the biofuel used, shown in table 3)

Benzo (a) pyrene is absent from both species of fishes used. Table 3)

Indeno(1,2,3-cd) pyrene was found only in firewood smoked *Horse markerel*, 0.005mg/kg. (table 3,) Dibenzo anthracene, highest was found in sawdust smoked *Horse markerel* (0.080mg/kg), followed by 0.030mg/kg in charcoal smoked *Horse markerel.* It is below traceable limit in scomber scombrus. (Table 3)

Benz (g,h,i), parylene in Table 3) shows that, sawdust smoked *Horse markerel* has the highest level (0.100mg/kg), followed by 0.050mg/kg in charcoal smoked *Horse markerel.* The lowest value is found in both charcoal smoked S*comber scombrus* and *Horse markerel*, firewood smoked, with the same similar value of 0.010mg/kg.

TABLE 3: INDIVIDUAL CONCENTRATION OF ALL PAHS IN SELECTED FISHES AND SMOKING MATERIALS

| | SAMPLES | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Raw Fish | | Charcoal Smoked | | Sawdust smoked | | Firewood smoked | |
| | Fish species | | Fish species | | Fish species | | Fish species | |
| PARAMEERS (mg/kg) | Scomber scombrus | Horse markerel | Scomber scombrus | Horse markerel | Scomber scombrus | Horse markerel | Scombersc ombrus | Horse markerel |
| Naphthalene | 0.000±0.000 | 0.030±0.042 | 0.025±0.035 | 0.030±0.042 | 0.155±0.205 | 0.055±0.064 | 0.015±0.021 | 0.045±0.021 |
| Acenaphthylene | 0.040±0.057 | 0.000±0.000 | 0.130±0.071 | 0.000±0.000 | 0.215±0.035 | 0.000±0.000 | 0.125±0.021 | 0.000±0.000 |
| Fluorene | 0.050±0.000 | 0.020±0.028 | 0.135±0.035 | 0.045±0.007 | 0.230±0.014 | 0.145±0.078 | 0.155±.035 | 0.100±0.057 |
| Acenaphthene | 0.040±0.028 | 0.065±0.064 | 0.000±0.000 | 0.085±0.021 | 0.140±0.028 | 0.170±0.071 | 0.075±.007 | 0.125±0.064 |
| Phenanthrene | 0.000±0.000 | 0.000±0.000 | 0.140±0.071 | 0.035±0.035 | 0.210±0.212 | 0.055±0.035 | 0.070±.071 | 0.070±0.028 |
| Anthracene | 0.000±0.000 | 0.000±0.000 | 0.200±0.000 | 0.090±0.028 | 0.260±0.000 | 0.120±0.028 | 0.245±.078 | 0.175±0.092 |
| Fluoranthene | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.055±0.049 | 0.150±0.141 | 0.140±0.141 | 0.140±.071 | 0.090±0.085 |
| Pyrene | 0.035±0.035 | 0.040±0.042 | 0.055±0.035 | 0.105±0.021 | 0.170±0.071 | 0.195±0.078 | 0.125±.078 | 0.145±0.049 |
| Benzo(a) anthracene | 0.000±0.000 | 0.000±0.000 | 0.160±0.057 | 0.065±0.035 | 0.395±0.148 | 0.235±0.092 | 0.170±.000 | 0.140±0.085 |
| Chrysene | 0.050±0.042 | 0.000±0.000 | 0.105±0.078 | 0.000±0.000 | 0.080±0.071 | 0.000±0.000 | 0.035±.035 | 0.000±0.000 |
| Benzo(a) pyrene | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±.000 | 0.000±0.000 |
| Indeno(1,2,3-cd) pyrene | 0.005±0.007 | 0.005±0.007 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±.000 | 0.005±.0007 |
| Dibenzo(a,h) anthracene | 0.005±0.007 | 0.010±0.014 | 0.000±0.000 | 0.030±0.028 | 0.000±0.000 | 0.080±0.057 | 0.000±.000 | 0.005±0.007 |
| Benz(g,h,i)parylene | 0.005±0.007 | 0.015±0.021 | 0.010±0.014 | 0.050±0.071 | 0.015±0.021 | 0.100±0.141 | 0.020±0.028 | 0.010±0.014 |



## DISCUSSION AND CONCLUSION

### 5.1 Concentrations of Individual PAHs in the Dried Fishes

Most of the individual PAHs were recorded in the smoke-dried fishes processed using saw dust and firewood smoke respectively. This may be attributed to the longer drying times as a result of the less heat and high smoke produced by saw dust and firewood leading to prolonged fish contact with the smoke, being the major source of the PAHs contamination.

Generally, all the fish samples processed using charcoal smoke recorded less PAHs. This may be

due to the short drying time as a result of the high heat and less smoke produced by the charcoal. In a similar study, Silva et al. (2011) also observed that at high temperatures, less smoke was produced and at lower temperatures, more smoke was produced during the smoking process. This suggests that smoke is actually the major source of PAHs contamination in the smoked fishes.

Concentrations of Total PAHs in the Smoked Fishes

The results of concentrations of total PAHs in the smoked fishes presented in table 1 revealed that all the samples dried using saw dust smoke recorded the highest levels varying between 1.295mg/kg to 2.020mg/kg followed by the samples dried using firewood with total PAHs content varying between 0.910mg/kg to 1.175mg/kg. The samples dried using charcoal smoke recorded the lowest total PAHs ranging from0.590g/kg to0.965g/kg. The raw samples (control) only recorded 0.230mg/kg in the *Scomber scombrus* and 0.185mg/kg in *Horse markerel*. The concentrations of PAHs in the fish varied with the smoke source. Similarly to the work of Ubwa et al.,(2015). The trend of the concentrations of the total PAHs of the fishes based on the processing methods revealed the following order: saw dust smoking > firewood smoking> charcoal smoking > control. The levels of the PAHs recorded may be attributed to the intensities of the smoke and heat generated by the smoking material which determine the drying duration of the fishes and hence, their contact time with the smoke. This finding also corroborates the report of similar study by Silva et al. (2011) that smoked fishes processed by charcoal gave the lowest level of total PAHs, followed by firewood method, while the saw dust method gave the highest level of total PAHs in the smoked fishes. The concentration of total PAHs detected in the raw samples of both *Scomber scombrus* may be attributed to contamination by PAHs from other sources such as oil spills into the fishes' water habitat, preservation, air deposition of smoke particles due to transportation and burning sources since the fish is sold in a market out in the open.

The total PAH level of smoked *Horse markerel* (mg/g) was lower than that of smoked *Scomber scombrus* (mg/g). This could be ascribed to the high fat content of the fish compared to that of beef. Akpan et al. (1994) reported that strong correlation exists between fish lipids and PAH compounds; since PAH compounds are stored in fatty fish tissue.

Based on the law of EU regulatory commission when an expert opinion from European Food Safety Authority questions the statement previously made by Scientific Committee on Food, on benzo (a) pyrene as a sole indicator of occurrence of PAH in food, they proposed another way of Indicating PAH with the use of ―the sum of PAH4 (the sum of 4 PAH, benzo (a) pyrene, benzo (a) anthracene, benzo (b) flourathane and Chrysene)‖ as a more suitable indicator for the occurrence of PAHs in food.

The Regulation committee, EU, reviewed the proposal and amends the regulation NO. 835/2011 which have a maximum value for benzo (a) pyrene, 0.005mg/kg, and in the amending regulation EU, No. 1881/2006, published 0.030mg/kg as the maximum levels of PAH in food to be declared carcinogenic or genotoxic.

TABLE 4: THE CONCENTRATION OF THE PAH4 FROM TABLE 3

| Carcinogenic PAH | Mean concentration mg/kg | | EU max. value (0.030mg/kg) | |
|---|---|---|---|---|
| | Scomber | Horse | Scomber | Horse |
| Benzo (a) pyrene | Not detected | Not decteted | below | below |
| Benzo (a) anthracene, | 0.395 | 0.235 | above | above |
| Anthracene | 0.260 | 0.175 | above | above |
| Chrysene | 0.105 | Not detected | above | below |

Conclusion

Polycyclic aromatic hydrocarbons (PAHs) were detected in two species of smoked fish obtained from a market in Southwestern part of Nigeria. Varying levels of PAH were observed in the smoked fish species with the highest total concentration of PAH in *Scomber scombrus.* This is due to its high oil content which is higher than that of *Horse markerel*, and the high lipophilicity level of PAHs in general.

The results also revealed that fish samples smoked using saw-dust had the highest concentrations of PAHs followed by firewood, and charcoal. The investigation of the aromatic compound distributions in all of the fish samples has underlined that there is a heterogeneous PAHs background pollution. All of degradative activities and their effects left an environmental footprint and needs attention. For a sustainable drying method, charcoal smoking produced the healthiest smoked fish product in terms of PAHs contamination. Thus, the use of sawdust should be discouraged, and increase in awareness on the toxicity and risk of PAHs. That will encourage better processing methods of foods by individuals in order to reduce the health risk.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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