

Effect of Processing Techniques on Polycyclic Aromatic Hydrocarbons Contents of Smoked Catfish (*Clarias gariepinus*) Processed in Keffi, Nasarawa State, Nigeria

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants produced by anthropogenic and natural activities. There are more than 100 PAHs, 16 are categorized as PAHs of health concern, and are used to determine the presence and levels of carcinogens in food. The study aimed at determining the effect of processing techniques on the contents of PAHs of smoked Catfish. Four groups of *Clarias gariepinus* each having three samples were used. Three groups were subjected to processing techniques (A. Open fire flame processing technique, B. Charcoal processing technique, and C. Aluminum foil using charcoal processing technique) while the 4th group labelled D was the negative control. PAHs contents were determined using High Performance Liquid Chromatography. The results were expressed as Mean±SD and the significant difference among the groups was assessed using the One-way analysis of variance. The results revealed 15 PAHs, of which 13 were priority PAHs: Acenaphthene, Acenaphthylene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo(a)anthracene, Benzo(k)fluoranthene, Benzo (b) fluoranthene, Dibenz(a,h)anthracene, Benzo(g,h,i)perylene. The highest mean priority PAHs was found in group A (19.96±2.34 µg/kg), while the lowest content was found in group C (4.25±0.89 µg/kg). Benzo[a]pyrene, Naphthalene, and Indeno(1,2,3-cd) pyrene were not detected in both the samples. PAH4 content (6.03±0.28, 1.92±2.41 and 0.56±0.57 µg/kg in group A, B and C respectively) were below the permissible limits (12 µg/kg). The study revealed that catfish is most contaminated by PAHs in group A, whereas group C is least contaminated.

Keywords: Priority PAHs, Smoked Catfish, HPLC, Aluminum Foil, Contamination, Sauki Fish Market Uke.

Introduction

Meat is one of the most significant foods in our daily diet for its valuable constituents and essential nutrients (Nadeem *et al.*, 2021). To improve the quality, safety, sensory attributes and shelf life of meat and meat products, various processing methods are employed (Biswas

et al., 2023). Processed meats, including fish and others, are often associated with industrially produced products that undergo curing and/or smoking processes, potentially contributing to the formation of carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) that can lead to adverse health effects (Geiker *et al.*, 2021).

Polycyclic aromatic hydrocarbons are a class of ubiquitous pollutants, eco-toxicants that are harmful to human health, with some known to be carcinogenic (Malarut & Vangnai, 2018). Polycyclic aromatic hydrocarbons are a group of more than a hundred organic compounds with two or more fused aromatic rings arranged in different configurations that are widespread in the environment as complex chemical mixtures (Zhang *et al.*, 2022a). These compounds are semi-volatile and highly lipophilic, being also resistant to heat, corrosion, and degradation (Zhang *et al.*, 2022a).

PAHs integrate the list of persistent organic pollutants and are released into the air, water resources, and soils via different natural (e.g., volcanic eruptions) but principally through several anthropogenic emission sources (Rovira *et al.*, 2021). PAHs are widely distributed in the environment and human exposure to them is unavoidable. A number of them, such as benzo[a]pyrene, are carcinogenic and mutagenic, and they are widely believed to make a substantial contribution to the overall burden of cancer in humans (White *et al.*, 2016). Humans are exposed to PAHs through inhalation, skin contact, and diet; however, dietary intake significantly impacts non-occupational exposure, with food consumption accounting for over 70% of exposure in non-smokers (Mallah *et al.*, 2022). Exposure to these compounds saturates the xenobiotic pathway in the body, leading to DNA damage and genomic instability (Doroodzani *et al.*, 2021).

PAHs are found in various foods, including smoked meats and vegetable oils, dried herbs, tea, and coffee. The PAHs in food mainly occur from food contamination or as a result of certain processing and cooking methods such as frying, grilling, baking, barbecuing, and smoking. Food processing techniques such as drying and high-temperature cooking methods (e.g., grilling, roasting, frying) are significant sources of PAH contamination in food (Masuda *et al.*, 2019). In this regard, several factors such as cooking duration, fuel type (e.g., coal, gas, wood, electrical), fat drainage, inherent properties of food materials (e.g., pH, composition, and moisture) and temperature used can affect the generation of PAHs in the food (Sharifiarab *et al.*, 2023). During these high-temperature (150–400 °C) thermal processes, proteins, fats, and carbohydrates in the food are pyrolyzed, oxidized, and polymerized to produce PAHs (Edna Hee *et al.*, 2024).

Classifications of PAHs are based on their molecular weights. PAHs with two or three fused rings are grouped as low molecular weight (LMW) while others with four or more fused rings are classed as high molecular weight (HMW). HMW PAHs persists in the environment because of their fractious nature, opposition to percolation, and low volatility. They are known to have carcinogenic, mutagenic, teratogenic, and hepatotoxic effects. The mutagenic or carcinogenic properties of PAHs are the main challenge to human health (Feng-Chen *et al.*, 2020). Due to their mutagenic, carcinogenic and teratogenic potencies

on human health, this group of ubiquitously distributed fused aromatic compounds have attracted particular concern and 16 PAHs were listed as priority pollutants (Tarafdar *et al.*, 2017). The International Agency for Research on Cancer (IARC) classified carcinogenic substances into four categories: Group 1 as carcinogenic to humans, Group 2A as probably carcinogenic to humans, Group 2B as possibly carcinogenic to humans, and Group 3 as not classifiable as carcinogenic to humans (Demers *et al.*, 2022). Benzo[a]pyrene (BaP) belongs to Group 1 and has been investigated for several decades due to its toxic effects, particularly with regard to genotoxicity and carcinogenicity.

The physiochemical properties of PAHs vary considerable with their molecular weight and structure. Distribution and consequent bio-accumulation of PAHs in the environment and organisms are strongly dependent on their physicochemical properties, namely molecular weight, non-polarity, and hydrophobicity, and on the type of interactions established with the surrounding media (Krzyszczak & Czech, 2021).

PAHs accumulate in some tissue, including the liver, lungs, and adipose tissue. In the liver, an accumulation location of enzymes, the BaP activation, and metabolism initiate and turn the potentially carcinogenic compounds into electrophilic genotoxic forms (Barangi *et al.*, 2020). Organisms exposed to this group are predisposed to oxidative damage. It causes DNA to break and produce carbonyl protein and fat peroxidation inside the cell (Liao & Yu, 2020).

Statement of the Problem

Environmental pollution due to PAHs has been a topic of interest to scientists because of their mutagenic and carcinogenic effects (Duan, *et al.*, 2018). Polycyclic aromatic hydrocarbons are a group of environmental contaminants, originating from incomplete combustion of organic matter, which have received much attention over the years because of their ability to cause cancer. Exposure to PAHs is a public health concern due to their many sources of production, frequency of detection in the environment, and known and suspected toxicities. The toxicity of different PAHs varies widely with forms of toxicity including carcinogenesis (Stading *et al.*, 2021), immunotoxicity (Yu *et al.*, 2022), cardiac and vascular toxicities (Ma *et al.*, 2022), neurotoxicity (Olasehinde & Olaniran, 2022), and teratogenesis (Geier *et al.*, 2018a). Carcinogenic components of wood smoke, such as PAH or PAH derivatives such as oxygenated PAH, which are formed as a result of insufficient combustion of organic materials, can cause potential health problems in smoked products (Öz, 2020). By this, an incidence that is capable of affecting negatively the safety and well-being or improvement in the health of the people is considered a public health problem.

Literature Review

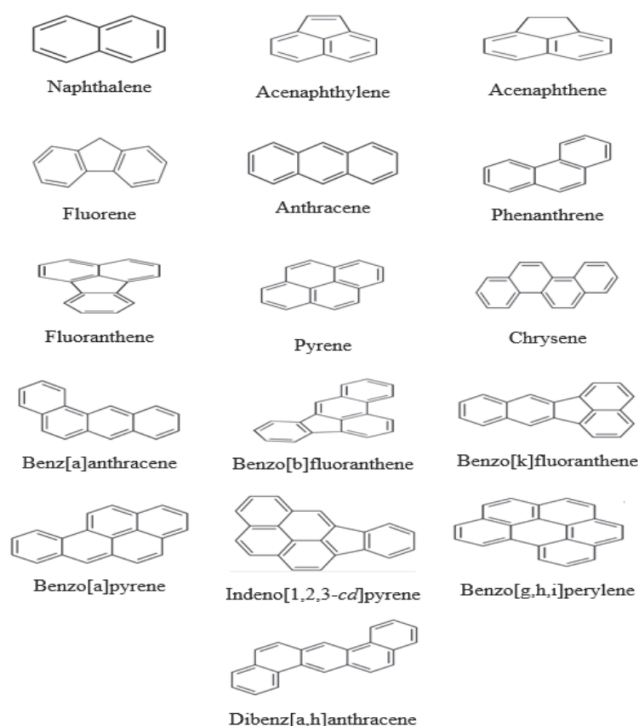


Figure 2.1: Chemical Structures of 16 US EPA's Priority PAHs (source: US EPA)

Table 2.1

PAHs Highest Permissible Limits in Food

S/N	FOODSTUFF	PAH	MAXIMUM LEVELS (µg/kg)
1	Dietary foods for special medical purposes intended specifically for infants	Benzo(a)pyrene of PAH ₄ Sum	1.0 1.0
2	Oils and fats (excluding cocoa butter and coconut oils) intended for direct human consumption or use as an ingredient in food	Benzo(a)pyrene of PAH ₄ Sum	2.0 10.0
3	Smoked meat and smoked meat products	Benzo(a)pyrene of PAH ₄ Sum	2.0 12.0
4	Smoked fish and smoked fishery products	Benzo(a)pyrene of PAH ₄ Sum	2.0 12.0
5	Infant formulae and follow-on formulae, including infant milk and follow-on milk	Benzo(a)pyrene of PAH ₄ Sum	1.0 1.0

FSA, (2015): Toxicology Factsheet Serrie

PAH₄ = (Benzo[a]pyrene, Benzo[a]anthracene, Benzo[b]fluoranthene, and Chrysene)

Novel and Reduction Strategies of PAHs from Processed Foods

1. Washing of smoked meat products

Washing of smoked meat products refers to the removal of skin or outer layer of the product, rinsing with lukewarm water, cleaning under running tap water etc. (Sava *et al.*, 2023). Few reports are available indicating that washing as a procedure is effective in reducing the PAHs content in smoked meat products. (Mahugija & Njale, 2018) observed the effect of washing of smoked products with purified lukewarm water (60°C, 2–3 min) to decrease the total PAHs content significantly.

2. Use of marinades to decrease PAHs

Marination is a universal practice used specially for improving the texture and quality attributes of thermo-processed meat products. Selection of appropriate marinade ingredients can reduce the level of PAHs in the end product by influencing the physio-chemical properties of the processed meat. The reason being, pretreatment of meat with a mixture of spices, fruits or vegetables or their extracts before thermal processing can inhibit the formation of carcinogenic components (Iko Afé *et al.*, 2020)

3. Application of edible coating

Edible coatings have been widely used to improve food quality and safety, such as salt reduction, for antimicrobial purposes, and to improve texture and appearances (Galus *et al.*, 2020). The edible coating may reduce the PAHs content by reducing oil uptake. The edible coating acts as a barrier to inhibit the oil particles penetrating into the food.

4. Application of Probiotics and Lactic Acid Bacteria (LAB)

Biological methods are now becoming promising alternatives over physical and chemical decontamination methods to eliminate carcinogenic and mutagenic compounds from foods. It is because of their GRAS (Generally Recognized as Safe) status, inexpensive nature, and nature-friendly properties. The reason being many microorganisms (bacteria, algae, and fungi) have the ability to utilize PAHs as carbon source required for their growth and development. In recent years, a novel environmentally friendly mitigation strategy to reduce the PAHs contamination in food has drawn the attention by biological degradation procedure with lactic acid bacteria and probiotics (Yousef *et al.*, 2022).

Ecotoxicity of PAHs

Most studies on experimental animals on individual PAHs, most especially on benzo[a]pyrene, have revealed various degree of toxicological effects, such as haematological effects, reproductive and developmental toxicity, immunotoxicity, (FSA, 2015) and possessing a teratogenicity potential (Hokkanen *et al.*, 2018). Their health implications such as carcinogenic and genotoxic (DNA-damaging) potential coupled with their ubiquitous nature in the environment has caused most concern among public health workers. PAHs are said to have teratogenic, haematological, and immunotoxic effects, (Abdel-Shafy & Mansour, 2016) exposing the yet unborn to health danger. Ecotoxicity of PAHs in the aquatic ecosystems is influenced by metabolism and photo – oxidation and are

said to be more toxic in the presence of ultraviolet light, possessing a moderate to high toxicity to aquatic organisms and birds (Abdel-Shafy & Mansour, 2016). Terrestrial organisms are also adversely affected as it is said to be toxic to invertebrates inhabiting the soil, affecting their development and reproduction (Abdel-Shafy & Mansour, 2016).

Research Questions

- i. Are PAHs found in smoked Catfish products?
- ii. Does processing techniques have effect on PAHs contents of smoked catfish?
- iii. Does the quality of catfish processing techniques differ?
- iv. Are levels of PAHs in processed Catfish within the maximum permissible limits?
- v. Can the risks of intake of PAHs be reduced?
- vi. Are PAHs found in other processed food products?

Aim of the Study

The aim of this work was to determine the effect of processing techniques on Polycyclic Aromatic Hydrocarbon contents of smoked Catfish processed in Keffi, Nasarawa state.

Specific Objectives

- i. To extract PAHs from traditional smoked Catfish
- ii. To determine the effect of processing techniques on PAHs contents of smoked Catfish
- iii. To assess catfish processing technique that is more hygienic and safer for human consumption
- iv. To compare the levels of PAHs on smoked Catfish using different techniques.
- v. To popularize strategies to reduce the risks of intake of PAHs in the flesh of smoked Catfish released for human consumption.
- vi. To understand the ubiquitous nature of PAHs in food products

Materials and Methods

Sampling

Three groups of three (3) Catfish (*Clarias gariepinus*) samples each processed using different traditional processing techniques as well as a group of three (3) raw Catfish samples were purchased from Sauki fish market Uke and Keffi area of Nasarawa State, with fish pond being the source. The groups were classified A-D as follows: A. Open fire flame processing technique, B. Charcoal processing technique using wood charcoal, C. Aluminum foil using charcoal processing technique, D. Raw catfish samples.

Study Area

The study on the effect of processing techniques on Polycyclic Aromatic Hydrocarbons contents in *Clarias gariepinus* smoked using different processing techniques in Keffi,

Nasarawa State was carried out from April, 2025 to November, 2025. Keffi is a local government in Nasarawa State located between Latitude 8° 49' N and Longitude 7° 52' E, in the middle belt region of Nigeria.

Sample Processing

Samples purchased were well dried using an Oven. In between the head and the tail regions of each sample oven dried, a central part which is the edible part mostly eaten by man was cut and the bones were removed after which the central parts of the samples cut were homogenized into powder form with the aid of a piston and mortar. The homogenized samples were then packaged in aluminum foil and labeled appropriately as grouped prior to extraction and analysis.

Standards and Reagents

Mixed PAH standards, acetonitrile, n-hexane, Silica Gel, anhydrous sodium sulphate, methanol, distilled water. All reagents and standards used for the analysis were of high HPLC analytical grade.

Materials and Apparatus Used

Aluminum foil paper, Mortar and piston, amber glass bottles, Whatman nylon filter membrane, Agilent 1260 infinity model HPLC system, Oven, centrifuge, micro pipette, analytical balance, hand gloves.

Extraction of PAHs

5g of each type of smoked dried fishes were weighed into amber glass bottles and homogenized for 5min. It was then extracted sequentially by ultrasonication using 25 ml of n-hexane for 1h at room temperature. After ultrasonication, the supernatant of the extracts was decanted into a vial and 15 ml of fresh solvent was added for another 1h of ultrasonication. The process was repeated with another 10ml of fresh solvent for 1h. The combined extracts (50ml) were centrifuged at 2500 rpm for 10min and the supernatant was decanted (Garcia-Falcon *et al.*, 1996). The supernatant was cleaned-up using solid phase extraction (SPE).

Silica Gel Column Clean-up

A slurry of 5g silica gel in n-hexane was transferred to a glass column (length 200 mm; diameter: 12 mm) and 1g anhydrous sodium sulphate was added on top. The excess of solvent was allowed to drain to the level of sodium sulphate, the concentrated sample extract was transferred to the column and eluted with 1 ml n-hexane. The n-hexane was evaporated and redissolved in 2ml methanol.

Chromatographic Analysis of the Selected Catfish Samples

The quantification of PAHs was performed using an Agilent 1260 infinity model HPLC system with a quaternary pump G1311B, vacuum degasser, Autosampler G1329B, a thermostatic column compartment G13164A, and Variable wavelength detector (VWD) G1314C. Separation of the PAHs was performed on an Agilent ZORBAX XDB-C8 Rapid Resolution Column with dimension 4.6mm×150mm, 5µm at ambient temperature (25±1°C) at a flow rate 1.0ml/min. Gradient elution using acetonitrile and water was employed. Peak detection and integration of data was carried out using chemstation software series. External calibration was carried out using mixed PAH standards. From the chromatogram, the retention times of the standards were used for the identification and quantization of the individual PAHs.

Statistical Analysis

The obtained data were expressed as mean±standard deviation and the significant difference among the group was assessed using the One-way analysis of variance (ANOVA) at 95% confidence level. For PAHs compounds with replicate samples less than three per processing technique, inferential statistical test (ANOVA) was not performed due to insufficient data for variance estimation, instead they were presented using descriptive statistics.

Results

Table 4.1

Priority PAHs Contents (µg/kg) for Clarias gariepinus Processed Using Different Processing Techniques

PAHs	Group A	Group B	Group C	Group D	F(df)	p-value
Naphthalene	ND	ND	ND	ND	NS	NS
Acenaphthene	2.27 ± 1.44	1.10 ± 0.15	0.68	2.64 ± 1.83	NS	NS
Acenaphthylene	2.18	ND	ND	ND	NS	NS
Fluorene	0.83 ± 0.43	1.34 ± 0.38	0.52 ± 0.30	0.68 ± 0.45	F(2,6) = 3.689	0.090
Phenanthrene	7.25	2.57 ± 1.24	2.37	1.38 ± 0.18	NS	NS
Anthracene	ND	0.77 ± 0.76	ND	0.27 ± 0.06	NS	NS
Fluoranthene	0.54	0.23 ± 0.24	0.06 ± 0.01	0.18	NS	NS
Pyrene	0.16 ± 0.13	0.08 ± 0.08	0.02 ± 0.00	0.07 ± 0.06	F(2,6) = 2.067	0.208
Chrysene	5.78	1.85 ± 2.42	0.49 ± 0.57	11.99	NS	NS
Benzo(a)anthracene	ND	0.08	ND	ND	NS	NS
Benzo(b)fluoranthene	0.26 ± 0.28	0.00	0.07	0.25	NS	NS
Benzo(k)fluoranthene	ND	ND	ND	0.08	NS	NS
Benzo(a)pyrene	ND	ND	ND	ND	NS	NS
Dibenz(a,h)anthracene	0.64	ND	ND	0.58 ± 0.40	NS	NS
Benzo(g,h,i)perylene	0.06 ± 0.06	0.06 ± 0.05	0.03 ± 0.01	0.10 ± 0.08	F(2,6) = 0.570	0.599
Indeno(1,2,3-cd) pyrene	ND	ND	ND	ND	NS	NS

PAHs	Group A	Group B	Group C	Group D	F(df)	p-value
Total Priority PAHs	19.96 ± 2.34	8.07 ± 5.32	4.25 ± 0.89	18.22 ± 3.06	-	-

Group A: Open fire flame processing technique; Group B: Charcoal processing technique; Group C: Aluminum foil using charcoal processing technique; Group D: Raw Catfish; ND: not detected; NS: not statistically tested. All values are expressed as Mean±SD except for those with only one value of which the results are presented without SD (n = 3).

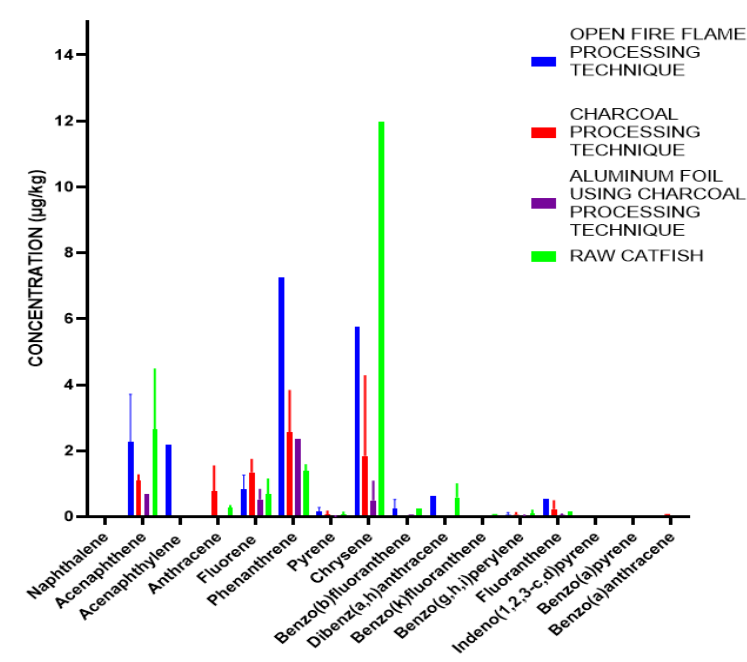


Figure 4.1: Concentration of 16 US EPA's Priority PAHs

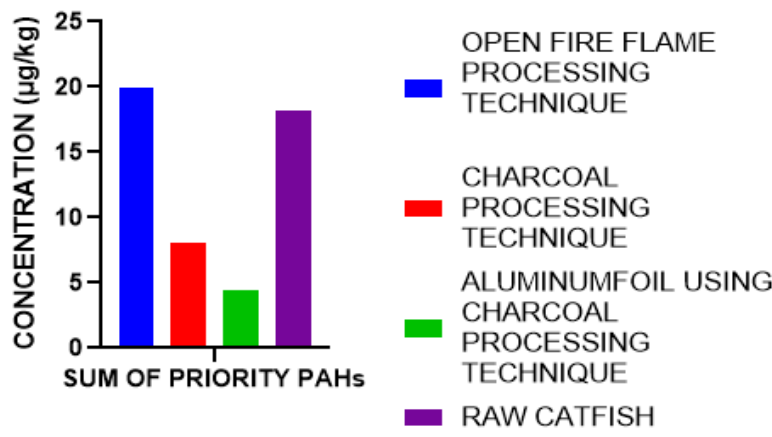


Figure 4.2: Concentration of Sum of Priority PAHs

Table 4.2

Priority PAH₄ Contents (μg/kg) for Clarias gariepinus Processed Using Different Processing Techniques.

PAHs	Group A	Group B	Group C	Group D
Benzo(a)pyrene	ND	ND	ND	ND
Benzo(a)anthracene	ND	0.08	ND	ND
Benzo(b)fluoranthene	0.25 ± 0.28	0.00	0.07	0.25
Chrysene	5.78	1.84 ± 2.41	0.49 ± 0.57	11.99
ΣPAH ₄	6.03 ± 0.28	1.92 ± 2.41	0.56 ± 0.57	12.24

Group A: Open fire flame processing technique; Group B: Charcoal processing technique; Group C: Aluminum foil using charcoal processing technique; Group D: Raw Catfish (Negative control); ND: not detected, ΣPAH₄: sum of the four indicator PAHs. All values are expressed as Mean±SD except for those with only one value of which the results are presented without SD (n = 3).

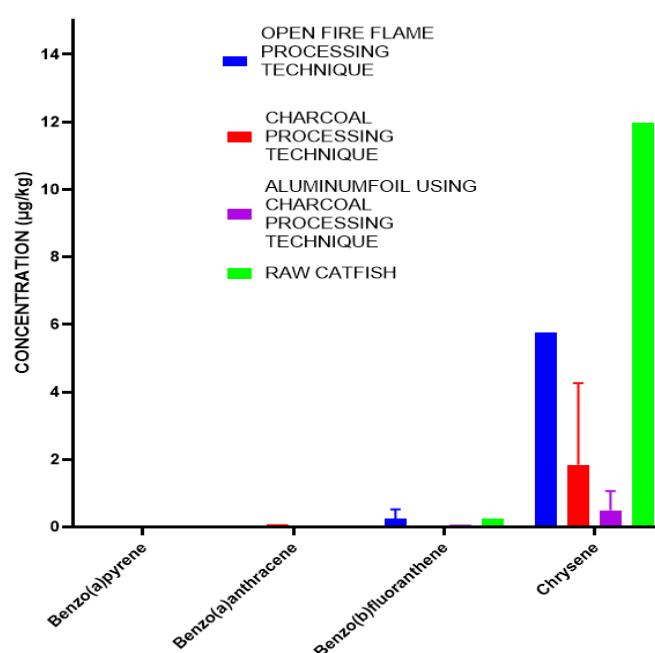


Figure 4.3: Concentration of Priority PAH₄

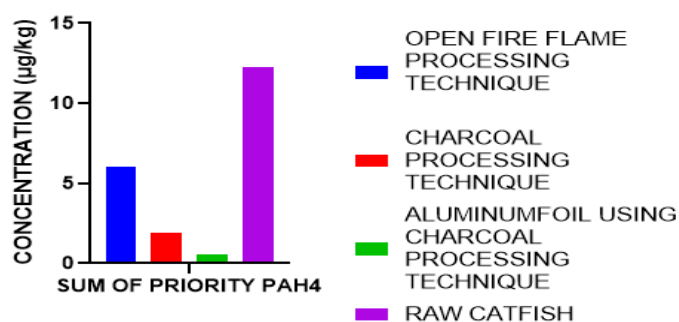


Figure 4.4: Concentration of Sum of Priority PAHs

Discussion of Results

During the study, fifteen (15) PAHs consisting of chrysene, benzo[a]anthracene, anthracene, phenanthrene, fluorene, acenaphthene, benzo(b)fluoranthene, pyrene, Benzo(g,h,i)perylene, acenaphthylene, Dibenz(a,h)anthracene, Benzo(k)fluoranthene, fluoranthene, 2-methylnaphthalene, and 1-methylnaphthalene were recorded from the processed catfish samples analyzed. Thirteen (13) are the priority PAHs while 2-methylnaphthalene and 1-methylnaphthalene are non-priority PAHs. The types of PAHs recorded in both the processing techniques were mostly high molecular weight (Fluoranthene, Pyrene, Chrysene, Benzo(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Dibenz(a,h)anthracene, Benzo(g,h,i)perylene) compounds. 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 agrees with the findings of Amos-Tautua *et al.*, 2013 who reported a high percentage of high molecular weight PAHs to that of low molecular weight. The low molecular weight PAHs detected include anthracene, fluorene, acenaphthene and phenanthrene compounds. Naphthalene, a low molecular weight compound was not detected in all the samples analysed. This negates the work of Kwaghihi *et al.*, 2018 who detected naphthalene in smoked *Clarias gariepinus*, from selected fish species in Benue State. The high molecular weight PAH, benzo[a]pyrene that was considered as indicator for carcinogenic PAHs which is the only polycyclic aromatic hydrocarbon with enough toxicological evidence to allow the setting of a guideline (Kwaghihi *et al.*, 2018), was not detected and quantified in both the samples analyzed. The result agrees with the work of Ongwech *et al.*, 2013 who also found that BaP was undetected in smoked *Lates niloticus* analysed from the markets in Uganda. Similarly, Qin *et al.*, 2012 reported that BaP was absent in both the control and smoked fish samples in Nigeria. This was in conformity with the studies by Hafez *et al.*, 2017. Also, a high molecular weight PAH, indeno[1,2,3-c,d]pyrene was not detected and quantified in all the samples analyzed which negates the finding by Juha, 2023.

Table 4.1 revealed different contents of the priority PAHs obtained on the processed catfish using different processing techniques. The results indicated 13 out of the 16 priority PAHs were detected on the processed catfish. The results revealed that the processed catfish in all the processing techniques were contaminated by PAH.

In group A (Open fire flame processing technique), all the 10 detected Priority PAHs were present in different concentrations (Acenaphthene 2.27 ± 1.44 $\mu\text{g/kg}$, Acenaphthylene 2.18 $\mu\text{g/kg}$, Fluorene 0.83 ± 0.43 $\mu\text{g/kg}$, Phenanthrene 7.25 $\mu\text{g/kg}$, fluoranthene 0.54 $\mu\text{g/kg}$, Pyrene 0.16 ± 0.13 $\mu\text{g/kg}$, Chrysene 5.78 $\mu\text{g/kg}$, Benzo(b)fluoranthene 0.25 ± 0.28 $\mu\text{g/kg}$, Dibenz(a,h)anthracene 0.64 $\mu\text{g/kg}$, and Benzo(g,h,i)perylene 0.06 ± 0.06 $\mu\text{g/kg}$) with Phenanthrene having highest content (7.25 $\mu\text{g/kg}$) followed by Chrysene (5.78 $\mu\text{g/kg}$) while Benzo(g,h,i)perylene recorded the least content (0.06 ± 0.06 $\mu\text{g/kg}$). This processing technique further recorded the sum of the 4 PAHs (Chrysene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(a)pyrene) (6.03 ± 0.28 $\mu\text{g/kg}$) which is below the maximum permissible limits (12.0 $\mu\text{g/kg}$) as adopted by the EU Scientific Committee on Food (SCF)

and the Food Safety Authority of Ireland (FSA, 2015). The group A (Open fire flame processing technique) is the most contaminated catfish processing technique and has the highest recorded sum of the priority PAHs ($19.96 \pm 2.34 \mu\text{g/kg}$) and the PAH₄ ($6.03 \pm 0.28 \mu\text{g/kg}$) respectively. The type of fuel that is used in the preparation and processing of food items generates PAHs. The high concentrations of PAHs could be attributed to the type of wood used during processing (Sampaio *et al.*, 2021). This implies that even though ΣPAH_4 is within the EU–SCF and the FSA set permissible limits ($12.0 \mu\text{g/kg}$), this technique still needs strict monitoring as was reported to possess major health risk (SFMP, 2017).

Group B (Charcoal processing technique) revealed 9 priority PAHs of different concentrations (Acenaphthene $1.10 \pm 0.15 \mu\text{g/kg}$, Fluorene $1.34 \pm 0.38 \mu\text{g/kg}$, Phenanthrene $2.57 \pm 1.24 \mu\text{g/kg}$, Anthracene $0.77 \pm 0.76 \mu\text{g/kg}$, Fluoranthene $0.23 \pm 0.24 \mu\text{g/kg}$, Pyrene $0.08 \pm 0.08 \mu\text{g/kg}$, Chrysene $1.85 \pm 2.42 \mu\text{g/kg}$, Benzo(a)anthracene $0.08 \mu\text{g/kg}$, Benzo (b) fluoranthene $0.00 \mu\text{g/kg}$, and Benzo(g,h,i)perylene $0.06 \pm 0.05 \mu\text{g/kg}$) with Phenanthrene having higher content ($2.57 \pm 1.24 \mu\text{g/kg}$) followed by Chrysene ($1.85 \pm 2.42 \mu\text{g/kg}$) while Benzo(g,h,i)perylene recorded the least content ($0.06 \pm 0.05 \mu\text{g/kg}$). This processing technique also recorded the sum total of priority PAHs ($8.07 \pm 5.32 \mu\text{g/kg}$) and PAH₄ ($1.92 \pm 2.41 \mu\text{g/kg}$) respectively, and is below the maximum permissible limits ($12 \mu\text{g/kg}$) as adopted by the EU Scientific Committee on Food (SCF) and the Food Safety Authority of Ireland (FSA), (2015). This technique is the second most contaminated fish processing technique.

Group C (Aluminum foil using charcoal processing technique) revealed 8 priority PAHs compounds of different concentrations: Acenaphthene $0.68 \mu\text{g/kg}$, Fluorene $0.52 \pm 0.30 \mu\text{g/kg}$, Phenanthrene $2.37 \mu\text{g/kg}$, Fluoranthene $0.06 \pm 0.01 \mu\text{g/kg}$, Pyrene $0.02 \pm 0.00 \mu\text{g/kg}$, Chrysene $0.49 \pm 0.57 \mu\text{g/kg}$, Benzo(b)fluoranthene $0.07 \mu\text{g/kg}$, and Benzo(g,h,i)perylene $0.03 \pm 0.01 \mu\text{g/kg}$, with Phenanthrene having highest content ($2.37 \mu\text{g/kg}$) followed by Acenaphthene ($0.68 \mu\text{g/kg}$) while Pyrene recorded least content ($0.02 \pm 0.00 \mu\text{g/kg}$). This processing technique also recorded the sum total of the priority PAHs (4.25 ± 0.89) and PAH₄ ($0.56 \pm 0.57 \mu\text{g/kg}$) respectively, which is below the maximum permissible limits ($12 \mu\text{g/kg}$) as adopted by the EU Scientific Committee on Food (SCF) and the Food Safety Authority of Ireland (FSA), (2015). The results further suggest that fish processing techniques may have impact on the level of PAHs contamination (Nnaji & Ekwe, 2018). This study presented a much lower level of PAHs contamination which negates the findings of SFMP (2017); Orudu & Peri, (2018), who reported higher concentrations of PAHs on processed fish but a similar trend was reported by Bwala & Imam (2021). The results indicated that group A was the most PAHs contaminated fish processing technique followed by Charcoal processing technique. This suggests that the distance between the heating chamber, fuel type, processing time, fat content, and the processing plates may have contributed to the contamination, and is in conformity with the studies of Bwala & Imam, (2021). Thus, it can be observed that processing technique is an important factor in the formation of PAHs in meat products. Both processing techniques can be utilized, but in

the open fire flame processing technique, contamination may be greater due to the deposition of PAHs produced by the incomplete combustion of materials used in food (Onopiuk *et al.*, 2021). All the priority PAHs compounds in all the samples analyzed were found to be below the EU permitted limit (12 µg/kg) in terms of PAH₄. PAHs were also reported by Kwaghihi *et al.*, 2018 in smoked *Clarias gariepinus*, from selected fish markets in Benue State, Nigeria and were within safety limits. Similarly, Ongwech *et al.*, 2013 also reported PAH values in smoked *Lates niloticus* from selected markets, Gulu District, Uganda and were within permissible limits, while non-priority PAH, 2-methylnaphthalene was found to be above the permissible limit (12 µg/kg) in both the samples analyzed.

The results showed that the mean contents of Phenanthrene in both the processing techniques were highest compared to other PAHs followed by Chrysene still except that in the aluminum foil using charcoal processing technique in which the mean content of Acenaphthene was rather next in concentration. Also, Benzo(g,h,i)perylene recorded the least mean contents in both groups A and B except in group C in which Pyrene recorded the least content.

Group D (raw Catfish) revealed 11 PAHs priority PAHs compounds of different contents as in table 4.1. Chrysene content was highest (11.99 µg/kg) compared to other priority PAHs compounds detected. This result of a high concentration of chrysene in catfish species is in agreement with the reports of Nilsen *et al.*, 2015; Obrist *et al.*, 2015. This group also recorded the sum total of the PAH₄ (12.24 µg/kg) as in table 4.2 and is above the maximum permissible limits (12 µg/kg) as adopted by the EU Scientific Committee on Food (SCF) and the Food Safety Authority of Ireland (FSA), (2015). The occurrence of PAHs in raw or fresh fish samples is a confirmation that the aquatic environment constitutes a sink for runoffs, treated domestic and industrial waste waters, crude wastes, and atmospheric condensate, which are potential sources of PAHs. This agrees with the findings of Hafez *et al.*, 2017 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.

This research showed that Open fire flame processing technique accumulated the highest content of priority PAHs, followed by Charcoal processing technique while the lowest content of PAHs was recorded in catfish processed in Aluminum foil using charcoal processing technique (Figure 4.2). The Aluminum foil (Zn and Al) was able to provide a shield /insolation against PAHs molecules from the heat source and against airborne PAHs molecules, thus rendering the content which is wrapped to be very safe. This finding buttress earlier observation by Zaruwa *et al.*, 2020, that the foil when used in food processing does not leach into the food material.

One-way ANOVA was used to evaluate the effect of processing techniques on individual PAHs with adequate replicates. There were no significant difference ($p > 0.05$) among the three processing techniques for fluorene ($F(2,6) = 3.689$, $p = 0.090$); pyrene ($F(2,6) = 2.067$, $p = 0.208$); benzo(g,h,i)perylene ($F(2,5) = 0.570$, $p = 0.599$). Other PAHs were not statistically tested due to insufficient data, instead descriptive statistics was used. It can be deduced from Figure 4.2 that Open fire flame processing technique recorded the highest mean

content of PAHs ($19.96 \pm 2.34 \mu\text{g/kg}$) followed by Charcoal processing technique ($8.07 \pm 5.32 \mu\text{g/kg}$) while the lowest content of PAHs was recorded in Aluminum foil using charcoal processing technique (4.25 ± 0.89). Therefore, in this study, the processed products are safe for human consumption because benzo[a]pyrene (BaP) was not detected in both the samples analyzed and the total PAH₄ does not exceed the maximum permissible limit ($12.0 \mu\text{g/kg}$) as adopted by the EU Scientific Committee on Food (SCF) and the Food Safety Authority of Ireland (FSA), (2015). These results negate the findings of El-Lahamy *et al.*, 2016, who reported that BaP was detected in cold and hot smoked catfish fillets.

Conclusion

Having analyzed all the samples, the study reveals that processing techniques affect the level of PAHs contents on processed Catfish. Open fire flame technique of fish processing was the most affected technique whereas aluminum foil using Charcoal processing technique was the least affected technique. Benzo[a]pyrene that was considered as indicators for carcinogenic PAHs was not detected in both the processing techniques. Also, the sum of priority PAH₄ contents in both the processing techniques were below the permissible limits by the European Food Safety Authority (EFSA). This study shows that Catfish processed in aluminum foil using charcoal processing technique could be safer and deemed fit for human consumption than those processed using Open fire flame processing technique and Charcoal processing technique.

Recommendations

Fish processors should be sensitized on the genotoxic and carcinogenic effects of PAHs on the health of consumers of smoked catfish and meat products. Furthermore, the awareness of polycyclic aromatic hydrocarbons presents in processed fish which are capable of causing various health conditions such as cancer and other related diseases in man was low. As such, there should be massive awareness by governments, extension agents, social media, and other relevant stakeholders to enlighten the public on the presence of PAHs in processed fish.

Limitations of the Study

This study was only limited to the effect of processing techniques but other variables like the type of fuel used in processing fish, fat content in the fish, sprinkling of cooking oil during processing, etc may have effect on the level of PAHs content, and therefore calls for further study on those other variables.

Conflicts of Interest

All authors have read and approved the manuscript and have no conflict of interest to declare.

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