Asian
Science
Bulletin

OPEN ACCESS

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in *Clarias gariepinus* and *Tilapia zillii* Smoked using Charcoal and Gas Smoking Kilns in Benue State

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ABSTRACT

Background and Objective: Fish is highly perishable of all staple commodities. Its perishability nature has necessitated fish preservation. Preservation of fish by smoking has been the major source of fish preservation in Nigeria. However, smoke is known to contaminate fish with polycyclic aromatic hydrocarbons, which are traced to carcinogenicity and mutagenicity. Preservation by smoking carried out by fish marketers in uncontrolled environments using firewood pulse a risk to consumers. Therefore, this study aimed to determine Polycyclic Aromatic Hydrocarbons (PAHs) in *Clarias gariepinus* and *Tilapia zillii* smoked using charcoal and gas smoking kilns in Benue State was carried out. **Materials and Methods:** Ten kilograms each of fresh *Clarias gariepinus* and *Tilapia zillii* were purchased from Wadata fish market landing site. Five kilograms of each fish sample were smoked using charcoal and gas-smoking kilns. The fish samples were then analyzed for Polycyclic Aromatic Hydrocarbons (PAHs) using gas chromatography coupled to a Hewlett packard 5972 mass selective detector. **Results:** The PAHs identified were of high molecular weight except for acenaphthalene, acenapthene and naphthalene, which were low molecular weight compounds. *Tilapia zillii* smoked using a charcoal smoking kiln showed low concentrations of PAHs and were undetectable in a gas smoking kiln. *Clarias gariepinus* recorded high concentrations of PAHs in both gas and charcoal smoking kilns, though all values were within safe limits as recommended by European Union (EU). **Conclusion:** Smoking of fish using a gas kiln resulted in lower levels of PAHs compared to the charcoal kiln. However, the PAH levels in both kilns were within the recommended limits as others were undetected. Therefore, the use of gas and charcoal as fuel sources for smoking fish is recommended as an alternative to the traditional method of firewood.

KEYWORDS

Tilapia zillii, *Clarias gariepinus*, polycyclic aromatic hydrocarbon, smoked, kiln, Benue State

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of molecules containing two or more fused aromatic rings made up of carbon and hydrogen atoms[1]. The chemicals are considered environmental

ISSN: 1813-0070 (Online)
https://doi.org/10.3923/asb.2024.242.248

Received: 23 Oct. 2023
Accepted: 19 Mar. 2024
Published: 30 Sep. 2024

contaminants and are carcinogenic in nature[2]. These products can be found in smoked fish products and the exposure to polycyclic aromatic hydrocarbons is a major concern for human health[3,4]. The PAHs are formed by incomplete combustion or pyrolysis of organic materials[5]. During intense thermal processing, the contamination occurs by direct pyrolysis of fish nutrients[6]. The PAHs are also deposited from smoke produced through incomplete combustion of different thermal agents[4].

Smoke acts as an effective bactericidal, bacteriostatic and antioxidant agent in fish preservation. It also provides a protective film on the surface of the smoked fish[7]. The preservation of food (such as meat and fish products) by curing it with wood smoke has been used since antiquity. As the generation of wood smoke is an example of incomplete combustion, undoubtedly PAHs are generated[8]. Originally the purpose was to preserve the food, partly by drying and partly by adding anti-microbiological constituents such as phenols from the smoke to the food. At present smoking is mainly used to achieve the characteristic taste and appearance of smoked food with preservation playing a minor role[1].

In Nigeria, traditional smoking has been a major means of preserving fish; hence electricity is not dependable for cold storage. This leads to high levels of PAHs contaminated fish products optioned in the markets for consumers to buy. Therefore, this study is aimed at determining the PAH residue level present in *Clarias gariepinus* and *Tilapia zillii* when smoked using charcoal and gas smoking kilns in Benue State and compare with the standard prescribed by the Codex Alimenterius Commission/EU and Nigerian Institute of Standard.

MATERIALS AND METHODS

Study area/duration: A study on the determination of Polycyclic Aromatic Hydrocarbons (PAHs) in *Clarias gariepinus* and *Tilapia zillii* smoked using charcoal and gas smoking kilns in Benue State was carried out from June 2020-December 2020. Benue State is located between Longitude 7°47` and 10°0` East; Latitude 6°25` and 8°8` North, in the middle belt region of Nigeria[9].

Sample collection and processing: Ten kilograms each of fresh *Tilapia zillii* and *Clarias gariepinus* samples were purchased from Wadata fish market, Benue State. Five kilograms of each fish sample were separated for both charcoal and gas smoking kilns. Each fish sample was de-gutted, washed and smoked using both gas and charcoal smoking kiln for 72 hrs. Thereafter, the samples were packaged separately using foil paper placed in bags, then transported to National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State for laboratory analysis.

Extraction and analysis for PAHs: The extraction process was carried out using the method described by Pagliuca *et al.*[10] with little modifications.

Solid-liquid extraction of PAHs: Five grams of anhydrous sodium sulphate and five pre-cleaned glass beads were added into a pre-cleaned extraction flask. Five grams of well-ground homogenized fish samples was placed inside the separatory funnel. About 20 mL of dichloromethane was then added and the separatory funnel was capped tightly. The flask was shaken vigorously until a slurry was formed. More Na_2SO_4 was added and shaken vigorously to produce a free-flowing finely divided slurry. The samples were extracted by the use of a centrifuge.

The solvent layer was pipetted into a collecting vial through a small glass funnel containing a layer of anhydrous sodium sulphate (Na_2SO_4) over a plug of glass wool. The extract was then filtered into a 25 cm^3 concentrator flask using a glass funnel packed with a plug of glass wool. The sample was extracted twice more using 5 cm^3 of dichloromethane and the extracts combined.

The combined extracts were transferred into a concentrator flask. Boiling chips were added to the concentrator flask and the extract was evaporated in a constant temperature hot water bath until the volume was reduced to approximately 1 cm^3, then removed and allowed to cool. The extract was collected and concentrated using a Kuderna-Danish concentrator. The extract was transferred into a vial fitted with a screw cap and stored in a refrigerator before clean up.

SPE clean-up procedure: The extracted samples were purified by passing them through a silica gel column prepared by loading 10 g of activated silica gel onto a chromatographic column (1 cm internal diameter) to about 5 cm. This was topped with 1 cm of anhydrous Na_2SO_4. It was then conditioned with dichloromethane. About 2 cm^3 of the concentrated extract was loaded and eluted with 20 cm^3 of dichloromethane. This method is able to remove the very polar lipids of the extract. Prior to analysis with GC/MS, the extracts obtained were preserved in an amber bottle to avoid oxidation.

GC-MS analysis: Shimadzu GCMS-QP2010 Plus Gas Chromatograph coupled to a Hewlett Packard 5972 Mass Selective Detector (Hewlett Packard L.P., Palo Alto, CA) was used to separate the compounds.

Conditioning of the GC: The SHIMADZU GCMS-QP2010 instrument was employed with a HP5MS column (30 m×0.25 µm×0.25 mm id), which featured a flow rate of 1.58 mL/min. The injection temperature was set at 250°C and the injection mode was set to split. The pressure was maintained at 108kPa and the total flow rate was 6.2 mL/min, resulting in a linear velocity of 46.3 cm/sec.

Conditioning of the MS: The time required for the solvent to be cut was 2.50 min. The speed at which the scan was conducted was 1250 m/sec^2. The starting point for the mass spectrum was 40.0 mass-to-charge ratio and the ending point was 600 mass-to-charge ratio.

PAHs identification and quantification: The PAHs were identified by comparing the retention times of the compounds in the samples to those of the PAH standards. A retention time match of ±1% was used for confirmation, as noted by Anyakora and Coker[11]. After identifying the elution times, the PAHs were confirmed by comparing their mass-to-charge (m/z) ratios to library database values, as reported by Anyakora and Coker[11].

The ions that were being monitored included Polycyclic Aromatic Hydrocarbons (PAHs) ranging from fluorene to benzo(g,h,i)perylene, which are among the 16 priority pollutants listed by the US Environmental Protection Agency (EPA). Additionally, the list of monitored ions included cyclopenta(c,d)pyrene, 5-methylchrysene, benzo(j)fluoranthene and dibenzo(a,l)pyrene, as well as dibenzo(a,e)pyrene, dibenzo(a,i)pyrene and dibenzo(a,h)pyrene.

Statistical analysis: The PAH concentrations were subjected to analysis of variance using GenStat (Discover Version). Fisher's Least Significant Difference (LSD) was used to separate the means at $p<0.05$.

RESULTS

PAHs identification: The PAHs identified from these study were acenaphthalene (ACPL), acenapthene (ACP), naphthalene (NAP), Benzo[g,h,i]perylene (BghiP), Dibenzo[a,h]anthracene (DBahA), Dibenzo [a,h]pyrene (DBahP) and Indeno[1,2,3-cd]pyrene (IP). All the PAH compounds detected were of high molecular weight (HMW, $\geq$5 ringed) except acenaphthalene (ACPL), acenaphthene (ACP) and naphthalene (NAP) which were low molecular weight (LMW, 2 to 4 ringed) compounds.

PAHs distribution in fish smoked using gas and charcoal kilns: The distribution of average PAH contents in *Clarias gariepinus* and *Tilapia zilli* smoked using gas kiln showed that PAHs were found at undetectable levels in *Tilapia zilli* whereas, *Clarias gariepinus* recorded acenapthene, benzo[g,h,i]perylene,

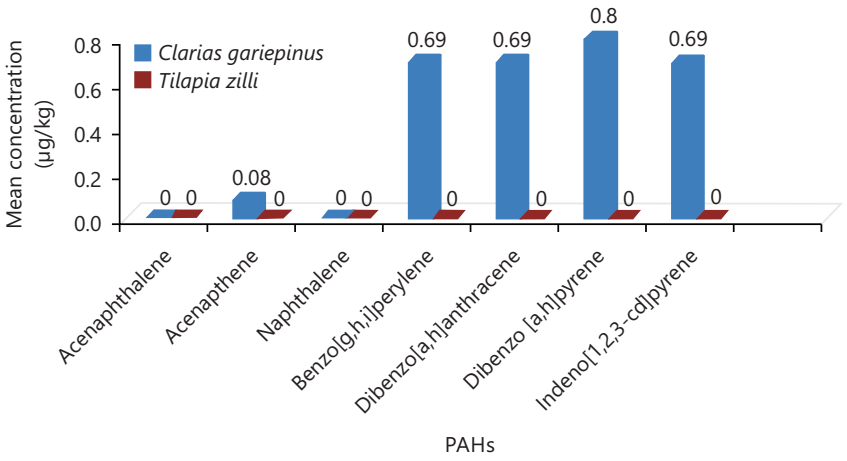

Fig. 1: PAH distribution in fish smoked using gas kiln

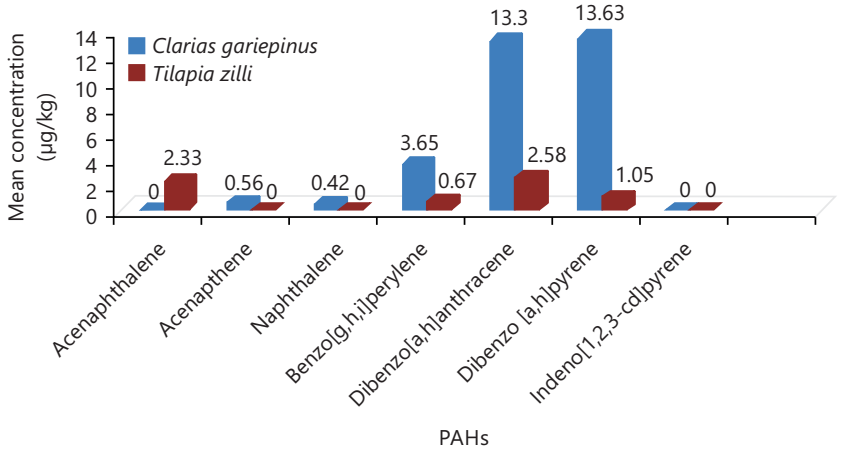

Fig. 2: PAH distribution in fish smoked using charcoal kiln

dibenzo[a,h]anthracene, dibenzo [a,h]pyrene and indeno[1,2,3-cd]pyrene at 0.08, 0.69, 0.69, 0.80 and 0.69 µg/kg, respectively. Though all the values were within the Europian Union (EU) recommended safety limits (Fig. 1).

The distribution of average PAH contents in *Clarias gariepinus* and *Tilapia zilli* smoked using charcoal kiln showed acenaphthene, naphthalene, benzo[g,h,i]perylene, dibenzo[a,h] anthracene and dibenzo [a,h]pyrene in *Clarias gariepinus* at concentrations of 0.56, 0.42, 3.65, 13.30 and 13.63 µg/kg, respectively. Whereas acenaphthalene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene and dibenzo [a,h]pyrene occurred in *Tilapia zillii* at concentrations of 2.33, 0.67, 2.58 and 1.05 µg/kg, respectively. Acenaphthalene, in *Clarias gariepinus*, acenapthene and naphthalene in *Tilapia zilli* and indeno[1,2,3-cd]pyrene in both fish samples were not in traceable limits. Therefore, all the fish samples were within the EU safety limits (Fig. 2).

The mean concentration of various PAHs present in both *Clarias gariepinus* and *Tilapia zillii* samples smoked using gas and charcoal kilns are shown in Table 1. The average total PAHs levels in *Clarias gariepinus* (2.95 µg/kg) and *Tilapia zilli* (0.00 µg/kg) smoked using a gas kiln were lower than that of the charcoal kiln (31.56 and 6.63 µg/kg) (Table 1).

In both kilns, *Clarias gariepinus* recorded the highest PAH level while *Tilapia zillii* recorded the lowest PAH level. However, in all the kilns, the PAH levels were within the European Union (EU) recommended limits.

Table 1: Summary of PAH concentration in smoked fish samples

	Mean concentration (µg/kg)			
	Gas kiln		Charcoal kiln	
PAH	*Clarias gariepinus*	*Tilapia zillii*	*Clarias gariepinus*	*Tilapia zillii*
Acenaphthalene	ND	ND	ND	2.33
Acenaphthene	0.08	ND	0.56	ND
Naphthalene	ND	ND	0.42	ND
Benzo[g,h,i]perylene	0.69	ND	3.65	0.67
Dibenzo[a,h]anthracene	0.69	ND	13.30	2.58
Dibenzo[a,h]pyrene	0.80	ND	13.63	1.05
Indeno[1,2,3-cd]pyrene	0.69	ND	ND	ND
Total	2.95	ND	31.56	6.63

ND: Not detected

Table 2: Interaction effects of PAH concentration of fish by species and smoking method

		PAH concentration (µg/kg)						
Fish species	Smoking method	ACPL	ACP	NAP	BghiP	DBahA	DBahP	IP
Clarias gariepinus	Gas kiln	0.00	0.08	0.00	0.69	0.69	0.80	0.69
	Charcoal kiln	0.00	0.56	0.42	3.65	13.30	13.63	0.00
Tilapia zillii	Gas kiln	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Charcoal kiln	2.33	0.00	0.00	0.67	2.58	1.05	0.00
	LSD (0.05)	NS	NS	NS	NS	NS	NS	NS

NS: No significant difference, ACPL: Acenaphthalene, ACP: Acenaphthene, NAP: Naphthalene, BghiP: Benzo[g,h,i]perylene, DBahA: Dibenzo[a,h]anthracene, DBahP: Dibenzo[a,h]pyrene and IP: Indeno [1,2,3-cd]pyrene

The interaction effects of PAH concentration of fish by species and smoking method show no significant difference ($p<0.05$) across the fish species and smoking methods as presented in Table 2.

DISCUSSION

Polycyclic Aromatic Hydrocarbons (PAHs) were determined in *Clarias gariepinus* and *Tilapia zillii* samples smoked using agas kiln and charcoal kiln in Benue State. The identified PAHs (acenaphthalene (ACPL), acenaphthene (ACP), naphthalene (NAP), Benzo[g,h,i]perylene (BghiP), Dibenzo[a,h]anthracene (DBahA), Dibenzo[a,h]pyrene (DBahP) and Indeno[1,2,3cd]pyrene (IP)), may be regarded as potentially genotoxic and carcinogenic to humans. Therefore, represents a priority group in the assessment of the risk of adverse health effects on consumption of these products either short or long-term effects[5]. This was agreed with the work of Kwaghvihi *et al.*[4] who also detected acenapthene (ACP), anthracene (ANT), phenanthrene (PHE), cyclopenta[c,d]pyrene (CPP) naphthalene (NAP), benzo[a]anthracene (BaA), chrysene (CHR), 5-methylchrysene (MCH), acenaphthalene (ACPL), and 2 methylnaphthalene (MNP) in smoked *Clarias gariepinus*, *Synodontis membranaceus* and *Mormyrus rume* from selected fish species in Benue State. The presence of acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[b]fluoranthene and indeno [1,2,3-cd]pyrene in smoked *Lates niloticus* from selected markets in Uganda was also confirmed by Ongwech *et al.*[12].

The PAH compounds detected in this study were of high molecular weight (HMW, $\geq$5 ringed) except for acenaphthalene, acenapthene and naphthalene which were low molecular weight compounds (LMW, 2 to 4 ringed). This could be due to the type of fuel used during the smoking process[13]. According to the observations of Akpan *et al.*[14] deciduous woods produce smoke with high concentrations of low molecular weight PAHs. Though low molecular weight PAHs were detected their concentrations were much lower than those of high molecular weight PAHs. This can be traced to the fact that the high molecular weight PAHs are more resistant to degradation both in the fish and the environment. This result agreed with the findings of Amos-Tautua *et al.*[15] who reported a high percentage of high molecular weight PAHs to that of low molecular weight. Similar PAH profiles in smoked fish from the Niger Delta and Ghana were also noted by previous research respectively[13,14].

Tilapia zillii smoked using a charcoal smoking kiln showed a low concentration of PAHs and in the gas smoking kiln, was undetectable. While *Clarias gariepinus* recorded high concentrations of PAHs in both gas and charcoal smoking kilns. This could be ascribed to the high-fat content of *Clarias gariepinus* compared to that of *Tilapia zillii*. According to Amos-Tautua *et al*.[15] strong correlation exists between fish lipids and PAH compounds; since PAH compounds are stored in fatty fish tissue. This was in agreement with the work of Newman *et al*.[16] that reported the average total PAH level of suya (14.83 µg/g) to be lower than that of roasted fish (Mackerel, *Scomber scombrus*) (63.43 µg/g).

Polycyclic aromatic hydrocarbon (PAHs) concentrations in all the samples were found to be within the European Union (EU) recommended safety limits. The PAHs were also reported by Kwaghvihi *et al*.[4] in smoked *Clarias gariepinus*, *Synodontis membraneceus* and *Mormyrus rume* from selected fish markets in Benue State, Nigeria and were within safety limits. More so, Newman *et al*.[16] reported PAHs in smoked bush meat in Kumasi Ghana were within safety limits. Similarly, Ongwech *et al*.[12] also reported PAH values in smoked *Lates niloticus* from selected markets, Gulu District, Uganda and were within permissible limits.

Benzo(a)pyrene is the most studied PAH and it is often used as a marker for PAHs in fish. So, its level was lowered from 5 to 2 µg/kg while the other PAHs were lowered from 30 to12 µg/kg[17]. Benzo[a]pyrene is the only polycyclic aromatic hydrocarbon with enough toxicological evidence to allow the setting of a guideline[4] but was found at undetectable levels in all the analyzed samples in the study. The result agreed with the work of Ongwech *et al*.[12] who also found that BaP was undetected in smoked *Lates niloticus* analysed from the markets in Uganda. Similarly, Qin *et al*.[17] reported that BaP was conspicuously absent in both the control and smoked fish samples in Nigeria.

Fish preservation by smoking carried out traditionally by fish marketers in uncontrolled environments using firewood pulse a risk to consumers. Therefore, this study recommends the utilization of other sources of fuel such as gas and charcoal in smoking of fish hence it has been compared favourably with the world standard for PAHs as recommended by the European Union (EU).

CONCLUSION

Smoking fish in gas kilns and charcoal kilns as practiced in the study resulted in a very low level of PAHs and in most cases, it is undetected compared to traditional smoking with firewood in an uncontrolled environment. Therefore, gas and charcoal are recommended as suitable sources of fuel for smoking of fish in other to reduce the potential health hazard posed to humans.

SIGNIFICANCE STATEMENT

This study holds substantial importance due to its multifaceted contributions. Firstly, it addresses a critical research gap by investigating Polycyclic Aromatic Hydrocarbons (pahs) in *Clarias gariepinus* and *Tilapia zillii* smoked using charcoal and gas smoking kilns in Benue State. Secondly, the study aimed at utilizing other sources of fuel such as gas and charcoal in smoking to compare with the world standard as recommended by the European Union (EU). The research findings indicate that the fuel sources examined produced safe products within the acceptable limits for Polycyclic Aromatic Hydrocarbons (PAHs) in both lean and fatty fish. This outcome is crucial for ensuring the safety of fish consumption and maintaining health standards. The PAHs are organic compounds formed during incomplete combustion and can be harmful to human health. Correct actions and adherence to safety standards are crucial to minimize risks associated with smoked fish consumption.

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