

Nutritional Composition and Health Risk Associated with Polycyclic Aromatic Hydrocarbons (PAHs) in Fresh and Smoked Fishes

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Abstract:

Apart from evaluating the nutritional contents of food, it is necessary to understand the possible health risk associated with food consumption. Polycyclic aromatic hydrocarbons are among the health risk factors that are found in food and are known to occur in high levels smoked food like fish. There is widespread occurrence of PAH in food products and they are toxic thus it is necessary quantify their levels in food to ensure people are not exposed to PAHs. The levels of nutrients, polycyclic aromatic hydrocarbons (PAHs) and health risks of three fish species Nile Tilapia fish (*Oreochromis niloticus*), Crevalle jack (*Caranx hippos*) and Silver catfish (*Chrysichthys nigrodigitatus*) obtained from two primary fish sale points in Lagos, Nigeria were investigated in this study. The fishes were prepared by smoking some of each of the species from each of the points of collection and then analysed for nutritional and PAH levels using standard protocols. The health risk via consumption was also calculated. The fresh non-smoked fishes had more moisture and carbohydrate than corresponding smoked fishes which had more protein, fibre, total fatty acid and PAH levels. Children had higher health index (HI) values than adults. All PAHs in fresh crevalle jack from Epe were carcinogenic, while fresh tilapia from Epe had no carcinogenic PAH and fresh silver catfish from Makoko contained no PAH. The toxicity equivalent quotient (TEQ), health indices (HI), and total polycyclic aromatic hydrocarbon (TPAH) values followed a certain trend across the different fish types and locations. Protein, fibre, fatty acid, and carbohydrate contents had a positive correlation with each other and with the TEQ, HI adult, HI children, TPAH, TCPAH (total carcinogenic polycyclic aromatic hydrocarbon), and %TCPAH of the fishes. Although smoked fishes had more nutrients, they also had higher health risk index values, which could lead to health problems compared to fresh fishes.

Keywords: Fishes; Health Risk; Nutrition; Polycyclic Aromatic Hydrocarbon; Toxic Equivalence

1- Introduction

Fishes are generally accepted sources of protein and other nutrients and are consumed in almost all parts of the world by various classes of people. Fish is considered an important source of low cost animal protein and nutrients (Allam *et al* 2020) and accounts for about 75% of daily animal proteins in developing countries (Mansour *et al* 2021; Maulu *et al* 2021a).

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Fishes are valuable sources of essential nutrients, especially high quality protein and fats (macronutrients), vitamins, and minerals (micronutrients) (FAO, 2020). Fish consumption has been associated with reduced risk of heart disease, colorectal cancer cognitive impairment and dementia, including Alzheimer's disease (Aglago *et al* 2020; Godos *et al* 2024). Compared to beef or chicken, fish is a preferred option for many people as they are relatively cheap and they have high quality of proteins for human consumption (Ali *et al*, 2021; Khalil *et al* 2021; Maulu *et al* 2021b). Fishes contain 18-20% protein, eight essential amino acids, 0.2 to 25% fat, minerals, vitamins essential for healthy living (Tacon *et al* 2020; Hasselberg *et al* 2020; Maulu *et al* 2021a). There can be bioaccumulation of toxic substances in fishes which can lead to poisoning of humans with some attendant health issues (Lee *et al* 2023). As fishes are perishable products which are prone to decomposition and fermentation (Maulu *et al* 2021a), there have been constant efforts to preserve them. One of the common methods of preserving fish meat is by smoking. Smoked fish is a popular form of fish product, but it may contain polycyclic aromatic hydrocarbons (PAHs), which can be harmful to human health (Tongo *et al.*, 2017) and have potential health risks. Despite the potential risks associated with PAHs in smoked fish, it remains a popular and culturally important food in many societies as it is a good source of protein, lipid and semi- essential fatty acids (Iko Afe *et al.*, 2020).

PAHs are a group of organic compounds with two or more fused aromatic rings with various structural forms and are widely distributed in the environment (Patel *et al* 2020; Sahoo *et al* 2020 Liu *et al* 2024). PAHs are hazardous organic pollutants that are ubiquitous in various environmental media, presenting significant health risks to humans and the environment. PAHs are hydrophobic organic pollutants that can be detected in all the compartments of the ecosystems (Jesus *et al.*, 2022). They are persistent organic pollutants that can originate from various sources, including combustion and petroleum products (Abdel-Shafy and Mansour 2016; Ali *et al.*, 2017). PAHs are also bioavailable and can accumulate in the tissues of aquatic organisms (Aigberua *et al.*, 2023) from which they can be transferred to the human food chain.

PAHs can enter the aquatic environment through various sources, such as atmospheric deposition, accidental oil spills, tanker operations discharge, oil leaks, domestic wastewater, and industrial effluents (Tongo *et al.*, 2017). Due to their high hydrophobicity and persistence, PAHs tend to accumulate in sediment, and their composition pattern can indicate their origin and potential risks to the aquatic environment (Keshavarzifard *et al.*, 2018). PAHs can enter the human body through different routes, with food being a major source for non-smokers and non-occupationally exposed individuals (Lu *et al* 2024). Food can become contaminated with PAHs from various sources, such as environmental pollution, industrial food processing, and some cooking practices (Dan *et al.*, 2020). Smoked fish is one of the significant sources of PAHs in human diets as they are in high levels in thermally processed foods liked smoked fish (Savin *et al* 2024).

Polycyclic aromatic hydrocarbons (PAHs) have been associated with various harmful effects on human health, including carcinogenesis, mutagenesis, immunosuppression, and/or capable of endocrine system disruption at concentrations higher than the maximum allowable limit (Adeniji *et al.*, 2019; da Silva Junior *et al*, 2021; Sampaio *et al.*, 2021; Shamsedini *et al.*, 2022). Long-term exposure to PAHs has been linked to various types of cancers such as skin, lung, bladder, and gastrointestinal cancers, as well as genetic mutations, cell damage, and cardiopulmonary-related mortality (Garcia *et al.*, 2014; Yu *et al* 2022; Mallah *et al* 2022; Dai *et al* 2023; Tartagline *et al* 2023). Some of the PAHs known for their carcinogenic, mutagenic, and teratogenic

activities include benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, coronene, dibenz[a,h]anthracene (C₂₀H₁₄), indeno[1,2,3-cd]pyrene (C₂₂H₁₂), and ovalene (Keshavarzifard *et al.*, 2018).

In addition to their carcinogenic properties, PAHs such as naphthalene can cause liver and kidney damage and failure, skin irritation, heart problems, acute lung dysfunction, jaundice and destruction of red blood cells when inhaled (Ali, 2019; Xu and Shu, 2014). Eye irritation, nausea, and vomiting are some of the short term consequences of PAHs exposure while long-term exposure poses risks of kidney and liver damage, difficulty breathing, and asthma-like symptoms (Venkatraman *et al* 2024). Exposure to PAHs may increase the risk of lung cancer as well as cardiovascular disease (CVD), including atherosclerosis, thrombosis, hypertension, and myocardial infarction (MI) and there has been a reported a relationship between PAH exposure, oxidative stress, and atherosclerosis (Mallah *et al* 2022). In addition, it has been reported that chronic PAH exposure can lead to respiratory diseases, as well as cardiovascular problems and immune system suppression (Montano *et al* 2025). Reactive intermediates in the body from metabolism of PAHs can lead to DNA damage and promote the development of various health conditions, particularly in environments with high exposure levels (Montano *et al* 2025). Male and female fertility can be compromised due to oxidative stress, DNA damage, and endocrine disruption as a result of PAH exposure (Montano *et al* 2025). Exposure to PAHs has also been linked to increased asthma emergency department visits in all age groups due to hypersensitivity to immunoglobulin E (IgE) substances (Vandenpls and Suojalehto, 2014; Hu *et al.*, 2021).

Several studies have shown the presence of PAHs in fishes to be due to two main sources namely bioaccumulation from the environment and contamination via food processing methods (Tongo *et al.*, 2017; Savin *et al* 2024). PAHs are present in a wide range of thermally processed food products thermally processed through roasting, grilling, smoking etc. (Onopiuk *et al.*, 2021; Wang *et al.*, 2022; Zhang *et al.*, 2021). The presence of polycyclic aromatic hydrocarbons (PAHs) residues in smoked fish above recommended levels can pose serious public health concerns (Lu *et al* 2024). Nauta *et al.* (2020) suggest that balancing the risks and benefits of food has become an important public health topic. To assess the health risks to humans, it is necessary to calculate the possibility of any severe health effects resulting from exposure to carcinogenic and/or non-carcinogenic substances over a particular period of time (Kamunda *et al.*, 2016). This research determined and compared the nutritional composition of fresh and smoked fishes and the associated health risks of PAHs in fish. The results obtained can assist in the choice of fish preservation and processing methods to achieve optimal nutrient intake with minimal health risks. The results can also increase public awareness of the potential health hazards associated with consuming PAH-contaminated food

2. Materials and Methods

2.1. Sample Collection

The fish used for this study were bought at the landing site of Epe Lagoon (freshwater) and Lagos Lagoon (Makoko) in Lagos, Nigeria (Figure 1) and were preserved in ice and transported to the chemical laboratory in a polythene bag. The fishes used in this study are Nile Tilapia fish (*Oreochromis niloticus*), Crevalle jack (*Caranx hippos*) and Silver catfish (*Chrysichthys nigrodigitatus*). The fish samples were identified and authenticated at the Aquaculture unit of the Marine Science Department, the University of Lagos.

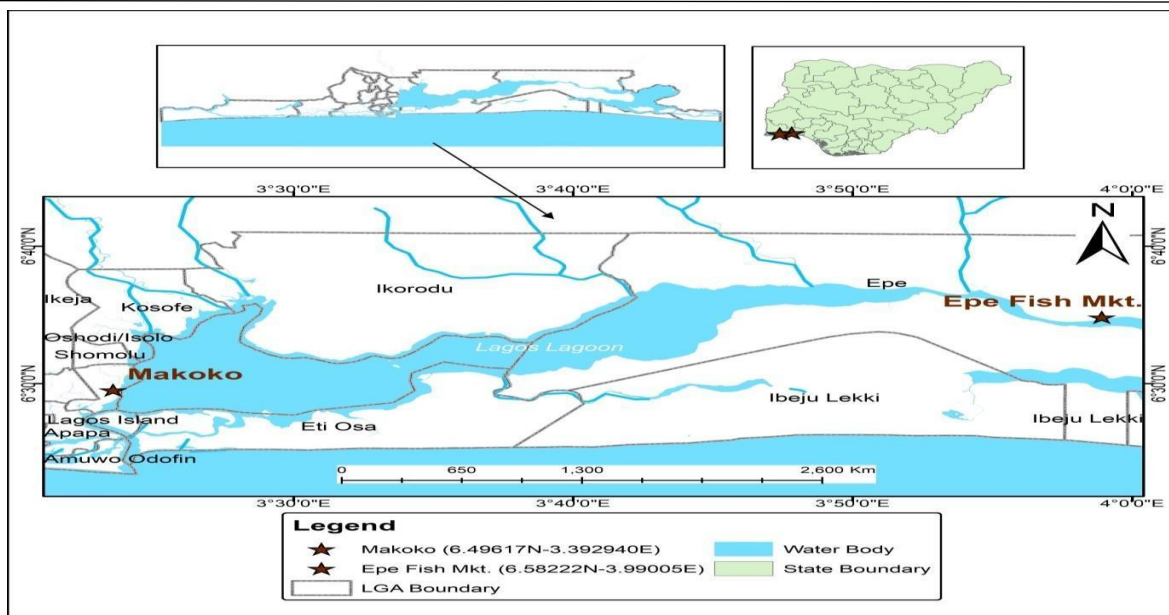


Figure 1: Map of Lagos, Nigeria showing the Epe Lagoon and Makoko Lagoon.

2.2. Sample Preparation

The fishes were thoroughly washed with tap and distilled water in order to remove hazardous contaminants and drained under folds of filter paper. Some of the samples from each fish species were smoked with firewood in a smoking klin in a regulated environment as was described in Adherr *et al* (2022). Each species of fish (raw and smoked) was dissected with a clean and sterilised knife to remove the guts, intestines, bones and other unwanted parts separately. The tissue samples were then placed separately and homogenized with an electric food blender and then stored in a deep freezer at -18°C .

2.3. Proximate Analyses of the Fish Samples

The nutritional composition of the fish samples was determined using the methods described in Ayanda *et al* (2019). The crude protein content was determined using the Kjeldahl method to determine nitrogen content, and using 6.25 as the conversion factor to get crude protein from total nitrogen. The moisture content of the fish samples was determined using the gravimetric method. The amount of moisture in each sample was calculated using equation 1

$$\% \text{ Moisture} = \frac{\text{Weight of Fresh sample} - \text{weight of oven dried sample}}{\text{weight of fish sample}} \times 100 \quad . \text{Eq 1}$$

The ash content was determined by heating the pre-dried samples used in the determination of moisture content to ash in a muffle furnace at 550°C for 4 hours. The percentage ash content was calculated as shown in equation 2

$$\text{Percentage Ash content} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100 \quad \dots\dots \text{Eq 2}$$

Crude fat content of each fish sample was determined as described in Das and Biswas (2019). 5g of ground samples were extracted with diethyl ether using soxhlet extraction. The extract was dried and the fat content was calculated using equation 3

$$\text{Percentage Fat content} = \frac{\text{Weight of Fat extract}}{\text{Weight of Sample}} \times 100 \quad \dots\dots \text{Eq 3}$$

2.4. Determination of Polycyclic Aromatic Hydrocarbon Content

Extraction of PAHs was carried out following the method described by Akinnusotu *et al* (2021). 10 g of the homogenized fish sample was thoroughly mixed with anhydrous Na₂SO₄ to dehydrate the sample. 20 ml of dichloromethane was added to the sample. Samples were covered with aluminium foil to prevent evaporation and sonicated to separate supernatants of extracts. Extracts were concentrated using an evaporator. Extracts were then cleaned up using a chromatographic column, moderately packed at the bottom with 1 cm glass wool. 2 g of silica gel and anhydrous Na₂SO₄ were added to the column while the column was pre-eluted with 20 ml dichloromethane. Extracts were then concentrated and collected in 2 ml vials.

Chromatographic analysis of the extract for PAHs content was carried out as described in Tongo *et al* (2017) using Gas chromatography (GC, Hewlett-Packard HP-5890 Series II with flame ionization detection (GC-FID)). The GC was programmed as follows: initial temperature of 60 °C for 2 min and ramped at 25 °C/min to 300 °C for 5 min and allowed to stay for 15 min giving a total run time of 22 mins. Compounds were identified by comparing the retention time of standards with that obtained from the extracts and individual analysis of PAHs was used for quantitation. The final quantification of each PAH was later recalculated into a dry weight basis of mg/kg by using equation 4:

$$\text{Quantity of PAH} = \text{GC reading (mg/L)} \times \frac{\text{Volume of Solvent}}{\text{Weight of sample}} \times 100 \quad \dots\dots \text{Eq 4}$$

The possible sources of the PAHs were estimated using the Ant/ (Ant+Phe), BaA/(BaA+Chr) and Fln/(Fln+Pyr) ratios as were described in Emoyan *et al.*, (2020) and Davies *et al* (2019).

2.5 Health Risk Assessment of the Fish Samples

The health risk assessment of the fish samples involved the determination of the estimated daily intake, hazard quotient, hazard indices and toxic equivalent quotient. Estimated daily intake (EDI) was calculated using equation 5 (Bogdanović *et al.*, 2019):

$$\text{EDI} = \frac{\text{Concentration of PAH} \times \text{Ingestion rate of fish per person}}{\text{Body Weight}} \quad \dots\dots \text{Eq 5}$$

(Ingestion Rate, 0.0548)

The hazard quotient for non-carcinogenic polycyclic aromatic hydrocarbons was calculated as described by Tongo *et al* (2018) as shown in equation 6;

$$\text{Hazard Quotient (HQ) for non-carcinogenic PAH} = \frac{\text{Estimated Daily intake}}{\text{Reference Dose}} \quad \dots \text{Eq 6}$$

The hazard index for each fish sample was calculated as the sum of the hazard quotient values as in shown equation 7:

$$\text{Hazard Index (HI)} = \sum_{i=1}^n \text{HQ ie (HQ}_1 + \text{HQ}_2 + \text{HQ}_3 + \dots + \text{HQ}_n) \quad \dots \text{Eq 7}$$

The toxic equivalent quotient (TEQ) was derived by adding the carcinogenic potencies of individual PAHs following equation 8:

$$TEQ = (B(a)Pteq1 + B(a)Pteq2 + B(a)Pteq3 + \dots + B(a)Pteqn) \dots Eq 8$$

B(a)Pteq is defined as the carcinogenic potencies of individual PAHs and was calculated as in equation 9

$$B(a)Pteq = \sum_{i=1}^n C_i \times TEF \dots \dots Eq 9$$

where TEF is defined as the toxicity equivalent factor and C_i is the concentration of parameter.

2.6 Statistical Analyses

Data obtained were statistically analysed using GraphPad Prism 9.0. Statistical analyses involved a two way analysis of variance (2 way ANOVA) and correlation study. A similarity study of the fishes was carried out using PAST software.

3.Results and Discussion

3.1 Nutritional Components of the Fish Samples

Table 1 provides the nutritional composition of the fish samples. The moisture content was higher in fresh fish samples compared to their smoked counterparts from the same locations. The fresh silver catfish from Epe had the highest moisture content (71.01%), while the smoked silver catfish from Makoko had the lowest moisture content (52.50%). The percentage of moisture in fish is a good indicator of the relative energy, fat and protein (Shabir *et al.*, 2018) as fishes with low moisture content usually have higher fat and protein content, as well as higher calorie density (Ahmed *et al* 2022). However, high moisture content in fishes can cause the degradation of polyunsaturated fatty acids, increase the fishes' vulnerability to spoilage by microorganisms, reduce fish quality for longer periods of preservation, play important roles in metabolic reactions and help to easily solubilize certain elements (Ayanda *et al* 2019). Thus the observed higher moisture content in fresh fishes compared to smoked fishes, suggesting that fresh fishes are more prone to spoilage and degradation of polyunsaturated fatty acids. The low moisture content in smoked fish confirms their ability to stay for an extended period suggesting that smoking is a good method of preserving fishes.

The knowledge of nutritional composition is essential for making informed decisions about food consumption, especially when specific nutrients are required (Ayanda *et al.*, 2019). The differential nutrient contents of the fishes we observed aligns with findings of Savin *et al* (2024). The variation in nutrient composition of the fishes may be due to differences in accessibility to water components and/or the fish's ability to consume and convert vital nutrients (Maulu *et al* 2021a). Additionally, exogenous and endogenous factors can influence the biochemical composition of fish (Ahmed *et al* 2022) thus the variations observed in this study.

The highest total fatty acid content was present in the smoked Tilapia from Makoko (14.50%), followed by the smoked Tilapia from Epe (14.00%). The lowest total fatty acid content was present in fresh crevalle jack from Makoko (3.70%), followed by fresh crevalle jack from Epe (4.90%).

Smoked crevalle jack from Epe had the highest protein content (20.02%), followed by smoked silver catfish from Epe (19.77%). The lowest protein content was found in fresh crevalle jack from Epe (15.04%), followed by fresh Tilapia from Epe (15.06%). Moisture protein, fat, and ash constitute the four basic constituents of fish meat. This study's nutrient composition pattern is

consistent with previous research (Ljubojevic *et al.*, 2016; Tiwo *et al.*, 2019). Higher levels of protein, fats, fibre and ash in the smoked dried fish could be attached to moisture loss due to drying and the attendant concentration of other nutrients in the fish (Tiwo *et al.*, 2019). Smoked silver catfish from Makoko had the highest carbohydrate content (0.83%), followed by fresh crevalle jack from Makoko (0.29%), while smoked crevalle jack from Epe had the lowest carbohydrate content (0.04%), followed by smoked tilapia from Makoko (0.05%).

In this study, we observed that different fishes can be selected based on their abundance of specific nutrients. For example, smoked fish are preferred for their high abundance of carbohydrates, proteins, total fatty acids, and fibre. Dietary fibre is essential in reducing the risk of coronary disease, hypertension, constipation, diabetes, and colon and breast cancer (He *et al.*, 2022). Fibre improves food bulk, appetite satisfaction, and promotes movement through the digestive system, preventing constipation (Eden and Rumambarsari 2020). Fresh crevalle jack from Makoko had the highest fibre content (5.65%), followed by smoked silver catfish from Makoko (4.55%). The lowest fibre content was found in fresh silver catfish from Epe (0.85%), followed by fresh tilapia from Makoko (1.33%).

The smoked Tilapia fish from Epe had the highest ash content (9.11%), followed by the fresh crevalle jack from Epe (8.05%), while the smoked silver catfish from Epe had the lowest ash content (2.00%), followed by the smoked silver catfish from Makoko (2.50%). The amount of ash present in food can be used to estimate the quantity of minerals present in the sample (Afify *et al* 2017). Therefore, the smoked tilapia from Epe and fresh crevalle jack from Epe, which we observed to have high ash content, may be said to contain more minerals than the other fish samples.

Table 1: Percentage Nutritional Components of the Fish Samples

Location	Fish	Moisture	Ash	Total Fatty Acid	Fibre	Protein	Carbohydrate
Epe	Smoked Tilapia	55.00	9.11	14.00	2.72	19.11	0.06
	Fresh Tilapia	69.08	6.11	7.19	2.36	15.06	0.22
	Smoked Crevalle Jack	56.50	6.30	13.20	3.92	20.02	0.04
	Fresh Crevalle Jack	70.00	8.05	4.90	1.79	15.04	0.21
	Smoked Silver Catfish	60.00	2.00	13.80	3.87	19.77	0.24
	Fresh Silver Catfish	71.01	4.00	6.30	0.85	17.71	0.12
Makoko	Smoked Tilapia	56.10	8.02	14.50	2.45	18.95	0.05
	Fresh Tilapia	70.06	7.01	6.05	1.33	15.25	0.20
	Smoked Crevalle Jack	58.33	6.12	12.40	3.99	18.44	0.24
	Fresh Crevalle Jack	68.90	5.95	3.70	5.65	15.49	0.29
	Smoked Silver Catfish	52.50	2.50	13.20	4.55	19.39	0.83
	Fresh Silver Catfish	67.90	6.71	7.20	2.75	15.13	0.21

3.2 The Polycyclic Aromatic Hydrocarbon Content of the Fish Samples

Table 2 presents the contents of polycyclic aromatic hydrocarbons (PAHs) in the fish samples. No polycyclic aromatic hydrocarbon was detected in the fresh silver catfish from Makoko, while the smoked silver catfish from Makoko had the highest total PAH content (7.034 mg/kg). The fish samples from Makoko generally had a higher total PAH content than those from Epe, except for smoked crevalle jack from Epe, which had a higher total PAH content (4.445 mg/kg) than the corresponding sample from Makoko (2.382 mg/kg). The varying PAHs levels in same fish type from different sources could be due to varying conditions in the habitats where they lived as has been stated by Khalili *et al* (2023). The total PAH content in each fish sample from each location was higher in the smoked fishes than in the fresh fishes. Naphthalene and acenaphthylene levels in all fish samples were below detectable levels. Only smoked silver

catfish from Makoko had benzo(ghi)perylene (0.381 mg/kg). Acenaphthene was only detected in smoked silver catfish from Epe (0.169 mg/kg), dry crevalle jack from Makoko (0.035 mg/kg), and smoked silver catfish from Makoko (0.306 mg/kg). Dibenzo(a,h)anthracene was detected only in smoked crevalle jackfish from Epe (0.129mg/kg), smoked tilapia from Makoko (0.047mg/kg) and smoked silver catfish from Makoko (0.434mg/kg). The naphthalene and the acenaphthylene contents of the fishes were significantly lower than the benzo(a)pyrene ($p = 0.0027$) and dibenz(a,h)anthracene ($p = 0.0252$) contents of the fishes. The acenaphthene contents of the fishes were significantly lower than the benzo(a)pyrene ($p = 0.0028$) and the dibenz(a,h)anthracene ($p = 0.0255$) of the fishes. The benzo(a)pyrene contents of the fishes significantly higher than the flouranthene, benzo(ghi)perylene and the phenanthrene contents of the fishes ($p = 0.003$), chrysene contents of the fishes ($p = 0.0032$) and the benz(j+k+b)flouranthene contents of the fishes ($p = 0.0049$)

The smoked silver catfish from Makoko had the highest total PAH content (7.034mg/kg) and the highest total carcinogenic PAH content (3.074mg/kg), followed by smoked tilapia from Makoko with a total PAH content of 4.515mg/kg and total carcinogenic PAH content of 2.698mg/kg. Fresh silver catfish from Makoko had no PAHs, while fresh tilapia from Epe had a total PAH content of 0.159mg/kg and no carcinogenic PAHs. All the PAHs in fresh crevalle jack from Epe were carcinogenic (100% TCPAH). The smoked tilapia from Makoko had the second-highest percentage of total carcinogenic PAHs (59.756%), while the fresh tilapia from Makoko had the lowest percentage of total carcinogenic PAHs (17.177%). The differences in PAH contents in different fishes from the same location and prepared in the same manner suggest that PAH accumulation can be species-specific (Savin *et al* 2024)

The amount of polycyclic aromatic hydrocarbons (PAHs) found in the fishes in this study is consistent with what has been reported in previous studies (Nwaichi and Ntorgbo, 2016). Factors such as fat and moisture contents, and the nature of the skin cover could contribute to the observed differences in PAH levels (Aksun Tümerkan 2022; Coroian *et al* 2023). Given that PAHs are known to be toxic, carcinogenic, and teratogenic and are associated with various health issues and infertility (da Silva Junior *et al*, 2021; Sampaio *et al.*, 2021; Shamsedini *et al.*, 2022; Montano *et al* 2025), the differential accumulation of PAHs by different fishes suggests that they have varying potentials to contribute to health risks for consumers. The PAHs found in the fresh fishes were likely from their growing environments, which could explain the differences observed in the PAHs of the fresh fishes from different locations (Tiwo *et al.*, 2019).

In this study, all samples except for fresh tilapia from Makoko and Epe and fresh silver catfish from Makoko contained BaP, suggesting that the fishes analyzed may have carcinogenic potentials. The percentage of total carcinogenic PAHs content observed in this study showed that only 58.3% of the fishes had a percentage of carcinogenic PAHs of 40% and above, while 8.33% (fresh crevalle jack from Epe) had all the PAHs as carcinogenic PAHs, indicating a high cancer risk associated with consuming such fish. Based on the classification by Howard *et al.* (2021), only fresh tilapia from Epe and fresh silver catfish from Makoko (TPAH < 0.200mg/kg) can be considered uncontaminated. Fresh crevalle jack from Epe (TPAH = 0.841mg/kg) is contaminated, while the other fish samples (TPAH >1.00mg/kg) are heavily contaminated

Table 2: Polyaromatic Hydrocarbons Contents of the Fish Samples (mg/kg)

PAH	EPE						MAKOKO					
	Fresh Tilapia	Smoked Tilapia	Fresh Crevalle Jack	Smoked Crevalle Jack	Fresh Silver catfish	Smoke d Silver catfish	Fresh Tilapia	Smoked Tilapia	Fresh Crevalle Jack	Smoked Crevalle Jack	Fresh Silver catfish	Smoke d Silver catfish
Nap	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AcPY	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AcP	0.000	0.000	0.000	0.000	0.000	0.169	0.000	0.000	0.000	0.035	0.000	0.306
Flu	0.000	0.000	0.000	0.709	1.072	0.042	0.000	0.207	0.005	0.161	0.000	0.387
Phe	0.000	0.000	0.000	0.551	0.728	0.460	0.265	0.439	0.230	0.328	0.000	0.892
Ant	0.000	0.409	0.000	0.738	0.000	0.550	0.000	0.620	0.268	0.349	0.000	0.839
Fln	0.068	0.000	0.000	0.410	0.199	0.998	0.570	0.340	0.176	0.213	0.000	1.198
Pyr	0.091	0.071	0.000	0.254	0.205	0.242	0.089	0.211	0.095	0.091	0.000	0.338
BaA	0.000	0.000	0.281	0.371	0.299	0.412	0.247	0.427	0.000	0.309	0.000	0.511
Chr	0.000	0.000	0.000	0.099	0.051	0.078	0.000	0.106	0.058	0.072	0.000	0.230
BaP	0.000	0.067	0.044	0.131	0.057	0.094	0.000	0.077	0.052	0.084	0.000	0.105
BbFL	0.000	0.202	0.137	0.220	0.173	0.122	0.000	0.955	0.178	0.287	0.000	0.212
BkFL	0.000	0.107	0.097	0.130	0.089	0.076	0.000	0.406	0.045	0.116	0.000	0.098
Ind	0.000	0.252	0.282	0.703	0.348	0.506	0.000	0.680	0.248	0.337	0.000	1.103
DBA	0.000	0.000	0.000	0.129	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.434
BP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.381
TOTAL PAH	0.159	1.108	0.841	4.445	3.893	4.980	1.438	4.515	1.355	2.382	0.000	7.034
TOTAL CPAH	0.000	0.628	0.841	1.783	1.017	1.288	0.247	2.698	0.581	1.205	0.000	3.074
%												
TCPAH	0.000	56.679	100.000	40.112	26.130	25.863	17.177	59.756	42.878	50.588	0.000	43.702

Nap = Naphthalene, AcPY = Acenaphthylene; AcP = Acenaphthene; Flu = Fluorene; Phe = Phenanthrene; Ant = Anthracene; FL = Fluoranthene; Pyr = Pyrene; BaA = Benzo{a}anthracene; Chr = Chrysene; BaP = Benzo{a}pyrene; BbFL = Benzo{b}fluoranthene; BkFL = Benzo{k}fluoranthene; Ind = Indeno{1,2,3-cd}pyrene; DBA = Dibenzo{a,h}anthracene; BP = Benzo{ghi}perylene

3.3 Source Characterization and Assessment of PAHs

The source of PAHs in the environment or medium can be understood with the analysis of PAH ratios among others (Emoyan *et al.*, 2020; Davies *et al.*, 2019). Table 3 shows petrogenic and combustion sources of the PAHs in the fish samples. All fish samples from both Epe and Makoko had BaA/(BaA + Chr) ratio values to be greater than 0.35, except for fresh tilapia from Epe, smoked tilapia from Epe, fresh crevalle jack from Makoko and fresh silver catfish from Makoko which were not applicable. Also, Ant/(Ant + Phe) ratio values for fish samples in Epe and Makoko were greater than 0.1 except for fresh tilapia from Epe, fresh crevalle jack from Epe, fresh silver catfish from Epe, fresh tilapia from Makoko, and fresh silver catfish from Makoko. The BaA/(BaA + Chr) and Ant/(Ant + Phe) ratios we obtained in the study were greater than 0.2 and 0.1, respectively, indicating wood combustion as the source of PAHs. Fresh tilapia and fresh silver catfish from Epe both have Fln/(Fln + Pyr) ratio values to be between 0.4 and 0.5, while smoked crevalle jack from Epe, smoked silver catfish from Epe, fresh tilapia from Makoko, smoked tilapia from Makoko, fresh crevalle jack from Makoko, smoked crevalle jack from Makoko and smoked silver catfish from Makoko had Flu/(Flu + Pyr) ratio values to be greater than 0.5. The Flu/(Flu + Pyr) ratio was between 0.4 and 0.5 for fresh tilapia and fresh silver catfish from Epe and greater than 0.5 for others, indicating wood combustion and petroleum combustion as the sources of PAHs, respectively.

Overall, the results suggest that wood combustion is the primary source of PAH contamination in fishes. Okedere and Eledhinafe (2022) have stated that food items processed via charcoal or wood smoking contain elevated levels of PAHs, and such levels increase progressively with smoking duration. Wood smoke also contains PAHs (Savin *et al* 2024), which could explain the

higher levels of PAHs observed in the smoked fish samples compared to their corresponding fresh ones, similar to the findings of other studies (Tiwo *et al.*, 2019; Bwala, 2023).

Table 3: Source Characterization and Assessment of PAHs.

LOCATION	FISH	BaA/(BaA + Chr)	Ant/(Ant + Phe)	Flu/(Flu + Pyr)
EPE	Fresh Tilapia	0.000	0.000	0.428
	Smoked Tilapia	0.000	1.000	0.000
	Fresh Crevalle Jack	1.000	0.000	0.000
	Smoked Crevalle Jack	0.789	0.572	0.617
	Fresh Silver Catfish	0.854	0.000	0.493
	Smoked Silver Catfish	0.841	0.545	0.805
MAKOKO	Fresh Tilapia	1.000	0.000	0.865
	Smoked Tilapia	0.801	0.585	0.617
	Fresh Crevalle Jack	0.000	0.538	0.649
	Smoked Crevalle Jack	0.811	0.516	0.701
	Fresh Silver Catfish	0.000	0.000	0.000
	Smoked Silver Catfish	0.690	0.485	0.780

*BaA = benz[a]anthracene, Chr = chrysene, Ant = anthracene, Phe = phenanthrene, Fln = fluoranthene, Pyr = pyrene

3.4 The Health Hazard Quotient (HQ) and Health Hazard Indices of the Fish Samples

Table 4 shows the Health Hazard Quotient (HQ) and Health Hazard Indices (HI) of the fish samples for adults and children. The Health Hazard Quotient values were higher in the smoked fishes compared to fresh fishes and were generally higher for children compared to adults. The highest Health Hazard Index values for both adults and children were observed in smoked silver catfish from Makoko (MSS), with HI values of 0.8937 and 4.1794, respectively (Table 3). Conversely, the fresh crevalle jackfish from Epe (EFC) had the lowest HI value of 0.0099 for adults and 0.0476 for children. The HI values for children were consistently higher than those for adults and smoked fish had higher HI values than fresh fish. However, in silver catfish from Epe, the HI value for children was higher in fresh fish (2.2853) than in smoked fish (2.2480), and a similar trend was observed for tilapia fish from Epe (0.2275 for fresh and 0.2243 for smoked). The tilapia fish from Makoko had higher HI values than those from Epe (both smoked and fresh). For crevalle jack, the fresh fish from Makoko (MFC) had higher HI values than the fresh fish from Epe and the reverse was true for the smoked fish. Regarding silver catfish, the smoked fish from Makoko had higher HI values than those from Epe, and the reverse was true for the fresh fish.

Hazard identification is used to determine whether exposure to a compound could have harmful effects on humans and whether the intensity of the effects will have public health significance (Shi *et al.*, 2020). This study's results indicate that no PAH had a hazard quotient (HQ) value equal to or greater than 1, which suggests that the individual PAHs in the fishes used in the study have a low risk of causing adverse health effects to humans (Ugwu *et al.*, 2022). Additionally, there is no likelihood of any non-carcinogenic effects resulting from consuming the fish samples used in this study. However, some of the fish samples had hazard index (HI) values greater than 1, which suggests that these fish samples could cause unacceptable effects or health risks in populations exposed to them. The higher HQ and HI values for children compared to adults in this study suggest that exposing children to the fish samples could lead to greater health risks than in adults (Abdel-Shafy and Mansour, 2016). Moreover, the health risks are likely to be greater in people exposed to smoked fish than in those exposed to fresh fish.

Table 4: The Health Hazard Quotient (HQ) and Health Hazard Indices of the Fish Samples in Adult and Children

PAH	EFT	EST	EFC	ESC	EFS	ESS	MFT	MST	MFC	MSC	MFS	MSS
Napadult	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
child	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
AcPYadult	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
child	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
AcPadult	0.0000	0.0000	0.0000	0.0000	0.0000	0.0350	0.0000	0.0000	0.0000	0.0067	0.0000	0.0617
child	0.0000	0.0000	0.0000	0.0000	0.0000	0.1600	0.0000	0.0000	0.0000	0.0333	0.0000	0.2883
Fluadult	0.0000	0.0000	0.0000	0.2150	0.3250	0.0125	0.0000	0.0625	0.0025	0.0500	0.0000	0.1175
child	0.0000	0.0000	0.0000	1.0050	1.5175	0.0600	0.0000	0.2925	0.0075	0.2275	0.0000	0.5475
Pheadult	0.0000	0.0000	0.0000	0.0223	0.0293	0.0187	0.0107	0.0177	0.0093	0.0133	0.0000	0.0360
child	0.0000	0.0000	0.0000	0.1040	0.1377	0.0870	0.0500	0.0830	0.0433	0.0620	0.0000	0.1683
Antadult	0.0000	0.0167	0.0000	0.0300	0.0000	0.0223	0.0000	0.0250	0.0110	0.0140	0.0000	0.0340
child	0.0000	0.0773	0.0000	0.1393	0.0000	0.1040	0.0000	0.1170	0.0507	0.0660	0.0000	0.1583
Flnadult	0.0200	0.0000	0.0000	0.1250	0.0600	0.3025	0.1725	0.1025	0.0525	0.0650	0.0000	0.3625
child	0.0975	0.0000	0.0000	0.5800	0.2825	1.4125	0.8075	0.4800	0.2500	0.3025	0.0000	1.6975
Pyradult	0.0275	0.0225	0.0000	0.0775	0.0625	0.0725	0.0275	0.0650	0.0300	0.0275	0.0000	0.1025
child	0.1300	0.1000	0.0000	0.3600	0.2900	0.3425	0.1250	0.3000	0.1350	0.1300	0.0000	0.4800
BaAadult	0.0000	0.0000	0.0025	0.0033	0.0026	0.0037	0.0022	0.0038	0.0000	0.0028	0.0000	0.0045
child	0.0000	0.0000	0.0116	0.0153	0.0123	0.0170	0.0102	0.0177	0.0000	0.0128	0.0000	0.0212
Chradult	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
child	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
BaPadult	0.0000	0.0058	0.0037	0.0117	0.0051	0.0080	0.0000	0.0066	0.0044	0.0073	0.0000	0.0095
child	0.0000	0.0277	0.0183	0.0540	0.0234	0.0387	0.0000	0.0321	0.0211	0.0350	0.0000	0.0438
BbFadult	0.0000	0.0018	0.0012	0.0020	0.0015	0.0011	0.0000	0.0085	0.0015	0.0026	0.0000	0.0019
child	0.0000	0.0083	0.0057	0.0091	0.0072	0.0050	0.0000	0.0395	0.0074	0.0119	0.0000	0.0088
BkFadult	0.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0000	0.0004	0.0000	0.0001	0.0000	0.0001
child	0.0000	0.0004	0.0004	0.0005	0.0004	0.0003	0.0000	0.0017	0.0002	0.0005	0.0000	0.0004
Indadult	0.0000	0.0023	0.0025	0.0062	0.0031	0.0045	0.0000	0.0061	0.0022	0.0030	0.0000	0.0098
child	0.0000	0.0104	0.0117	0.0291	0.0144	0.0210	0.0000	0.0281	0.0103	0.0139	0.0000	0.0456
DBAadult	0.0000	0.0000	0.0000	0.0117	0.0000	0.0000	0.0000	0.0043	0.0000	0.0000	0.0000	0.0387
child	0.0000	0.0000	0.0000	0.0533	0.0000	0.0000	0.0000	0.0197	0.0000	0.0000	0.0000	0.1796
BPadult	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1150
child	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5400
HI Adult				0.504				0.302				
	0.0475	0.0492	0.0099	8	0.4893	0.4808	0.2129	3	0.1135	0.1922	0	0.8937
HI Child				2.349				1.411				
	0.2275	0.2243	0.0476	7	2.2853	2.2480	0.9927	3	0.5256	0.8955	0	4.1794

* (MSS = smoked silver cat fish from Makoko, MFS = fresh silver catfish from Makoko, MSC = smoked crevalle jack from makoko, MFC = fresh crevalle jack from Makoko, MST = smoked tilapia from Makoko, MFT =fresh tilapia from Makoko, ESS = smoked silver catfish from Epe, EFS = fresh silver catfish from Epe, ESC = smoked crevalle jack from Epe, EFC = fresh crevalle jack from Epe, EST = smoked tilapia from Epe, EFT = fresh tilapia from Epe)

3.5 The Toxicity Equivalent Quotients of the PAHs in the Different Fish Samples.

Table 5 shows the toxicity equivalent quotients (TEQs) of polycyclic aromatic hydrocarbons in the different fish samples. Smoked catfish from Epe had the highest TEQ value of 0.7865, while fresh tilapia from Makoko had the least TEQ value of 0.0319. Generally, smoked fish had higher TEQ values than fresh fish. The TEQ values of the fishes from Epe were higher than those of the corresponding fishes from Makoko, except for smoked crevalle jack, where the TEQ value in fish from Epe (0.2510) was lower than that of the fish from Makoko (0.5340). The highest TEF was recorded for dibenzo(a,h)anthracene in smoked tilapia from Epe (0.4340) and the lowest TEF (0.0001) was recorded in pyrene in fresh and smoked crevalle jack from Epe, fresh and smoked tilapia from Makoko and fresh tilapia from Epe.

Applying the benzo(a)pyrene (BaP)-toxic equivalent factor (BaPTEF) to PAH concentrations can provide a more accurate risk assessment of environmental exposure to PAHs. Toxic equivalency factors are useful tools for regulating compounds with common mechanisms of action, such as

PAHs (Olayinka *et al.*, 2019). Higher toxic equivalent (TEQ) values indicate higher potential health effects (Olayinka *et al.*, 2019) and a greater potential to cause carcinogenic risks (Tongo *et al.*, 2017). In this study, the smoked silver catfish from Epe had the highest TEQ value, suggesting the highest potential health effects and a high potential for carcinogenic risk compared to the other fish and fish samples.

Table 5: The Toxicity Equivalent Quotients of the PAHs in the Different Fishes

PAH Components	Silver Catfish				Crevalle Jack				Tilapia			
	Makoko		Epe		Makoko		Epe		Makoko		Epe	
	Fresh	Smoked	Fresh	Smoke d	Fresh	Smoked	Fresh	Smoke d	Fresh	Smoke d	Fresh	Smoked
Nap	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	0.0000
AcPY	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	0.0000
AcP	0.0000	0.0002	0.0000	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	0.0000
Flu	0.0000	0.0000	0.0011	0.0004	0.0000	0.0007	0.0000	0.0002	0.0000	0.0000	0.000	0.0002
Phe	0.0000	0.0005	0.0007	0.0009	0.0000	0.0006	0.0002	0.0003	0.0000	0.0000	0.0003	0.0004
Ant	0.0000	0.0055	0.0000	0.0084	0.0000	0.0074	0.0027	0.0035	0.0000	0.0040	0.0000	0.0062
Fln	0.0000	0.0001	0.0002	0.0012	0.0000	0.0004	0.0002	0.0002	0.0001	0.0000	0.0006	0.0003
Pyr	0.0000	0.0002	0.0002	0.0003	0.0000	0.0003	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002
BaA	0.0000	0.0392	0.0225	0.0514	0.0191	0.0321	0.0200	0.0250	0.0226	0.0206	0.0189	0.0419
Chr	0.0000	0.0412	0.0229	0.0511	0.0281	0.0371	0.0000	0.0309	0.0000	0.0000	0.0247	0.0427
BaP	0.0000	0.0008	0.0005	0.0023	0.0000	0.0010	0.0006	0.0007	0.0000	0.0000	0.0000	0.0011
BbFL	0.0000	0.0940	0.0570	0.1050	0.0440	0.1310	0.0520	0.0840	0.0000	0.0670	0.0000	0.0770
BkFL	0.0000	0.0839	0.0447	0.0150	0.0112	0.1220	0.0342	0.0695	0.0091	0.0500	0.0078	0.0240
Ind	0.0000	0.0012	0.0017	0.0021	0.0014	0.0022	0.0018	0.0029	0.0000	0.0020	0.0000	0.0096
DBA	0.0000	0.0506	0.0348	0.1103	0.0282	0.0703	0.0248	0.0337	0.0000	0.0252	0.0000	0.0680
BP	0.0000	0.0000	0.0000	0.4340	0.0000	0.1290	0.0000	0.0000	0.0000	0.0000	0.0000	0.0470
Benzo(ghi)perylene	0.0000	0.0000	0.0000	0.0038	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Total TEQ	0.0000	0.3183	0.1864	0.7865	0.1320	0.5340	0.1366	0.2510	0.0319	0.1690	0.0523	0.3186

Nap = Naphthalene, AcPY = Acenaphthylene; AcP = Acenaphthene; Flu = Fluorene; Phe = Phenanthrene; Ant = Anthracene; FL= Fluoranthene; Pyr = Pyrene; BaA = Benzo{a}anthracene; Chr = Chrysene; BaP = Benzo{a}pyrene; BbFL = Benzo{b}fluoranthene; BkFL = Benzo{k}fluoranthene; Ind = Indeno{1,2,3-cd}pyrene; DBA = Dibenzo{a,h}anthracene; BP= Benzo{ghi}perylene

3.6 Relationship Between the Nutritional Composition, Total PAH and Health Indices of the Fish Samples

Figure 2 shows the relationship between the nutritional values and the total polycyclic aromatic hydrocarbons (TPAH) and toxicity equivalent quotient (TEQ) values. The protein, fibre, fatty acid, and carbohydrate contents of the fish samples had a positive correlation with each other and with TEQ, HI adult, HI children, TPAH, TCPAH, and %TCPAH values. The moisture contents of the fish samples had a minor positive relationship with ash (10%) and a negative relationship with other nutrients and risk parameters. Apart from the moisture content of the fishes, the ash content had a positive correlation with %TCPAH values and a negative correlation with other nutritional and health risk parameters.

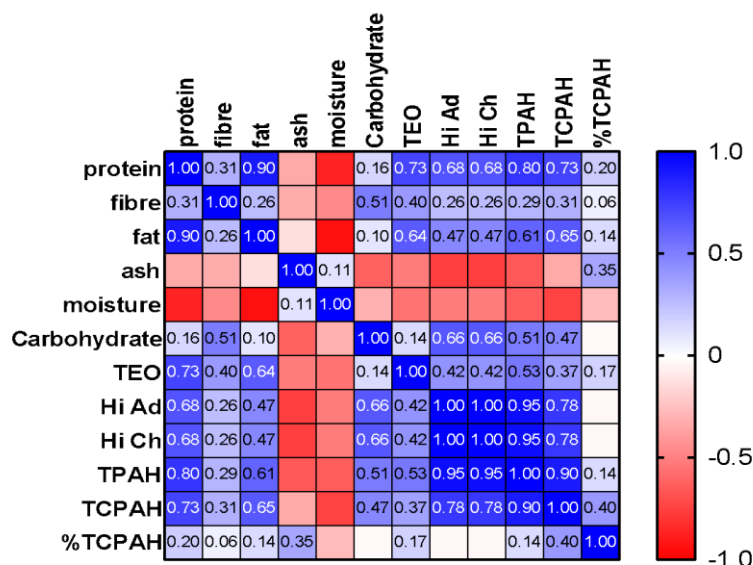


Figure 2: Heat map of the correlation of the nutritional, total polycyclic aromatic hydrocarbons, health hazard indices and toxicity equivalent quotient values of the fish samples.

3.7 Phylogenetic Relationship of the Fish Nutritional and Toxicity Indices

The fish samples formed two similar clusters, with the fresh fish samples forming one cluster and the dried fish samples forming the other cluster (figure 3). In the fresh fish cluster, the fresh silver catfish sample from Epe had the lowest level of similarity to the others, while the fresh silver catfish from Makoko and fresh tilapia from Epe had the highest level of similarity. In the smoked fish cluster, the smoked catfishes had a higher level of similarity and a lower level of similarity with the others.

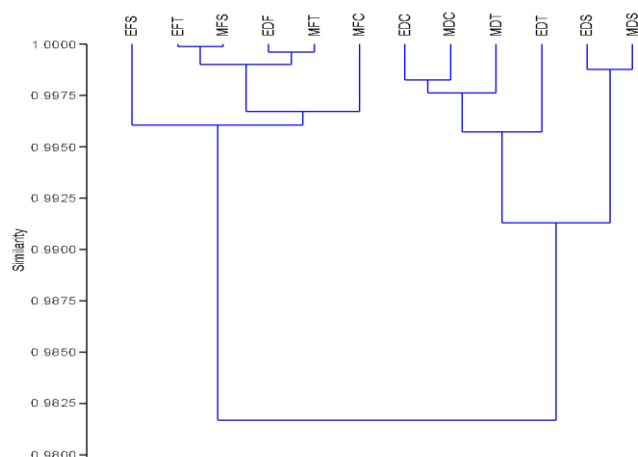


Figure 3: Similarity Cluster of the Fishes from Epe and Makoko. (MSS = smoked silver catfish from Makoko, MFS = fresh silver catfish from Makoko, MSC = smoked crevalle jack from Makoko, MFC = fresh crevalle jack from Makoko, MST = smoked tilapia from Makoko, MFT =fresh tilapia from Makoko, ESS = smoked silvexr catfish from Epe, EFS = fresh silver catfish from Epe, ESC = smoked crevalle jack from Epe, EFC = fresh crevalle jack from Epe, EST = smoked tilapia from Epe, EFT = fresh tilapia from Epe)

4. CONCLUSION

The hazard indices, total PAH, and total CPAH were highest in smoked silver catfish from Makoko, while fresh silver catfish from Makoko had the least TEQ, hazard indices, total PAH, Total CPAH, and %TPAH values. The moisture content was higher in fresh fishes than in smoked fishes. Protein, fibre, fat, and carbohydrate contents of the fishes were positively correlated with each other and with the TEQ, hazard indices, TPAH, TCPAH, and %TCPAH of the fishes. Overall, the study results demonstrate that while smoked fishes had higher nutrient content than their fresh counterparts, the PAH content and associated health risks were higher in smoked fishes. Thus, consuming smoked fish could pose more significant health risks than fresh fish. The protein content in smoked fish from Epe was higher than those from Makoko. The method of fish preparation significantly impacts the level of PAH, nutrient content, and associated health risks. The close clustering of the fishes prepared in the same manner highlights this fact. Observations from this study showed a similarity between the total PAH and toxicity equivalent, indicating that fish processing has a considerable impact on the nutritional content and health risk indices. The positive correlation between TPAH and fatty acid content in the fishes could be attributed to the lipophilic nature of PAH, leading to their accumulation in tissues with high fatty acid content

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CONFLICT OF INTEREST

The authors declare that they have no competing interests, financial or otherwise, that could potentially influence the results or interpretation of this study. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR'S CONTRIBUTION

Kelechi L. Njoku contributed to the study design, data collection, data analysis, interpretation of results, and drafting of the manuscript. Motunrayo M. Ajetunmobi participated in data collection, data analysis, and manuscript revision. Anjolaoluwa T. Ajayi was involved in data analysis, interpretation of results, and manuscript revision. All authors have read and approved the final version of the manuscript and agreed to be accountable for the content of the work.

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