International Journal of Multidisciplinary Research and Growth Evaluation.

Studies on polycyclic aromatic hydrocarbon (PAH) concentration on smoke dried African Catfish (*Clarias gariepinus*) Using Difernet Localized Smoke Drying Equipment

Okeke PA [1*], Ichoku CC [2]
[1] Department of Fisheries and Aquaculture, Faculty of Agriculture, Nnamdi, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[2] Department of Fisheries and Aquaculture, Faculty of Agriculture, Nnamdi, Azikiwe University, Awka, Anambra State, Nigeria

* Corresponding Author: **Okeke PA**

Article Info

ISSN (online): 2582-7138
Volume: 04
Issue: 03
May-June 2023
Received: 25-03-2023;
Accepted: 21-04-2023
Page No: 207-217

Abstract
The study of the Polycyclic Aromatic Hydrocarbons (PAH) concentration of smoke dried *Clarias gariepinus*, using different smoking methods (Smoking Drum, Chorkor and Smoking kiln), with hardwood as source of energy, to determine which traditional method produced the best and safest smoke dried fish in terms of lowest PAH concentration for human consumption. The PAH components were determined using Gas Chromatography/Mass Spectrometer (GC/MS) analysis. The results obtained were statistically analysed using SPSS (version 20.0) windows software. The PAH mean concentration and standard error mean (S.E.M), were calculated for each method and results were subjected to one way ANOVA at 0.05% significant level. There were significant difference ($p<0.05$) in the PAHs mean concentration values of (Pyrene, Benzo(g-h)perylene, 1-2 Benzanthracene, Benzo(a)Pyrene, Dibenzyl(a-h)anthracene, Fluorene, Benzo(k)Fluoranthene, Phenanthrene (0.57±0.66, 0.44±0.15, 0.41±0.28, 0.37±0.28, 0.27±0.03, 0.18±0.20, 0.13±0.26, 0.13±0.16) respectively, in fish smoked dried with smoking kiln, and those smoked with chorkor and drum fo PAH mean concentration of (0.25±0.50, 0.30±0.14, o.23±0.27, 0.25±0.20, 0.21±0.29, o.13±0.21, 0.09±0.18, 0.07±0.15 and (0.38±0.52, 0.23±0.19, 0.20±0.33, 0.33±0.12, 0.14±0.17. 0.09±0.18, 0.02±0.03) respectively. There was no significant difference in ($p>0.05$) in the PAH mean concentration values of Acenaphthylene and Naphthalene of fish samples smoked dried with chorkor and drum with (0.13±0.15, 0.16±0.20) and (0.12±0.13, 0.16±0.20) respectively, while Anthracene and kylene were absent in the fish samples smoked dried using all the methods. The PAH content of all the fish samples, were within the permissible limits of 2ppb set by the European Commission Regulation and are safe for human consumption. Benzo(a)pyrene (BaP)as a marker, did not exceed the maximum permissible limit and therefore safe for human consumption. It is recommended that fish processors should be sensitized on the genetoxic and carcinogenic effects of PAHs on the health of consumers of smoked dried fish.

DOI: https://doi.org/10.54660/.IJMRGE.2023.4.3.207-217

Keywords: Polycyclic Aromatic Hydrocarbon, *Clarias gariepinus*, Smoke dried, Chorkor, Drum, Smoking kiln, Hardwood

Introduction

There is a tremendous increase in fish production through aquaculture in Sub-Saharan Africa, especially in the West African countries like Ghana, Nigeria, Togo, Beni Republic etc. This is consequence of improvement in fish technology, transportation, communication, population increase and increase in demand for fish products. It is estimated that 20 % - 50% of fish produced or captured in remote communities and hinterland of many tropical countries perish or spoils before it reaches the final

consumers, due to poor handling, preservation and processing practices adopted by the fisher-folks, processors and fish sellers. These days, attention is being given to fish preservation and processing with the intention to achieving extended shelf-life and improve the fish quality. There is need for commensurate interest to be shown in the technology of fish processing in order to meet up with the consumer's taste, there by enhance fish utilization, marketing and reduction of post harvest losses (Eyo, 2001) [16]. There has been a concerted effort in Nigeria by the government and stake holders in agriculture to improve the protein in-take of her citizens through the consumption of animal (fish) protein. This they believed can be achieved through aquaculture (fish rearing in ponds and other receptacles), which is proving to be effective in the face of rising cost of other sources of animal (chicken, pork, beef, mutton) proteins and the dwindling fish catch from the wild, caused by high cost of production in livestock industries, diseases, water pollution, use of obnoxious fishing methods (dynamite and poison), over fishing, flooding, erosion, tsunami and climate change (Okeke *et al.*, 2014. To prolong shelf life and improve fish quality, adequate interest must be shown in the technology of fish processing to meet consumer's taste and thereby enhance fish utilization and improved marketing of the catch (Eyo, 2001) [16]. It is obvious that the potentials of aquaculture in fisheries development, considering the enormous wet-able-land in Nigeria is not in doubt. In response to the national call for fish culture, Nigerians has embraced fish farming through the rearing of fish in earthen and concrete ponds, reservoirs and cages. The most preferred cultured species is the *Clarias gariepinus* of the family claridae. The preference the catfish *C. gariepinus* is because of these numerous attributes such as; ability to thrive in a wide range of environmental conditions, hardiness, disease resistance, fast growth rate, acceptability of artificial feed, ability to propagate in captivity, palatability and consumers acceptability. The hardiness of *C. gariepinus*, makes it an ideal species for high intensive culture, without prerequisite pond aeration or high-water exchange rate (VASEP, 2005) [46]. Fish is highly susceptible to deteriorate immediately they are captured or harvested. Once the fish dies, a number of physiological and microbial activities commence, which reduces the quality of the fish (Okonta and Ekelemu, 2005 and Adibe *et al.*, 2018) [36, 1]. The fragile and perishable nature of fish, makes it to requires proper handling, prompt preservation and processing once caught, to increase its shelf-life, retain the quality and nutritional status. The best way to avoid fish spoilage and loss of quality is keep harvested or captured fish alive until it's about to be preserved or processed (FAO, 2005) [17]. Immediately a fish dies, a complicated series of chemical and bacteria activity begin to take place within the fish and if not controlled, the fish may be spoilt within 12 hours in a tropical environment. It is estimated that out of the 128.8 million tons of fish produced annually, about 20 million tons are lost due to inability to transform the freshly harvested and captured fish into stable acceptable product, and distributed to those who need them in good quality and at affordable prices (FAO, 2005) [17].

The importance of fish as a cheap source of animal protein and income generator for many people cannot be over emphasised. Fish production is in its ascendency in Nigeria consequence of expansion of aquaculture industries brought about by various development programs of the government to encourage private sector participation, thereby making fish food available to the teeming population. The increase in fish production, has led to the need for good preservation and processing methods to prevent post harvest losses. As a result of this, it became pertinent to study the various traditional methods of fish processing by way of smoking. It is estimated that 70 - 80 % of captured and cultured fish produced in Nigeria are consumed in smoked dried form. The advantages of smoking fish are manifold. Fish smoking prolongs shelf life, enhances flavour and increases utilization in soups and sauces. It reduces wastages in time of bumper catches and/or harvest and allows storage for the lean season. It also increases protein availability to people throughout the year and makes fish easier to handle (pack, transport and market). Fish is consumed by a large number of people because of its palatability, flavour and availability. It gives protein improved nutrition because it has high biological value in terms of high protein retention in the body. It also contains some bioactive compounds with therapeutic properties that are beneficial to human health (Foran *et al*., 2005, Akinola *et al*., 2006 and Nnaji *et al*., 2010) [19, 4, 33].

Polycyclic aromatic hydrocarbons (PAHs) are a group of fused benzene-ring compounds formed during various domestic and industrial combustion as well as natural phenomenon such as volcanic eruption (Freeman and Cattell, 1990., Cappacioni *et al*., 1995) [20]. They have been classified as hazardous compounds of environmental concern due to their carcinogenicity and mutagenicity (Martson *et al.*, 2001., Koyano *et al*., 2001; Liu and Korenga, 2001 and Simko, 2002) [20, 41]. Consequently, sixteen PAHs compounds including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, indeno (1, 2, 3 - c d) pyrene, dibenzo (a, h) anthracene and benzo (g, h, i)perylene have been listed as priority pollutants (European Commission, 2005 and JECFA, 2005) [25]. Polycyclic aromatic hydrocarbons (PAHs) consist of a versatile group of organic compounds that have at least two or more aromatic rings joint together (Commission of European Communities, 2002; Simko, 2002a) [41]. They are fat soluble and chemically stable compounds that are classified as human carcinogens (CEC, 2002). Several metabolic pathways may result in reactive intermediates inducing mutagenic or carcinogenic processes of PAHs (European Food Safety Authority, 2008). The carcinogenic capacity varies, despite having similar structural properties, those with four to six fused rings, such as Benzopyrene (BaP), are effective carcinogens belonging to Group 1 carcinogens according to the International Agency for Research on Cancer (IARC, 2016) [24]. Additionally, PAHs have teratogenic, haematological, and immune-toxic effects, and their concentrations in food should therefore be as low as reasonably achievable (ALARA principle) (Purcaro *et al.*, 2013; and WHO, 2016) [38].

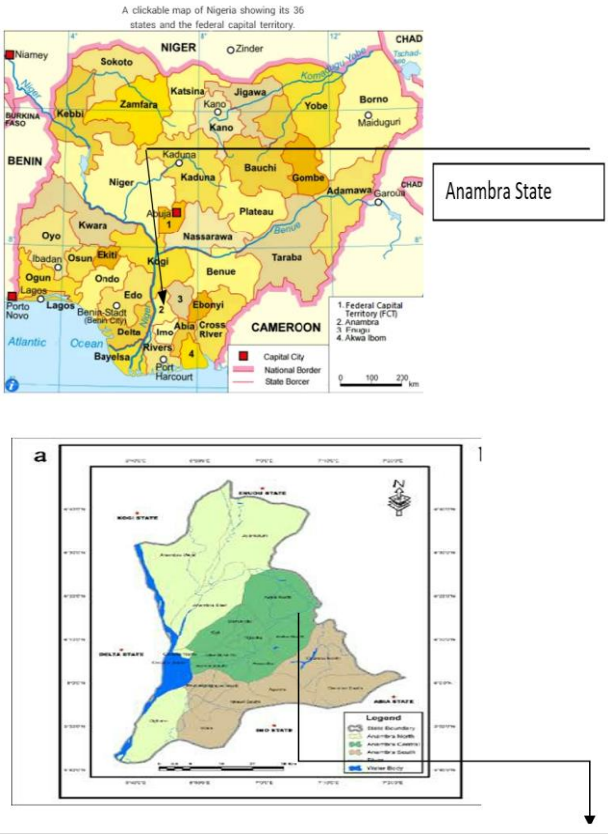
PAHs are formed during the incomplete combustion of organic matter, and they are widely distributed in the environment via air (Scientific Committee on Food, 2016) [40]. Industry, traffic, smoking, forest fires, and volcanic eruptions generate PAHs, and humans are consequently mainly exposed by inhalation, skin contact, and ingestion. Despite also being environmental contaminants, PAHs are formed in food processing, such as drying, grilling, roasting, and smoking (Purcaro *et al.*, 2013; Rose *et al.*, 2015) [38, 39]. For non-smokers, the diet appears to be the main source of PAH exposure (Bansal and Kim, 2015). Food smoking is one

of the oldest preservation methods and is still widely used (Stołyhwo and Sikorski, 2005). Smoking is mainly used to obtain the desired colour, flavour, aroma, and appearance in the smoked food and also, for preservation purposes (Fasano *et al.*, 2016 and Okeke *et al.*, 2020) [35]. Traditional smoking is generally performed by the formation of smoke from wood (Duedahl-Olesen *et al.*, 2010; Purcaro *et al.*, 2013) [12, 38]. Smoke is generated as a result of thermal pyrolysis of wood, when access to oxygen is limited (Purcaro *et al.*, 2013) [38]. PAHs and other chemical compounds occur in smoke particles, which can migrate into the food product being smoked (Wretling *et al.*, 2008). Wood smoke contains a combination of antioxidant and antimicrobial chemicals (e.g., phenols, carboxylic acids, aldehydes, and acetic acids), but also some harmful compounds, such as PAHs (Visciano *et al.*, 2008 and Essumang *et al.*, 2013 and Lingbeck *et al.*, 2014) [47, 14, 29]. PAHs are potential health hazards associated with smoked foods, in which they typically occur as a complex mixture (Stołyhwo and Sikorski, 2005 and Purcaro *et al.*, 2013) [38]. In Nigeria, smoking with direct and indirect techniques is widely used in the processing of meat and fish products. For direct smoking, smoke is generated from an open fire in the same chamber as the smoked product, whereas in indirect smoking, the smoke is generated in an external chamber separated from the food and the smoke is led to the product from the external smoke generator (Duedahl-Olesen *et al.*, 2010; Codex Alimentarius Commission, 2017) [12]. Along-side the smoking technique, the type of smoking process (grilling, roasting, smoking, burning and drying), the distance between the food (fish) and the smoke source, the exposure time, and temperature have impact on the formed PAH levels (Purcaro *et al.*, 2013) [38]. Fish smoking involves the exposure of freshly caught or harvested fish to open flame from dried wood for a period of about 1 or 2 hours to reduce its moisture content, thereafter allowed to air dry. However, this processing method may have negative impacts on consumer health due to the fact that smoking may lead to the deposition of Polycyclic Aromatic Hydrocarbons (PAHs) on smoked fish. The concentrations of PAHs in foods, particularly smoked fish, are affected by the type and composition of wood, temperature of smoke and smoking time (Karl and Leinemann, 1996; Guillen *et al.*, 2000) [27, 22]. It may be inferred that PAHs found in cooked or prepared foods, originated from pyrotic source arising from incomplete combustion of component of the foods or materials used in preparing or cooking the food. PAHs are environmental contaminants, originating from incomplete combustion of organic matter (Jira *et al.*, 2006) [26]. They are formed when complex organic substances are exposed to high temperature or pressure or by the incomplete combustion of woods, coal or oil (Groova *et al.*, 2005; Wretling *et al.*, 2010) [21, 49]. Food can be contaminated by PAHs that are present in air, soil, or water, or during food processing and cooking. PAHs are also found in water though they are hydrophobic especially heavy (PAHs). It is estimated that nearly 70% of PAHs are consumed with food, including the consumption of smoked fish. Out of the several hundreds of PAHs known, only sixteen have been identified as priority PAHs, because they have been considered to be more harmful to man than the others (Andrzej and Zdzislaw, 2005; Anyakorah and Cooker, 2006) [6]. Therefore, this study seeks to investigate the Polycyclic Aromatic Hydrocarbon content of smoke dried *Clarias gariepinus* smoked with hardwood using traditional smoking methods.

Materials and Methods

Study Area

This research was carried out in Awka South and North Local Government Areas of Anambra State, Nigeria. They situate in the Geo-coordinates of longitude 6.2220^0 N and latitude 7.0821^0 E on the map as shown in figure 1 below.



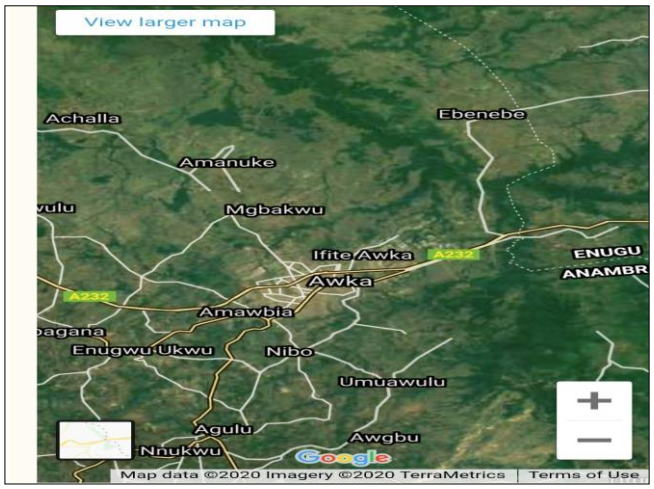


Fig 1: Map of Awka North and South L.G.A. of Anambra State

Sample collection

The fish samples were procured from locations that practice any of the methods of smoke drying. A total of 45kg of *C. gariepinus* was used in this research. These smoking processes were carried out three times at rate of 5kg per replicate using the three different smoking methods (Drum, Chorkor and Kiln).

Fish smoking process

The fish were processed by degutting and removing all the internal organs. Also, the morphological appendages (pectoral fins, pelvic fins, anal fin, dorsal fin, caudal fin and barbells) were removed. They were cut into steaks of 200 gm. weight. They were washed with clean water and properly rinsed. They were then soaked in 10% brine water for 30 minutes. They were then evenly spread on the wire gauze or the rack of the smoking equipment. The fish steaks were watched very closely to prevent the samples from burning or charring. To prevent such from happening, the fish samples were turned regularly at interval. In the case of smoking kiln, the racks were inter-changed. Where by the rack closet to burning chamber is taking to the top while the one at the top is taking down. All the racks are inter-changed in that order. The fish samples were allowed to smoke dry for a period of 8 hrs. at a temperature between 300 and 500^0C. At this time, the moisture content of fish samples were between 9 and 11%. The fish samples were allowed to cool down properly and packaged before they were taken to laboratory for PAHs analysis.

Smoking equipment

Chokor: It is traditional equipment used to smoke dry fish. It is built with red mud soil. Their sizes and shapes vary from locality to locality. The heights are basically the same. The features are wall made with thick red mud, a small door or window by the side through which woods are introduced into the burning chamber and the wire gauze placed at 45cm above the bottom of the chamber.

Plate 1: Picture of a Chorkor

Plate 2: Inside of Chorker chamber

Drum oven: This is made from emptied coal tar drum or any metal drum. The features are a window or door cut at the case for introducing the woods into the burning chamber, the metal wall and the wire gauze or mesh is placed at 45 – 60 cm above the base of the chamber as shown in the picture below.

Plate 3: A smoking Drum

Smoking kiln: This is constructed with metal in form of a cupboard. The height varies depending on the capacity. The features include a burning chamber at the base where wood are introduced. The smoking chamber where combustion pyrolysis occurs is 60 cm above the base and rack partitioning starts from this height and racks placed 45 cm apart. The rack partitions and compartments are constructed in such a way that the rack can easily be removed during the smoking process. The smoking chamber has doors which are always closed when fish are being smoked.

Plate 4: A metal smoking kiln.

PAH laboratory analysis

The fish samples after cooling were stored in a well labelled polyethylene bag and kept in a dry cool place prior to analysis. The lipid extraction of fish samples was done at Springboard Laboratory, Awka, where the GC/MC analysis of the extracted solution were carried out, using Soxhlet

Extraction Method

Soxhlet extraction method: 10.0 gm of homogenized fish muscle were weighed and mixed thoroughly with 5.0 gm of anhydrous sodium sulphate in a laboratory crucible until a complete homogenate was obtained. The extraction was carried out using a Soxhlet extractor apparatus which consists of a 250 cm^3 round bottomed flask, condenser and an extractor tube, seated in a temperature-controlled heating mantle. The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of the Soxhlet extraction unit. The extraction was carried out as recommended by USEPA 3540 method, using 150 cm^3 dichloromethane for 16hrs. (USEPA, 1996). The extract was concentrated to 2cm^3 using a Fischer brand rotary evaporator in a water bath that was pre-set to a temperature of 35°C and stored in an amber bottle and kept in a refrigerator to aoxidation of the extract prior to clean up.

Sample purification

The extracted samples were purified by passing them through a silica gel column prepared by loading 10.0gm of activated silica gel (100-200 Mesh) onto a chromatographic column (1cm to 5cm internal diameter). This was topped with 1cm of anhydrous A_2SO_4 and then conditioned with dichloromethane. 2 cm^3 of the concentrated extract was loaded and diluted with 20 cm^3 of dichloromethane. This method was able to remove the very polar lipids off the extract. Prior to analysis with Glass Chromatography/Mass Spectrometer (GC/MS), the extracts obtained were preserved in an amber bottle to avoid oxidation.

Gas Chromatography/Mass Spectrometer (GC/MS) analysis

Gas Chromatograph equipped with auto sampler connected to an Agilent 5975MSD mass spectrometric detector was used. 1µl of sample solution was injected in the pulsed spilt less mode onto a 30 mm x 0.25 mm id DB5 MS coated fused silica column with a film thickness of 0.15 µ m. Helium was used as the carrier gas and the column head pressure was maintained at 20 psi to give constant flow 1ml/min. the operating conditions were pre-set, pulse time 0.90 min, purge flow 50 cm^3, purge time 1min, and injection temperature 300 °C. The column temperature was initially held at 55°C for 0.4 min, increased to 200°C at a rate of 25°C/min, then to 280°C at a rate of 8°C/min. and to a final temperature of 300°C at a rate of 25°C/min. and held for 2 min at transfer line of 320°C. The mass spectrometer (MS) condition was electron impact positive ion mode. The PAHs identification time was based on retention time since each of the PAHs has its separate retention time in the column. Those with lower retention times were first identified then followed by those with longer retention times. The GC/MS was calibrated with calibration standard concentration. PAHs were identified by comparing the retention times of the peaks with those obtained from standard mixture of PAHs. The standards were supplied by the instrument manufacturers.

Statistical Analysis

The results obtained, were statistically analysed using SPSS (version 20.0) windows software. Mean concentration and standard error of the mean (S.E.M) were calculated for each parameter. The results were subjected to one-way ANOVA at 0.05% level of significant

Results

Table 1, shows the mean values of PAH concentrations (mg/kg) in smoked *C. gariepimus* samples smoked with different smoking equipment (chorkor, smoking kiln and drum). It shows that Kylene and Anthracene were not presence in all the fish samples analysed. It also showed that fish sample smoked with chorkor, smoking kiln and drum, recorded highest and lowest PAH mean values concentration of Benzo(g-h-i)perylene (0.30±0.14) and phenanhthrene (0.07±0.15), Pyrene (0.57±0.66) and Fluoranthene (0.09±0.18) Pyrene(0.38±0.52) and Benzo9K0fluoranthene (0.02±0.03) respectively.

Table 1: Mean PAH concentrations (mg/kg) of *C. gariepinus* smoked dried using Chorkor, smoking kiln and drum

PAHs	Chorkor	Smoking Kiln	Drum
Acenaphthylene	0.13±0.15^{a}	0.00±0.00^{b}	0.12±0.13^{a}
Naphthalene	0.16±0.20^{a}	0.00±0.00^{b}	0.16±0.20^{a}
1-2 Benzanthracene	0.23±0.27^{b}	0.41±0.28^{a}	0.20±0.33^{b}
Acenaphthrene	0.22±0.15^{a}	0.14±0.16^{a}	0.18±0.13^{a}
Benzo(a)pyrene	0.25±0.20^{b}	0.37±0.28^{a}	0.33±0.12^{a}
Kylene	0.00±0.00*	0.37±0.28^{a}	0.00±0.00*
Pyrene	0.25±0.50^{c}	0.57±0.66^{a}	0.38±0.52^{b}
Benzo(g-h-i) perylene	0.30±0.14^{b}	0.44±0.15^{a}	0.23±0.19^{b}
Fluorene	0.09±0.18^{b}	0.18±0.20^{a}	0.09±0.18^{b}
Fluoranthene	0.09±0.18^{a}	0.09±0.18^{a}	0.00±0.00*
Phenanthrene	0.07±0.15^{b}	0.13±0.16^{a}	0.00±0.00*
Dibenzyl(a-h) anthracene	0.21±0.29^{b}	0.27±0.03^{a}	0.14±0.17^{c}
Benzo(k)fluoranthene	0.13±0.21^{a}	0.13±0.26^{a}	0.02±0.03^{b}
Anthracene	0.00±0.00*	0.00±0.00*	0.00±0.00*

a, b, c, values on the same row with the same superscripts are not significantly (p>0.05)

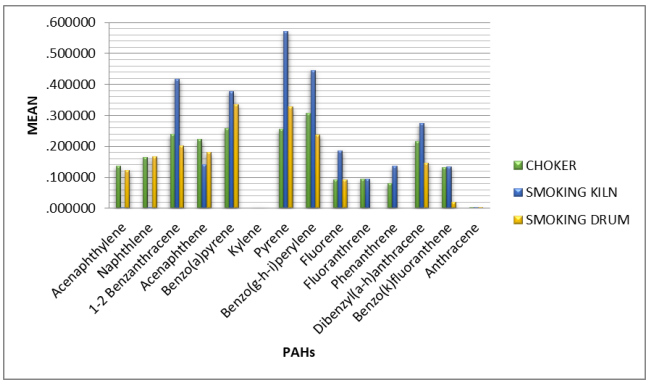


Fig 1: PAH mean value concentration of smoked dried *C. gariepinus*

Figure 1 shows the mean value concentration of PAH in *C. gariepinus* smoked dried with traditional and improved methods. It showed that there were no presence of Kylene and Anthracene in all the three smoking methods studied.

Discussion

In this era of increase in aquaculture production in Nigeria and of the fact that 70% of domestic fish produced are processed through one form of smoking methods or the other. It became pertinent that these various methods of smoke drying fish are studied because of the risk associated with PAH particles deposited by smoke on the fish. Although the PAH and other chemical products of wood pyrolysis, have some beneficial effects on the shelf life and organoleptic qualities of smoked dried fish, about 15 PAHs have been identified as having negative effect on the health of the consumers when their concentration are above the permissible limit recommended by European Union Scientific Committee on food as having genotoxic and carcinogenic effects as reported by Stolyhwo and Sikorski (2005) [43]. Fish smoking is one of the oldest food preservation technologies, which had been used to achieve good organoleptic properties (flavour, texture, taste and colour) because of the source of energy (hardwood), whose smoke possessed bacteriostatic, bactericidal and antioxidant effects, products from pyrolysis of hard wood (Eyo *et al.*, 2001 and Okeke *et al.*, 2018) [16]. Stolyhwo and Sikorski (2005) [43] reported that hardwood smoke contain about 100 PAHs and alklated derivatives and many of them are carcinogenic and Benzo(a)Pyrene (BaP) is regarded as a marker of carcinogenic PAH in smoke and smoked dried fish when it exceeds 2.0 gm/kg of maximum limit standard. While in this research, out of the fourteen (14) targeted Poly aromatic Hydrocarbon (PAH) sorted for, only twelve were found to be presence in the three smoking methods with the exception of Kylene and Anthracene. Also, this study shows no presence of Acenaphthylene and Naphthlene in *C. gariepinus* smoked dried with Smoking kiln and no presence of Flouranthrene and Fluorene on fish sample smoked dried with drum. From the results, it can be observed that Benzo(g-h-i) perylene, Dibenzyl(a-h) anthracene, Benzo(a)pyrene and 1-2 Benzanthracene were highest in fish samples smoked using the smoking kiln methods than samples from chokor and drum methods. Pyrene concentration was significantly higher ($p<0.05$) in samples smoked with smoking kiln with mean value of 0.57±0.66 gm/kg followed by samples from drum and chokor with values of 0.38±0.52 gm/kg and 0.25±0.50 gm/kg respectively. This contradicts the findings of Hafez *et al.* (2017) [23] who reported Anthracene to be present in all the samples of fish he studied. All the 14 targeted PAHs were detected in all the smoked samples except for Kylene and Anthracene which were not detected in any of the smoke dried fish samples. The Benzo(a)Pyrene (BaP) mean values concentrations in this study are (0.25±0.20, 0.37±0.28 and 0.33±0.12) gm/kg^{-1} and are not within the range of 1.5 and 10.5 µg/ kg^{-1} observed in a study of BaP concentrations in four different fish samples from the Niger delta area of Nigeria as reported by Anyakorah *et al.* (2008), which mean (BaP) concentrations values were above the permissible limit and PAH EU limit in the samples. This can be attributed to the presence of naphthalene in the samples, the fish rearing process and possibly through the ingestion of PAH contaminated fish feed. This agrees with the findings of Hafez *et al.* (2017) [23] who found out in his studies, that high level of PAHs in fish muscle can result from fish rearing process, possibly through the ingestion of PAH contaminated fish feed. Therefore, in this study, the smoked products are safe for human consumption because BaP detected does not exceed the maximum permissible limit of 2.0 ppb/kg as set by the European Commission Regulation (Olayemi *et al.*, 2011) [37]. These results are in agreement with the findings of El-Lahamy *et al.* (2016) [13], who reported that BaP was detected in cold and hot smoked catfish fillets. This research also showed smoking kiln accumulated the highest level of PAHs as shown in table 1 and figure 1, followed by smoking drum while the lowest concentration of PAHs was recorded in fish samples smoked with chorkor. The particular reason for this circumstance, is that smoking kiln have holes

in the bottom plate, allowing direct contact with the smoke chamber and also due to the circulating nature of heat and smoke within the smoking chamber and no escape openings like in both drum and chorkor oven were smoke had ways of escape from the smoking fish samples. The result from this research, shows that there were significant difference ($p<0.05$) in the mean values of PAH concentration of all the fish samples smoked dried with chokor, smoking kiln and drum methods. From the table 1, it was observed that (Kylene and Anthracene) were not significantly different, (Acenaphthylene, Benzo(k)fluoranthene, Fluoranthrene, Fluorene, Nephthalene, Acenaphthrene and Phenanthrene) are not significantly different. Dibenzyl (a-h-) anthracene, Benzo(g-h-i) perylene, 1-2 Benzanthracene and Benzo(a)pyrene were significantly different and occurred highest in all the three smoking methods while pyrene is significantly different from the other hydrocarbons in the various smoking methods. More so, it was observed that there was significant difference ($p<0.05$) in the level of hydrocarbons in the three smoking methods (Chokor, smoking kiln, smoking drum). From the result, it was observed that the level of PAHs on the fish samples, using different smoking methods were in these order; Chorkor (Benzo(g-h-i)perylene > Benzo(a)pyrene > Dibenzyl(a-h)anthracene>1-2 Benzanthracene > Anthracene > Kylene). Smoking kiln (Pyrene > Benzo(g-h-i)perylene > 1-2Benzanthracene > Benzo(a)pyrene > Dibenzyl(a-h)anthracene > Anthracene > Kylene. While smoking drum it occurred in this order (Benzo(a)pyrene>pyrene > Benzo(g-h-i)perylene>1-2 Benzanthracene > Acenaphthylene> Anthracene > Kylene). It can be deduced from the table 1, that smoking kiln recorded the highest level of PAHs followed by smoking drum and chorkor in that order. Muiculis *et al*.(2011) reported heavily smoked fish from traditional kilns, especially their outer part, contain up to 50gm/kg of BP wet weight while the meat of mild hot smoked fish, from smoke houses supplied with conditioned wood smoke from external generator contain only about 0.10 gm/kg or less. The level PAH of traditionally smoked fish products is higher in comparison with industrially smoked fish products which exceeded maximum level of PAH established in the amended E. C. Regulation NO.1881/2006. Akpan *et al*. (1994) also recorded that traditional drum smoked fish samples had high BP and PAH levels of fluorine, Anthracene, Benzo(k)fluoranthracene, Benzo(a)pyrene and Benzo(g-h-i)perylene, which exceeded the EU maximum permissible limited of 5.0 gm/kg for BP.

Conclusion

In conclusion, the safety of smoked fish can be controlled by measuring benzo (a) pyrene level, which is one of the most carcinogenic PAHs. European Commission has limited the maximum acceptable concentrations of benzo (a) pyrene at 2 ppb/kg^{-1} for smoked fish and smoked fishery products, excluding bivalve molluscs. The presence of higher levels of PAHs in the smoked samples indicates a higher tendency of these PAHs to become deposited as pyrolytic residues during the smoking process, which Essumang *et al*. (2013) reported the health challenges of PAH on human health to include; Growth retardation, low birth weight, small head circumference, low intelligent quotient, damage DNA in unborn children, disruption of endocrine system, thyroids and steroids. Skin changes, early menopause due to the destruction of ova and breast, lung and other forms of human cancer. Therefore, it could be inferred from the findings that the smoking process generally increases the PAH levels in the fish samples. Therefore, it can be deduced from the fish samples smoked using different smoking methods that, the most efficient processor among the three is smoking kiln followed chorkor and drum.

Recommendation

In the light of the negative health implications of Polycyclic Aromatic Hydrocarbo (PAH) on the health of consumers when consumed continuously at a level above the permissible limit Therefore, it is recommended that Public health authorities (Anambra State Ministry of Agriculture and Rural Development, Anambra State Ministry of Health, Federal Ministry of health, National Agency for Food Drugs Administration and Control-NAFDAC etc.) and other stake holders, should by of control engage fish processors in training and also, set standards for fish processing and preservation in Anambra State and Nigeria, due to the associated public health risks associated with poorly smoked dried fish. Sensitize fisher-folks and the consumers of the health risks associated with it continuous consumption of badly smoked dried fishes.

APPENDIX

Moisture Content

Appendix 1: Moisture Content

Sample Name	% Moisture Content
Sample 1A	7.711
Sample 1B	6.738
Sample 1C	5.668
Sample 1D	5.109
Sample 2A	5.017
Sample 2B	7.427
Sample 2C	5.362
Sample 2D	5.532
Sample 3A	7.348
Sample 3B	6.146
Sample 3C	6.680
Sample 3D	5.966

Ash Content

Appendix 2: Ash Content

Sample Name	% Ash Content
Sample 1A	8.716
Sample 1B	9.222
Sample 1C	5.061
Sample 1D	6.411
Sample 2A	6.096
Sample 2B	5.044
Sample 2C	9.295
Sample 2D	6.495
Sample 3A	7.276
Sample 3B	7.489
Sample 3C	8.000
Sample 3D	6.747

Fibre Content

Appendix 3: Fibre Content

Sample Name	%Fibre Content
Sample 1A	3.310
Sample 1B	3.722
Sample 1C	5.035
Sample 1D	4.348
Sample 2A	6.008
Sample 2B	4.502
Sample 2C	5.801
Sample 2D	5.049
Sample 3A	3.288
Sample 3B	3.685
Sample 3C	3.988
Sample 3D	4.493

Fat Content

Appendix 4: Fat Content

Sample Name	% Fat Content
Sample 1A	10.544
Sample 1B	11.146
Sample 1C	11.920
Sample 1D	15.422
Sample 2A	14.141
Sample 2B	17.154
Sample 2C	19.791
Sample 2D	16.012
Sample 3A	15.203
Sample 3B	16.022
Sample 3C	15.422
Sample 3D	15.471

Protein Content

Appendix 5: Protein Content

Sample Name	% Protein Content
Sample 1A	19.950
Sample 1B	16.800
Sample 1C	19.600
Sample 1D	22.400
Sample 2A	21.000
Sample 2B	20.300
Sample 2C	19.250
Sample 2D	21.000
Sample 3A	14.000
Sample 3B	16.450
Sample 3C	17.150
Sample 3D	20.700

Carbohydrate Content

Appendix 6: Carbohydrate Content

Sample Name	% Carbohydrate Content
Sample 1A	49.769
Sample 1B	52.372
Sample 1C	52.724
Sample 1D	46.310
Sample 2A	48.798
Sample 2B	45.580
Sample 2C	40.501
Sample 2D	45.922
Sample 3A	52.887
Sample 3B	49.402
Sample 3C	48.011
Sample 3D	45.623

Polycyclic Aromatic Hydrocarbons

Sample 1

Appendix 7: Sample 1 PAH

components	1A mg/ml	1B mg/ml	1C mg/ml	1D mg/ml
Acenaphthylene	0.0015	0.2990	0.2499	--
Naphthalene	0.2383	0.000	0.4198	--
1-2 Benzanthracene	--	0.1513	0.1719	0.6308
Acenaphthene	0.3285	0.2708	0.2709	0.6308
Benzo(a) pyrene	0.5042	0.2355	0.2911	--
Kylene	--	0.00	--	--
pyrene	--	0.0036	--	1.0158
Benzo (g-h-i) perylene	0.1655	0.39977	0.4627	0.2014
Fluorene	--	--	0.0030	0.3668
Fluoranthrene	0.3741	--	--	--
Phenanthrene	0.3169	--	--	--
Dibenzyl (a-h) anthracene	0.2333	--	--	0.6332
Benzo (k) fluoranthene	0.0800	--	--	0.4468
Anthracene	--	--	--	0.0018

Sample 2

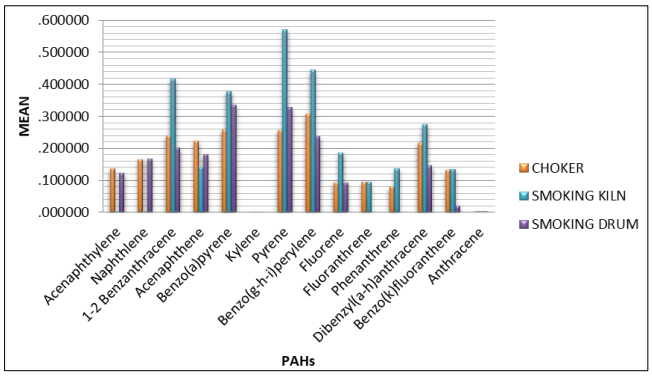
Appendix 8: Sample 2 PAH

components	2A mg/ml	2B mg/ml	2C mg/ml	2D mg/ml
Acenaphthylene	--	0.0018	--	0.0009
Naphthalene	--	--	--	0.005
1-2 Benzanthracene	0.6200	0.2346	0.6981	0.1199
Acenaphthene	0.0056	0.3285	--	0.2285
Benzo(a) pyrene	--	0.5045	0.3434	0.6612
Kylene	--	--	--	--
pyrene	1.0170	--	1.2865	--
Benzo (g-h-i) perylene	0.2118	0.5285	0.5168	0.5285
Fluorene	0.3804	0.0049	0.3585	0.0040
Fluoranthrene	--	0.3742	--	0.0025
Phenanthrene	--	0.3170	--	0.2251
Dibenzyl (a-h) anthracene	0.2608	0.2331	0.3141	0.2855
Benzo (k) fluoranthene	0.5368	0.00	0.0038	0.00
Anthracene	0.0020	--	0.0039	--

Sample 3

Appendix 9: Sample 3 PAH

components	3A mg/ml	3B mg/ml	3C mg/ml	3D mg/ml
Acenaphthylene	0.0100	0.2801	0.2011	--
Naphthalene	0.2552	0.00	0.4112	--
1-2 Benzanthracene	--	0.1155	0.1601	0.6901
Acenaphthene	0.3115	0.1508	0.2600	--
Benzo(a) pyrene	0.5012	0.2623	0.2821	0.3521
Kylene	--	--	--	0.00
pyrene	--	0.3001	--	1.0085
Benzo (g-h-i) perylene	0.1601	0.3325	0.4507	--
Fluorene	--	0.0022	--	0.3622
Fluoranthene	--	--	--	--
Phenanthrene	--	--	--	--
Dibenzyl (a-h) anthracene	0.2341	--	--	0.3526
Benzo (k) fluoranthene	0.0744	--	--	0.0082
Anthracene	--	0.0008	--	0.0031



Appendix 10: barchart representing concentration of PAHs in different smoking method

Plate 1: image showing a Chokor Kiln

Plate 2: Image showing a Chokor Kiln

Plate 3: Image showing fish being smoke dried using drum drying Method

Results

4.1 Proximate composition of analysed fish samples

Table 1 presents the proximate composition of analyzed fish samples. The result revealed significant differences ($p<0.05$) in the fiber and fats contents of the sample while moisture content, crude protein, ash content, and carbohydrate content among the samples were not significant ($p>0.05$). Moisture content was low (5.8±1.0) in the smoking kiln compared to corresponding chorkor and drum. The highest moisture content was recorded in drum (6.5±0.6).

Table 1: Mean proximate composition of analyzed fish samples

Samples	Protein	fats	fibre	moisture	ash	carbohydrate
Chorkor	19.6 ± 2.2^{a}	12.2 ± 2.1^{b}	4.1 ± 0.7^{ab}	6.3 ± 1.1^{a}	7.3 ± 1.9^{a}	50.2 ± 2.9^{a}
Kiln	20.3 ± 0.8^{a}	16.7 ± 2.3^{a}	5.3 ± 0.6^{a}	5.8 ± 1.0^{a}	6.7 ± 1.8^{a}	45.2 ± 3.4^{a}
Drum	17.0 ± 2.7^{a}	15.5 ± 0.3^{a}	3.8 ± 0.5^{b}	6.5 ± 0.6^{a}	7.3 ± 0.5^{a}	48.9 ± 3.0^{a}

Note: significant difference between different smoking methods as against the proximate compositions

4.2 Mean PAH concentrations (mg/kg) in smoked fish samples

From the table below, it was observed that (Kylene and Anthracene) were notsignificantlydifferent, (Acenaphthylene, Benzo (k) fluoranthene, Fluoranthrene, Fluorene, Nephthalene, Acenaphthrene and Phenanthrene) are not significantly different.Dibenzyl(a-h-) anthracene, Benzo(g-h-i) perylene, 1-2 Benzanthracene and Benzo(a)pyrene were significantly different and occurred highest in all the three smoking methods while pyrene is significantly different from the other hydrocarbons in the various smoking methods.More so, it was observed that there was significant difference ($P<0.05$) in the level of hydrocarbons in the three smoking methods (Chokor, smoking kiln, smoking drum).

Table 2: Mean PAH concentrations (mg/kg) in smoked fish samples

PAHs	Chorkor	Smoking Kiln	Drum
Acenaphthylene	0.13 ± 0.15^{a}	0.00 ± 0.00^{b}	0.12 ± 0.13^{a}
Naphthalene	0.16 ± 0.20^{a}	0.00 ± 0.00^{b}	0.16 ± 0.20^{a}
1-2 Benzanthracene0.	23 ± 0.27^{b}	0.41 ± 0.28^{a}	0.20 ± 0.33^{b}
Acenaphthrene	0.22 ± 0.15^{a}	0.14 ± 0.16^{a}	0.18 ± 0.13^{a}
Benzo(a)pyrene	0.25 ± 0.20^{b}	0.37 ± 0.28^{a}	0.33 ± 0.12^{a}
Kylene	$0.00\pm0.00^{*}$	$0.00\pm0.00^{*}$	$0.00\pm0.00^{*}$
Pyrene	0.25 ± 0.50^{c}	0.57 ± 0.66^{a}	0.38 ± 0.52^{b}
Benzo(g-h-i) perylene0.	30 ± 0.14^{b}	0.44 ± 0.15^{a}	0.23 ± 0.19^{b}
Fluorene	0.09 ± 0.18^{b}	0.18 ± 0.20^{a}	0.09 ± 0.18^{b}
Fluoranthene	0.09 ± 0.18^{a}	0.09 ± 0.18^{a}	$0.00\pm0.00^{*}$
Phenanthrene	0.07 ± 0.15^{b}	0.13 ± 0.16^{a}	$0.00\pm0.00^{*}$
Dibenzyl(a-h) anthracene	0.21 ± 0.29^{b}	0.27 ± 0.03^{a}	0.14 ± 0.17^{c}
Benzo(k)fluoranthene0	13 ± 0.21^{a}	0.13 ± 0.26^{a}	0.02 ± 0.03^{b}
Anthracene	$0.00\pm0.00^{*}$	$0.00\pm0.00^{*}$	$0.00\pm0.00^{*}$

($\sum$mPAH) = 14, significant difference of PAHs between the smoking methods .From the result, it was observed that the level of PAHs in the smoking methods is in this order smoking kiln (Pyrene>Benzo(g-h-i)perylene>1-2Benzanthracene> Benzo(a) pyrene> Dibenzyl (a-h)anthracene> Anthracene> Kylene), for smoking drum it occurred in this order (Benzo(a)pyrene>pyrene> Benzo(g-h-i)perylene>1-2 Benzanthracene> Acenaphthylene> Anthracene> Kylene) while for Chorkor (Benzo (g-h-i) perylene> Benzo(a) pyrene> Dibenzyl (a-h) anthracene>1-2 Benzanthracene > Anthracene > Kylene) respectively.

Also, it can be deduced from the table that smoking oven recorded the highest level of PAHs followed by smoking drum and chorkor had the lowest.

References

1. Adibe AC, Okeke PA, Arinze OM. Evaluation of yhe Organoleptic Properties of Clarias gariepinus Smoked with Natural and Artificial Spices. Journal of Biomedicine and Nursing. 2018; 4(4):92-96.
2. Akpan V , Lodovici M, Dolora P. Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from three Nigerian cities. Bulletin of Environmental Contamination and Toxicology. 1994; 53:246-253.
3. Akpan V, Lodovici M, Dolora P. Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from three Nigerian cities. Bulletin of Environmental Contamination and Toxicology. 1994; 53:246-253.
4. Akinola OA, Akinyemi AA, Bolaji BO. Evaluation of traditional and solar fish D\drying systems towards enhancing fish storage and preservation in Nigeria. Journal of Fish International, Pakistan. 2006; 3 (4):44-49.
5. Akintola SL, Brown A, Abdullahi B, Osowo OD, Bello BO. Effects of Hot Smoking and Sun Drying Processes on Nutritional Composition of Giant Tiger Shrimp (Penaeus monodon, Fabricius, 1798) Pol. Journal of Food and Nutritional Science. 2013; 63(4):227-237.
6. Andrzej S, Zdzislaw ES. Polycyclic aromatic hydrocarbons in smoked fish - a critical review. Journal of Food Chemical. 2005; 91:303-311.
7. Anyakora C, Coker H. Determination of polynuclear aromatic hydrocarbons (PAHs) in selected water bodies in the Niger Delta.African Journal of Biotechnology. 2006; 5:2024-2031.
8. Anyakora C, Arbabi M, Coker H. A screen for benzo (a) pyrene in fish samples from crude oil polluted environments. American Journal Environmental Science. 2008; 4:145-150.
9. Bansal V, Kim K. Review of PAH contamination in food products and their health hazards, Environment International. 2015; 84:26-38.
10. Codex Alimentarius Commission. Code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking anddirect drying processes, CAC/RCP, 2017, 68, http:// www.codexalimentarius.org/download/standards/11257 / CXP_068e.pdf.
11. Commission of the European Communities. Commission regulation (EC) no 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs, Official Journal of the European Union. 2002; 88:29-38.
12. Duedahl-Olesen L, Christensen JH, Højgård A, Granby K, Timm-Heinrich M. Influence of smoking parameters on the concentration of polycyclic aromatic hydrocarbons (PAHs) in Danish smoked fish. Food Additives & Contaminants: Part A. 2010; 27(9):1294-1305.
13. El-Lahamy AA, Khalil IK, El-Sherif SA, Award AM. The influence of smoking method on the levels of polycyclic aromatic hydrocarbons (PAHs) in smoked catfish (Clarias gariepinus) fillets. Int J Adv Res. 2016; 4:1529-1538.
14. Essumang DK, Dodoo DK, Adjei JK. Effect of smoke generation sources and smoke curing duration on the levels of polycyclic aromatic hydrocarbon (PAH) in different suites of fish, Food and Chemical Toxicology. 2013; 58:86-94.
15. European Food Safety Authority. Polycyclic Aromatic Hydrocarbons in Food: Scientific Opinion of the Panel on Contaminants in the Food Chain. The EFSA J. 2008; 724:1-114.
16. Eyo AA, Mdaihli M. Assessment of post-harvest losses in Nigeria fishery: the Kainji Lake Model. In: 14th Annual Conference of the Fisheries Society of Nigeria (FISON), Ibadan, Nigeria, pp. 203-212. Biologist. Rep. FAO/ UNDP(TA). 2001; 2771, p. 196.
17. FAO. Post-harvest changes in fish. In: FAO Fisheries and Aquaculture Department, Food and Agriculture Organization, Rome, Italy, 2005, http://www.fao.org/fishery/topic/12320/en
18. Fasano E, Martınez-Carballo IE, Simal-Gándara J. Profiling, distribution and levels of carcinogenic polycyclic aromatic hydrocarbons in traditional smoked plant and animal foods, Food Control. 2016; 59:581-590.
19. Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ. Risk-based consumption advice for farmed Atlantic and wild Pacific salmon contaminated with dioxins and dioxin-like compounds. Environmental Health Perspect. 2005; 33:552-556.
20. Freeman DJ, Cattell FRC. Wood burning as a source of atmospheric PAHs, Environ. Sci. Technol. 2001; 24:1581-1585.
21. Groova N, Monteau F, Le Bizec B, Feidt C, Andre F, Rychen G. Determination of phenanthrene and hydoxyphenanthrenes in various biological matrices at trace levels using gas chromatography coupled to mass spectrometry. J. Anal. Tox. 2005; 29:175-181.
22. Guillen MD, Sopelana P, Partearroyo MA. Polycyclic aromatic hydrocarbons in liquid smoke flavourings obtained from different types of wood, J. Agric. Food Chem. 2000; 48:5083-5087.
23. Hafez NE, Award AM, Ibrahim SM, Mohamed HR. Safety assessment of polycyclic aromatic hydrocarbons (PAHs) in cold smoked fish (Mugil cephalus) using GC-MS, Journal Food Processing Technol. 2017; 8:688. Doi. 10.4172/2157-7110.1000688
24. International Agency for Research on Cancer. Chemical agents and related occupations. A review of human carcinogens. IARC Monographs on the evaluation of carcinogenic risks to humans, 2016, <https://monographs.iarc.fr/wpcontent/uploads/2018/06 /mono100F.pdf.>
25. JECFA. Joint FAO/WHO Expert Committee on Food Additives. Sixty-Fourth meeting, Rome, 2005, 8-17 February, JECFA/64/SC.
26. Jira W, Ziegenhal K, Speer K. PAK in geräucherten Fleischer zeugnissen.Untersuchungennach den neuen EU- Anforderungen. EU-Anforderungen. Fleischwirtschaft. 2006; 86(10):103-106.
27. Karl H, Leinemann M. Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns, Z. Lebensm. Unters. Forsch. 1996; 202:458-464.
28. Koyano M, Mineki S, Tsunoda Y, Endo O, Goto S, Tadhairo I. Effect of fish (mackerel pike) boiling on

polycyclic aromatic hydrocarbons contamination of suspended particulate matter in door air. J. Health Sci. 2001; 47:452-459.
29. Lingbeck JM, Cordero P, O'Bryan CA, Johnson MG, Ricke SC, Crandall PG. Functionality of liquid smoke as an all-natural antimicrobial in food preservation, Meat Science 2014; 97(2):197-206.
30. Liu X, Korenga T. Concentrations and Health risk of polycyclic hydrocarbons in tea. Food. Chem. Toxicol. 2001; 43:41-48.
31. Martson CP, Pereira C, Ferguson J, Fischer KH, Olaf Dashwood W, Baird WM. Magmatic fumaroles: hints of catalytic and thermal reactions, Bulletin of Volcanology. 1995; 56:593-600.
32. Miculis J, Valdovska A, Šterna V, Zutis J. Polycyclic aromatic hydrocarbons in smoked fish and meat, Agronomy Research. 2011; 9(2):439-442.
33. Nnaji JC, Ekwe NP. Effect of smoking on polycyclic aromatic hydrocarbons (PAHs) concentrations in catfish and tilapia muscles, Journal of Applied Sciences and Environmental Management. 2010; 22(2):293-297.
34. Okeke PA. Studies on the Effects of Stocking Densities on Growth Perfprmance and Survival Level of Heterclarias (Hybrid) International Journal of Agriculture and Biosciences. 2014; 3(3):136-140.
35. Okeke PA, Adibe AC, Ezenwenyi JU, Ogbonnaya HF, Moghalu K. Evaluation of The Effect Anthonatha macrophylla (hardwood) Dialium guineense (softwood) and gas oven on the nutrient composition and the organoleptic properties of smoked dried Clarias gariepinus. Biomedecine and Nursing. 2020; 6(2):65-69. Doi: 10.7537/marsbnj060220.07.
36. Okonta AA, Ekelemu JK. A preliminary study of micro-organisms associated with fish spoilage in Asaba, Southern Nigeria, Proceedings of the 20th Annual Conference of The Fisheries Society of Nigeria (FISON), Port Harcourt, 2005, 14-18 November, pp. 557-560.
37. Olayemi FF, Adedayo MR, Bamishaiye EI, Awagu EF. Proximate composition of catfish (Clariasgariepinus) smoked in Nigerian stored products research institute (NSPRI) developed kiln, International Journal of Fisheries and Aquaculture. 2011; 3(5):96-98.
38. Purcaro G, Moret S, Conte LS. Overview on polycyclic aromatic hydrocarbons: occurrence, legislation and innovative determination in foods, Talanta. 2013; 105:292-305.
39. Rose M, Holland J, Dowding A. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting, Food and Chemical Toxicology. 2015; 78:1-9.
40. Scientific Committee on Food. Opinion of the Scientific Committee on food on risks to human health of polycyclic hydrocarbons in food, expressed on 4 December, Report No. SCF/CS/CNTM/PAH/29 Final, Scientific Committee on Food, Brussels, Belgium, October 2016. <https://ec.europa.eu/food/files/safety/docs/sci-com scf out153 en.pdf.>
41. Simko P. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives, Journal of Chromatography B. 2002a; 770 (1-2):3-18.
42. Simko P. Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavourings, Molecular Nutrition and Food Research. 2002b; 49(7):637-647.
43. Stolyhwo A, Sikorski EZ. Polycyclic aromatic hydrocarbons in smoked fish- a critical review. Food chemistry. 2005; 91(2):303-311.
44. United State Environmental Protection Agency. Method 3540C: Soxhlet Extraction test method for evaluating solid waste. USEPA, Washington DC, 1996.
45. USEPA. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Risk Assessment and Fish Consumption Limits, 2000, 3rd ed., vol. 2. EPA 823-B-00-008.
46. VASEP. Vietnam Association of Seafood Exporters and Producers. Weekly Aquaculture Trade Journal. October 7, 2005.
47. Visciano P, Perugini M, Conte F, Amorena M. "Polycyclic aromatic hydrocarbons in farmed rainbow trout (Oncorhynchus mykiss) processed by traditional flue gas smoking and by liquid smoke flavourings,"Food andChemical Toxicology. 2008; 48(5):1409-1413.
48. World Health Organization. Evaluation of certain food additives and contaminants in WHO food additive report series," vol. 28, October 2016, http://www.inchem.org/ documents/jecfa/jecmono/v28je18.html.
49. Wretling S, Eriksson A, Eskhult GA, Larson B. Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. J. Food Comp. Anal. 2010; 23(1):264-272.