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Polycyclic Aromatic Hydrocarbon (PAH) Levels in *Clarias gariepinus* Dried with Traditional and Modern Smoking Kiln

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Abstract

The study compared the polycyclic aromatic hydrocarbon content of *Clarias gariepinus* dried by traditional and modern smoking methods at the teaching and research farm of the Department of Fisheries and Aquaculture Management, Nnamdi Azikiwe University Awka, Nigeria. The traditional method involved using firewood while the modern method involved using charcoal charged smoking kiln to dry the fish. Laboratory analysis of 16 polycylic aromatic hydrocarbons was done by gas chromatography. The traditionally smoked fish contained significantly higher ($P<0.05$) concentrations of fluorene (0.63µg/kg), anthracene (9.02µ/kg), Phenanthrene (3.34 µg/kg), pyrene (55.05 µg/kg) and benzo (a) anthracene (17.55 µg/kg) than the modern smoked fish. The average PAH concentration in traditionally smoked fish (14.568 µg/kg) was significantly higher ($P<0.05$) than the average PAH concentration in the modern smoked fish (4.404 µg/kg). However the maximum acceptable concentration for most PAHs was not exceeded in the smoked fish samples, therefore fish studied was declared wholesome for human consumption. Recommendation of efforts geared towards processing fish with methods using charcoal smoke instead of wood smoke was upheld in order to reduce the levels of PAHs in smoke dried fish.

Keywords: Polycyclic Aromatic Hydrocarbons (Pahs); *Clarias gariepinus*; Traditional Smoking Method; Modern Smoking Methods; Gas Chromatography

Abbreviations: PAHs: Polycyclic Aromatic Hydrocarbons; WHO: World Health Organization; SEM: Standard Error of Mean.

Introduction

Catfish consumption is increasingly becoming popular among Nigerians. It can be consumed in different ways. Due to its perishable nature, many processing and preservative methods are employed to extend its shelf life in Nigeria. Such methods include freezing, drying and smoking. However, smoking is the most common and practicable method of preservation [1,2]. In less developed and developing countries, traditional smoking kiln is very much practiced especially in the tropics due to the sophisticated nature, erratic supply and cost of modern smoking equipment [3]. Smoking enhances the flavour, taste and keeping quality of fish, however consumption of smoked fish is one of the major sources of polycyclic aromatic hydrocarbons (PAHs) in humans. Consumption of these PAHs at certain levels

becomes detrimental to human health. Polycylic aromatic hydrocarbons (PAHs) are large class of organic compounds containing two or more fused aromatic rings without heteroatoms [4,5]. PAHs occur in curing smoke [6] and are known to accumulate on smoked meat [7]. In 2001, PAHs ranked 9th on the list of most threatening compounds to human health [8]. The compounds are lipophilic, chemically stable and poorly degraded by hydrolysis.

The majority of PAHs are readily metabolized and broken down in mammals though; some are bioaccumulated especially in organisms higher up the food chain [9]. They occur in every type of environment as complex mixtures that originate from environmental sources, industrial food processing of drying and smoking, packaging materials and certain alimentary practices [10]. The two processes that may explain formation of PAHs during combustion are pyro synthesis and pyrolysis. Lower hydrocarbons form PAHs by pyro synthesis, while higher alkane present in fuels and plants tissues forms PAHs by pyrolysis. The pyrolysis of organic matter such as fat, carbohydrates and proteins at temperatures above 200_{o}c promotes PAHs formation as well as the yield of lipids dripping in direct contact over the flame at intense heat, a commonly used method for moisture removal of food for better conservation [11].

Food safety is of growing concern globally and PAHs residues present in smoked fish above recommended levels could pose serious public health problem. Consumption of these PAHs at certain levels becomes detrimental to human health [12]. Different mechanisms expose people to PAHs in humans. The majority of people are mostly exposed to PAHs through food sources. Because of the consequences PAHs have on the environment and public health, they have attracted a lot of attention. According to various studies, exposure to the PAHs can cause damage to the brain system and important organs including the liver and kidney as well as cancer, mutation, reproductive abnormalities, immunosuppression, and growth retardation. As a result, they interfere with organism survival [13]. Although PAHs have received much attention in developed countries, studies in developing countries are scarce and limited. With rapid population growth in ,most parts of the country and the multiple anthropogenic activities in most of the of the country which include domestic wastes , industrial discharges and oil spillage, as well as the use of firewood, grasses and other materials for fish smoking processes, serious contamination of fish by PAHs could be expected [14].

The levels and compositions of PAHs in dried food substances vary greatly depending on the materials and techniques used for the drying and hence the compositions in different areas/countries are variable. In Nigeria, traditional smoking kilns and upgraded modern smoking kilns are the two most widely utilized fish drying methods [15]. The PAH content of the dried products may be significantly impacted by a variety of drying processes [16]. Therefore there is need to look into the different energy sources in fish smoking to identify which is the most suitable and minimizes PAHs release. Hence the need for this research work which aims at comparing the PAHs content of *Clarias gariepinus* smoked dried by the traditional and modern smoking methods. Therefore the objectives of this research work is to assess the levels of the following PAHs (Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene, Chrysene, Benz (a)anthracene, Benzo (K) Fluoranthene, Benzo(b) Fluoranthene, Benza (a) pyrene, Indono (1,2,3) pyrene) in smoke dried fish using traditional and modern methods and to compare their levels to the World health organization (WHO) maximum (safety) permissible limit of PAHs in food for human consumption.

Materials and Methods

The study was carried out in the teaching and research farm of the Department of Fisheries and Aquaculture Management, Faculty of Agriculture, Nnamdi Azikiwe University Awka. The PAH evaluation was done at the central laboratory of the Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria. Four matured *Clarias gariepinus* weighing 1.2kg each were harvested from one of the ponds in the departmental fish farm. They were stunned with salt, gutted, washed and brined in 10% saline, cut and spiced with salt. The cuts were 16 pieces and 8 pieces were smoked dried by using the traditional method of burning wood with a metal basket containing the cut fish while the other 8 pieces were smoked dried using the modern smoking kiln (oven) using charcoal. PAHs extraction was carried out by the method of Wrething S, et al. [17]. Homogenized sample weighing 5g was weighed into a 250ml Erlenmeyer flask and 10ml of distilled water was added. The supernatant was transferred into a new bottle. Acetone hexane of 1:2 measuring 10ml was added to the residue and shaken for 10minutues. The mixture was centrifuged at 3000rpm for 10minutes. The supernatant obtained was passed through anlydrous sodium sulphate, before 50ml of hexane was added to the residue. The mixture was shaken and placed in ultrasonic for 10mintues and the supernatant obtained was passed through anhydrous sodium sulphate. The sample obtained after passing through anhydrous sodium sulphate was concentrated by rotary evaporator, transferred into a test tube. The mixture was centrifuged at 2000rpm for 5minutes. The supernatant (hexane layer) was transferred into a new tube. The supernatant was made up to 10ml with hexane. The 10ml supernatant was concentrated to approximately 1ml, 300µl of methanol was added and column chromatography was done. Elution of sample carried was with 10ml of 1:4 (diethylether 20% and Hexane 20%).

Eluate was concentrated to 2ml and transferred into a sample vial and stored in a refrigerator for analysis. Target analytes included sixteen non-alkylated PAHs. Isolation, identification and quantification of the 16 priority pollutants by Stout SA, et al. [18] which follows a standard procedure of organic extraction, sample clean-up and analysis using Agilent G.C. 7890A FID detector type.

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM) and difference between the two groups were considered significant at 5% level of significance using one way analysis of variance SPSS version 20.

Results

The concentration of PAHs (µl/kg) in *Clarias gariepinus* smoked with modern and traditional kiln is presented in Table 1. The results of the analysed *Clarias gariepinus* samples showed PAH levels ranged from non-detectable (n.d) levels to 55.05µg/kg of smoked *Clarias gariepinus*. The table showed that fluoranthene content of 6.75 µg/kg and dibenz (a,b) anthracene content of 4.03 µg/kg were present only in the fish smoked with modern kiln. Acenaphthylene concentration of 3.35 µg/kg detected in the fish smoked with modern kiln was significantly higher than that detected in the traditionally smoked fish (2.36 µg/kg). The traditionally smoked fish contained significantly higher concentrations of fluorene (0.63 µg/kg), anthracene (9.02 µg/kg); Phenanthrene (3.34 µg/kg); Pyrene (55.05 µg/kg) and benzo (a) anthracene (17.55 µg/kg) than the modern kiln smoked fish. Naphthalene, acenaphthene,Chrysene, , benzo (b) fluoranthene, benzo (a)pyrene, indeno (1,2,3-cd) pyrene and benzo (g,h, i) perylene were not detected in any of the smoked *Clarias gariepinus* samples. The average PAH concentration in traditionally smoked fish (14.568 µg/kg) was significantly higher ($p<0.05$) than the average PAH concentration in the modern smoked fish (4.404 µg/kg) at 5% level of significance ($P<0.05$).

S/N	Polycyclic Aromatic Hydrocarbons (PAHs)	Conc. of PAHs in *Clarias G.* µg/kg. Modern Smoking Kiln	Conc. of PAHs in *Clarias G.* µg/kg. Traditional Smoking Kiln	WHO Standard
1	Naphthalene	-	-	ALARA
2	Acenaphthylene	3.35 ± 0.063	2.36 ± 0.859	ALARA
3	Acenaphthene	-	-	ALARA
4	Fluorene	0.53 ± 0.028	0.63 ± 0.225	ALARA
5	Anthracene	8.60 ± 0.049	9.02 ± 0.294	ALARA
6	Phenanthrene	1.46 ± 0.025	3.34 ± 0.368	ALARA
7	Fluoranthene	6.75 ± 0.041	-	ALARA
8	Pyrene	0.58 ± 0.044	55.05 ± 0.232	ALARA
9	Chrysene	-	-	ALARA
10	Benzo (a) anthracene	9.94 ± 0.043	17.55 ± 0.071	ALARA
11	Benzo (k) fluoranthene	1.67 ± 0.025	3.61 ± 0.043	ALARA
12	Benzo (b) fluoranthene	-	-	ALARA
13	Benzo (a) pyrene	-	-	ALARA
14	Indeno (1,2,3-cd) pyrene	-	-	ALARA
15	Dibenz (a,b) anthracene	4.03 ± 0.318	-	ALARA
16	Benzo (g, h, i)perylene	-	-	ALARA
	Average PAHs	4. 404	14.658	
	p-value	0.05	0.05	

ALARA means: As Low as Reasonably Achievable

Table 1: Concentration of PAHs (µl/kg) in *Clarias gariepinus* smoked with modern and traditional kiln (SEM).

Discussion

Generally, the consumption of traditionally smoked fish is 3.3 times higher than the modern ones, resulting in concentration of PAH consumed, thereby posing a greater public health risk of cancer than consuming fish smoked with modern kiln. Akpambang VOE, et al. [19] investigated PAH levels in some smoked/ grilled fish and meat products

commonly consumed in Nigeria and reported heavy contamination with fluorene, anthracene, phenanthrene, pyrene and benzo (a) anthracene in amounts that exceeded the limit of 5mg/kg by European commission. Phenanthrene is known to inhibit and affect the fluid balance of the body and promotes the abnormal functioning of the body nerves and muscles.

Benzo (a) anthracene, the most toxic PAH was found in significantly higher amounts in traditionally smoked fish suggesting danger to human health. Ongwech A, et al. [20] determined PAHs in smoked Lates niloticus from three markets in Gulu district of Uganda and detected the following PAHs in fish samples; acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo (b) fluoranthene and indeno (1,2,3-cd) pyrene. Generally most of the samples analysed had EPAH4 levels within the maximium acceptable risk limits of 30µg/kg as recommended by European commission regulations hence the fish could be deemed fit for human consumption.

Tongo I, et al. [21] studied human health risk assessment of polycyclic aromatic hydrocarbons in smoked fish species from markets in southern Nigeria. They detected significantly higher concentration of benzo (a) pyrene in *Clarias gariepinus* and *Ethmalosa fimbriata* when compared to the baseline value of 0.05mg/kg. Visciano P, et al. [22] studied the polycyclic aromatic hydrocarbons in farmed rainbow trout (*Oncorhynclms mykiss*) processed by traditional fuel gas smoking and by liquid smoke flavourings. These compounds, anthracene, fluoranthene, pyrene, benz (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene and benz (g,h,i) perylene were detected in all fish samples and no significant difference (P>0.05) was found between the two smoking techniques, except for chrysene and benzo(b) fluoranthene. They concluded that PAHs found in rainbow trouts fillets could be considered as a consequence of environmental pollution and that mild smoking process did not affect their concentrations.

Lorenzo JM, et al. [23] studied the polycyclic aromatic hydrocarbons in two Spanish traditional smoked sausage varieties "Androlla" and "Botillo", and reported that total mean levels of PAHs detected were higher in "Androlla" (36.45 µg/kg) than in "Botillo" (29.39 µg/kg) although no significant differences (P>0.05) were observed. They also reported correlation statistical analysis (P>0.01) showed that benzo (a) Pyrene (BaP) was a good marker of 6 International Agency for Research on Cancer Classification (IARC) possible and probable carcinogenic PAHs in "Androlla" (RBaP/6IARC=0.63) and in "Botillo" samples (RBap/6IARC=0.96).

In this study, the highest concentrations of PAHs which include pyrene (55.05µg/kg) and benza (a) anthracene (17.55µg/kg) were observed in traditionally smoked fish. The findings of this study agrees with the findings of Akpambang VOE, et al. [19] who studied the polycyclic aromatic hydrocarbons in commonly consumed Nigerian smoked/grilled fish and meat and concluded that smoking and or grilling when carried out with traditional methods involving direct contact with wood combustion fumes was responsible for high contamination levels with carcinogenic poycyclic aromatic hydrocarbons (PAHs). They also reported that samples that were smoked or grilled using traditional systems which use a wood fire were heavily contaminated with benzo (a) pyrene at levels ranging from 2.4 to 31.2µg/kg. Lower contamination levels were found in samples smoked or grilled in the laboratory using a charcoal fire (BaP from 0.7-2.8 µg/kg). Moreover, Lorenzo JM, et al. [23] reported that the conditions of smoking have a major impact on the quantity of PAHs produced during the processing of fish. Traditional smoking methods usually involve placing the food directly over the smoking wood, which produces smoke at the bottom of the oven. In the contemporary improved smoking kiln, smoke is produced in a separate chamber and fed into the smoking chamber where the items are placed. Better control over the smoking process is encouraged by this method.

Conclusion and Recommendation

The results from this study revealed that the highest concentrations of PAHs which include pyrene (55.05µg/kg) and benza (a) anthracene (17.55µg/kg) were observed in traditionally smoked fish. This demonstrates the need for establishing legal limits of PAHs in traditionally smoked foodstuff in Nigeria and possible risk management action, as the impact of the consumption of traditionally and modern smoked fish is assessed to be significantly different in overall PAH intake. However the maximum acceptable concentration for most PAHs was not exceeded in the smoked fish samples. It was therefore concluded that fish studied was fit for human consumption. Efforts should be directed at processing methods using charcoal smoked instead of wood smoked to reduce the levels PAH in smoked dried fish.

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