

LEVEL OF POLYCYCLIC AROMATIC HYDROCARBONS CONCENTRATION IN SMOKED CATFISH (*Clarias gariepinus*) USING AFRICAN MESQUITE TREE (*Prosopis africana*) WOOD DERIVATIVES AS FUEL

*ONIMISI, M. M., M. M. SALAUDEEN AND C. OBAJE

Department of Fisheries and Aquaculture, Prince Abubakar Audu University, Anyigba, Kogi State

*Correspondence author: onimisimeriyamohmero@yahoo.com, 07032078313

ABSTRACT

The use of wood for thermal processing of fish may enhance desirable organoleptic attributes but may also allow the promotion of undesirable chemical compounds such as polycyclic aromatic hydrocarbons. The study was conducted to compare the polycyclic aromatic hydrocarbons content of smoked African catfish (*Clarias gariepinus*) using the charcoal and firewood of the African mesquite tree (*Prosopis africana*). The total polycyclic aromatic hydrocarbons content, and the sum of the four new polycyclic aromatic hydrocarbons markers (PAH4) namely; benzo(a)pyrene, benzo(a)anthracene, chrysene and benzo(b)fluoranthene were determined in the smoked fish products. The study revealed that the total polycyclic aromatic hydrocarbon was higher in the fire wood sample ($105.75 \pm 13.69 \mu\text{g/kg}$) as compared to the charcoal sample ($74.78 \pm 6.45 \mu\text{g/kg}$). PAH4 content was also higher in the firewood sample ($37.23 \pm 9.15 \mu\text{g/kg}$) as compared with the charcoal sample ($24.58 \pm 2.77 \mu\text{g/kg}$). All the samples exceeded the maximum limits set by the European Commission for PAH4 and Benzo(a)pyrene levels in smoked fish products. Control measures such as reducing the smouldering temperature and time, discarding the outer layer of the smoked fish or the Hazard Analysis Critical Control Points (HACCP) may be applied during fish smoking to reduce the level of polycyclic aromatic hydrocarbons present in the smoked products.

Keywords: Smoked Catfish, Charcoal, Firewood, Polycyclic aromatic hydrocarbons, PAH4

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are common environmental contaminants. They are ubiquitous and are formed during the incomplete combustion of carbonaceous materials (Suchanova *et al.*, 2008). Polycyclic Aromatic Hydrocarbons are compounds that have been the subject of concern in recent years due to their toxic potential (ASTDR, 2009). The International Agency for Research on Cancer (IARC, 2010), has evaluated the carcinogenicity of some polycyclic aromatic hydrocarbons based on evidence in human and experimental animals.

Benzo(a)pyrene is the most studied polycyclic aromatic hydrocarbons and it is often used as a marker for polycyclic aromatic hydrocarbons in ambient air and food. However, four Polycyclic Aromatic Hydrocarbons (PAH4) are used as markers considering their levels of toxicity and carcinogenicity namely, benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene (EC, 2005; EC, 2011).

Fish smoking is the most commonly used method of processing fish in Nigeria. A lot of new technologies have been developed to smoke fish; however the use of wood and wood derivatives is still widely practiced for reasons related to its appearance, aroma and taste (Bolorunduro, *et al.*, 2005; George, *et al.*, 2014 and Adeyeye, *et al.*, 2015). An average fish processor in Nigeria uses any type of wood just to ensure that their fish is smoked without critically evaluating the wood components

as well as their effects on the fish, the processors and fish consumers. Woods used in fish smoking have different properties and can have effects on human health (Diagne, 2002; Clucas and Ward, 2008; Atanda *et al.*, 2015 and Bede-ojimadu & Orisakwe, 2020). It is therefore expedient to continue seeking information as regards these properties to ensure safety of what is consumed. This study seeks to determine and compare the levels of one of such properties (polycyclic aromatic hydrocarbons) in the charcoal and firewood of a commonly used hardwood species (*Prosopis africana*) of fish smoking in Nigeria.

The study addresses safety and health effects of the different materials used in processing smoked fish, with special attention on the use of firewood and charcoal. This will to a large extent create awareness and raise consciousness among the fish processors and consumers on health implications of type of materials used for fish processing.

MATERIALS AND METHODS

Study Area

The study was carried out at the Fisheries and Aquaculture fish farm of the Prince Abubakar Audu University, Anyigba, Kogi State. Charcoal production and smoking of the fish samples were all done at the farm while analysis of Polycyclic Aromatic Hydrocarbons in the smoked fish samples was carried out at the central laboratory of Nigerian Institute for

Oceanography and Marine Research, Victoria Island, Lagos, Nigeria.

Sample Collection

Very dried wood samples of *Prosopis africana* were obtained from Green Alley Farms at Ojuwo-ojonuchegbo in Dekina Local Government Area of Kogi State, Nigeria and transported to the Kogi State University fish farm. The leaves and stems of the tree were collected for identification and taxonomic classification at the Department of Forestry and Wildlife, Prince Abubakar Audu University. Samples of fresh African Catfish (*Clarias gariepinus*) were also obtained from the same fish farm and used for the study.

The method of traditional charcoal production as outlined by Agboola, 2004 was followed procedurally. Charcoal produced from the wood (*Prosopis africana*) were then packed and stored in sacks.

Smoking Procedure

An improved fish smoking kiln fabricated by the Department of Fisheries and Aquaculture, PAAU, Anyigba (Plate 1) was used to smoke the fish. It consists of two compartments and is designed to work with charcoal or with firewood directly.



Figure 1: Fabricated smoking kiln by the Department of Fisheries and Aquaculture, PAAU

Twenty-four fresh samples of 300-350g *Clarias gariepinus* were used for the smoking procedure. They were randomly divided into two equal groups. One group was smoked with the charcoal produced from *Prosopis africana* and was labeled sample A. The second group was smoked with the firewood of *Prosopis africana* and was labeled sample B.

Analysis of Polycyclic Aromatic Hydrocarbons

Modified method 51991 for determination of Polycyclic Aromatic Hydrocarbons (PAHs) and Aliphatic Hydrocarbons in fish by Thermo Fisher Scientific used for PAHs analysis in the fresh and smoked fish samples was adopted. The fish samples were homogenized in a ceramic mortar, and approximately 5 g of each sample was taken for extraction. Extraction of the homogenized samples were conducted in a bottle prewashed with dichloromethane before adding the sample and extracting with hexane (50ml) and shaken for 1 hour on mechanical shaker. The crude extracts were

filtered using simple filtration method and concentrated to a volume of nearly 2 ml using a rotary vacuum evaporator. The concentrates were purified by short-column silica gel chromatography using dichloromethane as the eluting solvent. The eluates were further reduced to a final volume (2 ml) using nitrogen gas and reconstitute with 2ml of chromatographic grade isooctane. The purified extracts (in sealed vials) were kept in the fridge until analysis by GC- FID (Simko 2005).

On analysis, GC-FID was used for the detection of polycyclic aromatic hydrocarbons. The purified sample extracts were kept in the oven. Hydrogen and compressed air was used as ignition gases for the chromatograph with an initial temperature of 60°C for one minute then increased to 21°C and 32°C for fifteen minutes subsequently. Sixteen individual polycyclic aromatic hydrocarbons were determined with their levels recorded. Also, total polycyclic aromatic hydrocarbons for each sample with dilution factor

were also recorded. Final PAH values were calculated using the formula below:

$$\text{Final Result} = \frac{\text{GC result of analyte from chromatogram} \times \text{dilution factor}}{\text{Weight or Volume of sample taken for extraction}}$$

Packaging and Storage of Smoked Fish

Smoked fish samples from each of the two methods were randomly selected and pounded in a mortar differently. Replicates of each sample were made in triplicates and packaged in sealed air-tight polyethylene bags and were all labeled. These were taken for chemical analysis of Polycyclic Aromatic Hydrocarbons.

RESULTS

The results of sixteen identified PAH's analyzed in *C. gariepinus* smoked with charcoal and firewood

from *Prosopis africana* are shown in table 1. Thirteen PAH were detected in the samples of fish smoked with charcoal of *Prosopis africana* while only twelve were detected in the samples smoked with its firewood. A sum of sixteen PAHs identified in samples of *C. gariepinus* smoked with charcoal was found to be $74.78 \pm 6.45 \mu\text{g/kg}$ while total in samples smoked with firewood was found to be $105.75 \pm 13.69 \mu\text{g/kg}$.

Table 1: Occurrence and levels ($\mu\text{g/kg}$) of PAHs in smoked *Clarias gariepinus* using charcoal and fuel wood from *Prosopis africana*

PAHs	Charcoal ($\mu\text{g/kg}$)	Firewood ($\mu\text{g/kg}$)
Naphthalene	399 ± 0.31	4.06 ± 1.14
Acenaphthalene	2.48 ± 0.49	1.23 ± 0.71
Acenaphthene	2.07 ± 0.39	ND
Flourine	2.19 ± 0.01	1.06 ± 0.61
Phenanthrene	3.44 ± 0.67	6.80 ± 3.25
Anthracene	26.00 ± 7.91	48.07 ± 35.27
Fluoranthene	2.61 ± 0.65	2.50 ± 0.04
Pyrene	2.47 ± 0.22	1.56 ± 0.90
Benzo(a)anthracene	9.85 ± 0.41	5.58 ± 0.88
Chrysene	6.42 ± 0.22	4.35 ± 0.00
Benzo(k)fluoranthene	4.95 ± 0.13	3.23 ± 0.00
Benzo(b)fluoranthene	4.96 ± 0.99	23.00 ± 1.68
Benzo(a)pyrene	3.35 ± 0.75	4.30 ± 0.52
Dibenzo(a,h)anthracene	ND	ND
Indeno(1,2,3-cd)pyrene	ND	ND
Benzo(g,h,i)perylene	ND	ND
TOTAL	74.78 ± 6.45	105.75 ± 13.69

Values are mean \pm SD of triplicate values. ND - not detected.

The results of four PAHs identified by the European Commission to be the new markers of toxicity of PAHs in smoked fish for *Clarias gariepinus* smoked with charcoal and firewood are shown in table 2. The

four PAHs analyzed were benzo(a)anthracene, chrysene, Benzo(b)fluoranthene and benzo(a)pyrene.

Table 2: Levels of PAH4 in smoked *Clarias gariepinus* using charcoal and firewood from *Prosopis africana*

PAH4	Charcoal	Fuel wood
Benzo(a)anthracene ($\mu\text{g/kg}$)	9.85 ± 0.41	5.58 ± 0.88
Chrysene ($\mu\text{g/kg}$)	6.42 ± 0.22	4.35 ± 0.00
Benzo(b)fluoranthene ($\mu\text{g/kg}$)	4.96 ± 0.99	23.00 ± 1.68
Benzo(a)pyrene ($\mu\text{g/kg}$)	3.35 ± 0.75	4.30 ± 0.52
TOTAL ($\mu\text{g/kg}$)	24.58 ± 2.77	37.23 ± 9.15

Values are mean \pm S.D of triplicate values.

In checking the safety of smoked fish consumed, the European Commission (EC) has set standard limits for the sum of PAH markers (PAH4) and benzo(a)pyrene as sole marker of toxicity in smoked

fish. Table 3 shows the comparison of PAH4 values recorded in *C. gariepinus* smoked with charcoal and firewood from *Prosopis africana* with the maximum limits set by EC in smoked fish.

Table 3: Comparison of levels of PAH markers (PAH4) in smoked *Clarias gariepinus* using charcoal and fuel wood from *Prosopis africana* with recommended levels by European Commission

	PAH4	Benzo(a)pyrene (µg/kg)
Charcoal (µg/kg)	24.58	3.35
Fuel wood (µg/kg)	37.23	4.30
EC maximum limit (µg/kg)	(30.0 until 31/08/2014) (12.0 as from 01/09/2014) EC, 2005 and EC, 2011	(5.0 until 31/08/2014) (2.0 as from 01/09/2014) EC, 2005 and EC, 2011

DISCUSSION

The study as shown in Table 1 showed that samples of fish smoked with firewood from *Prosopis africana* contained significantly ($P < 0.05$) higher levels of polycyclic aromatic hydrocarbons when compared to those smoked with its charcoal. This confirms the study of Suchanova *et al.*, 2008 who reported the release of PAH compounds during incomplete combustion of carbonaceous materials. Derache, 2009 also reported that PAHs may be released during the burning of wood thus this study confirms it as some of the PAHs present in charcoal may have been burnt off leading to its low amount as compared to fuel wood. Silva *et al.* (2011) had earlier reported variations in values of PAHs in different fuel sources which include sawdust, firewood, charcoal and oven dried. Onyemaechi, *et al.* (2017) also reported variations in values of PAHs in fish smoked with different wood types. Forsberg, 2017; Salaudeen *et al.*, 2017 and Racovita *et al.*, 2020 reported different levels of PAHs in smoked fish using different smoking systems, temperature and duration of exposure to smoke.

The study as shown in Table 2 shows that samples of *C. gariepinus* smoked with firewood from *Prosopis africana* contained higher levels of PAH4 as compared to *C. gariepinus* smoked with its charcoal. The results of this research further confirmed the position of Dauda *et al.*, 2020 who opined that different wood derivatives might have different effects on quality and safety of consuming smoked fish. This result therefore implies that fish smoked or barbecued directly on firewood and with more smoke would pose more risks of toxicity and carcinogenicity to consumers.

The result therefore reveals that *C. gariepinus* smoked with charcoal of *P. africana*, and *C. gariepinus* smoked with firewood of *P. africana* had PAH4 levels exceeding the new limits set by EC for smoked fish. Benzo(a)pyrene levels for *C. gariepinus* smoked with charcoal and firewood of *P. africana* also exceeds the new limit. It was recommended by Salaudeen, *et al.*, 2017 to remove the skin of smoked fish before consumption to reduce the concentration of PAHs and PAH4 in the smoked fish.

CONCLUSION

The study concludes that the level of PAHs present in smoked fish is dependent on the type of wood derivative used. It is also conclusive that fish smoking with charcoal is safer compared to firewood as it contains lesser concentration of PAHs. Neither the charcoal nor the firewood of *Prosopis africana* meets the current standards for PAHs in smoked fish. However, its charcoal has significantly lower concentration of PAHs when compared with its firewood.

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