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Polycyclic aromatic hydrocarbon (PAHs) and amino acid profile in *Oreochromis niloticus* along four fishing villages of Sokoto State, Nigeria

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ABSTRACT

This study aims to examine the amino acid profile and concentration of polycyclic aromatic hydrocarbons (PAHs) in fresh and smoked *Oreochromis niloticus* from four fishing communities (Wurno, River Rima, Romo Lake, and Shagari Reservoir) in Sokoto State, Nigeria. The amino acid profiles and PAHs of the samples were determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Data collected were subjected to analysis of variance. There were significant differences ($p<0.05$) in both essential and nonessential amino acids of both fresh and Smoked *O. niloticus* caught from four distinct aquatic environments. Proline (5.483±0.05g/100g protein and 5.024±0.05g/100g protein) recorded the highest mean value in fresh fish caught from Romo and Wurno respectively, likewise leucine composition (5.320±0.05 and 5.19±0.058g/100g protein) from fresh fish of Shagari reservoir and Wurno water body respectively. Wurno smoked fish have the highest levels of Arginine, Lysine, Alanine, Glycine and Aspartic acid which are crucial for growth, protein synthesis, energy metabolism and collagen structure. While all four locations provide a good source of protein, Wurno and River Rima appear to offer a more balanced and higher concentration of essential amino acids. The most abundant PAHs in fresh fish samples is Chrysene (CHRY), consistently showing the highest concentration across all four landing sites, with high mean concentration at the River Rima, Romo, and Shagari sites (>21 µg/kg). Consequently, the results show an enormous increase in PAHs concentrations in smoked fish compared to the fresh *O. niloticus* samples. For example, Naphthalene concentrations, which were around 2 µg/kg in fresh fish, skyrocketed to a maximum of 48.743 µg/kg in smoked *O. niloticus.* There were also high levels of Dibenzo [a, h] anthracene (9.434±0.80 µg/kg) and Anthracene (22.946±0.410 µg/kg). The findings strongly suggests that the traditional smoking methods used in these locations are a major source of PAHs contamination and require attention from public health and regulatory bodies.

Keywords: Amino acid; PAHs, *Oreochromis niloticus*

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INTRODUCTION

Fish is a nutrient-rich, flavourful, and easily digestible food source (Khanjani and Sharifinia, 2024). It is widely popular among diverse segments of the global population, especially in developing nations (Belton *et al.,* 2018). It is believed that approximately 60% of individuals in many developing regions rely on fish for more than 30% of their intake of animal protein. In contrast, about 80% of people in developed countries derive less than 20% of their animal protein from fish. (Tran *et al.,* 2022).

Nonetheless, as recognition of the nutritional advantages of fish consumption grows and fish prices subsequently increase, these statistics are swiftly shifting. Fish is also a valuable source of all the essential amino acids, offering particularly prominent levels of lysine, which is notably deficient in grains. Consequently, incorporating fish into a varied diet can enhance the amino acid profile and elevate the overall protein quality of the diet. (Aragão *et al.,* 2022)

Fish is a critical component of the diet for many populations around the globe due to its high-quality protein, essential amino acids, and beneficial fatty acids, including omega-3 fatty acids which are vital for human health (Nazir *et al.,* 2021). The nutritional value of fish is influenced by numerous factors including species, habitat, diet, and post-harvest processing methods such as smoking (Hasselberg *et al.,* 2020)

The Nile tilapia (*Oreochromis niloticus*) is another widely farmed species due to its adaptability and palatability (Levina *et al.,* 2021). Fish stands out as a nutritious and safe choice that's highly recommended for maintaining good health. It's a fantastic addition to our diets, which are mostly sustained by crops grown on land, providing a key source of animal protein, beneficial fats, minerals, and vital micronutrients (Balami *et al.,* 2019). The protein found in fish is particularly easy for our bodies to absorb and is packed with the essential amino acids necessary for the growth and health of infants, children, and pregnant women. (Maulu *et al.,* 2021). That's why more people, especially those living in rural, riverine, and coastal communities, are increasingly turning to fish as a preferred food source. It's important to note that the nutritional value of fish can vary greatly. This depends on the species, the season, the region where the fish is found, its age, stage of maturity, and the availability of food. Freshwater and saltwater fish also differ in their nutritional makeup, and even fish from the same habitat can have different chemical compositions (Ahmed *et al.,* 2022).

Smoking is a traditional method of preserving fish in Nigeria, and it has been shown to affect the nutritional quality of the fish (Adeyeye *et al.,* 2019). While smoking can enhance flavour and prolong shelf life, it may also lead to alterations in fatty acid profiles and the degradation of some amino acids (Li *et al.,* 2021).

Polycyclic aromatic hydrocarbons (PAHs) are a group of highly hydrophobic and organic compounds consisting of two or more fused aromatic rings, are ubiquitous in the environment (Stolyhwo & Sikorski, 2005) Generally, the number of PAHs generated during the thermal food processing might cause by many parameters, such as temperature, duration of the treatment, distance from the source of heating, fat content, oxygen accessibility, and the type of combustible used (Stolyhwo & Sikorski, 2005). Besides, researchers need to know most of these foodstuffs (e.g., cereals), oils and fats and even fish or large amounts of fishery by-products and by-catch that are produced annually as fish silage and shrimp meals are being used as feed ingredients in the formulation of feed in the aquaculture industry. From a toxicological point of view, future research efforts should focus on the concentration of PAHs in most of the feed ingredients commonly used in the formulation of feed for farmed fish.

Chromatography is an important analytical tool that allows for the separation of components in a gas mixture. GC is a common type of chromatography used to separate and analyze compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components and relative amounts of different components of a mixture. GC can also be used to prepare pure compounds from a mixture (Advanced Gas Chromatography - Progress in Agricultural, Biomedical and Industrial Applications, 2012).

MATERIALS AND METHODS

Study Area

This research was carried out in the Fish Processing Unit, Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto state is in the Sudan savannah zone in the extreme Northwestern part of Nigeria, between Latitude 13^{o} 07'45.12" N and Longitudes 5^{0}12' 18" E, at an altitude of 290.169m above sea level. It shared a common border with the Niger Republic to the North, Kebbi State to the Southwest, and Zamfara State to the East (Mamman *et al.,* 2000).

Tarana water body, Araba of Barayar Zaki village, Kwargaba ward of Wurno Local Government Area in Sokoto State, Nigeria. It has an area of 685 km2 and a population of 162,307 at the 2006 census (HASC, 2006). The postal code of the area is 842. River Rima is within Wamakko Local Government Area, situated in the northwestern Sudan savanna region of Nigeria. The specific geographic coordinates of Sokoto State, where the river is located, are between latitudes 10.8°N and 13.58°N and longitudes 3.30°E and 7.13°E (Adejuwon, 2018). Tambuwal (Romon Sarki) is in the region of Sokoto. Sokoto's capital Sokoto, is approximately 116 km away from Romo Sarki. It has an area of 923,768.0 km² and a population of 195,874,740 people. A latitude of 12°13'21.1"N (12.2225400°) and a longitude of 4°36'10.0"E (4.6027800°). The Shagari Dam falls between latitudes 12^{O} 43.57'5" N and 12^{O} 73.26'30" N and Longitudes 5^{O} 07'070 "E and 5^{O} 118'615" E it is located southeast of Sokoto city.

Experimental Fish

A total of 36 fresh fish (*Oreochromis niloticus*) samples with an average weight of 200-500g were sampled from wild caught directly from the anglers at the bank of the river/dams (Shagari Earth Dam, Romon Sarki Lake of Tambuwal, River Tarana of Barayar Zaki village in Wurno Local government, and Rima River). Smoked-dried samples were purchased from fish processors at the respective villages.

Traditional Fish Smoking

Fish samples were purchased from the four sampling areas, following the method described by Crapo (2011); fish were carefully cleaned with water to remove slime and blood. The fish was eviscerated, leaving the skin on the fish. Some fish samples were bent into horseshoe shape so that no parts would get overheated, and the fish were smoked for 18 hours. The mud Banda kiln temperature was adjusted as needed throughout the smoking

period to maintain the temperature. The hands, utensils, and work surfaces were cleaned when transferring fish.

***Defatting sample*:** The sample was defatted using chloroform/methanol mixture of ratio 2:1. 1.0 g of the sample was put in extraction thimble and extracted for 1 hour in soxhlet extraction apparatus.

***Extraction and derivatization of amino acids*:** The defatted sample of 100.0 mg with 1 g of fine quartz sand was thoroughly ground in a thick ceramic dish and homogenized with 5 ml distilled water. After centrifugation for 5 min, 0.5 ml of the supernatant was passed slowly through a separating funnel filled with wools and eluted with 4M NH_4OH.

The derivatization method included esterification of the carboxylic function step, by adding 200 ml butanol=HCl 3M, for 1h at 110^0C, followed by acetylation of the amine functional group using 100 ml trifluoroacetic anhydride, for 20min at 60^0C. After the esterification and acetylation reactions, the excess solvents were removed by nitrogen. The dried derivatives were dissolved in 500 ml ethyl acetate and analyzed by GC–MS (Desmarchelier *et al*., 2020).

Analysis of Amino Acid

All solvents and reagents used were of analytical grade. The GC–MS analysis was performed using a Varian 3800/4000 gas chromatograph/mass spectrometer. The samples were injected into the gas chromatograph on an Agilent capillary column, 30 m x 0.25 mm, 0.25µm film thickness, using a temperature program from 70^0C, 2min, and 50 °C/min to 110^0C, 10^0C/min to 290^0C, and 16^0C/min to 300^0C. The flow rate of nitrogen, the carrier gas, was 1mL/min. The following conditions were followed: transfer line temperature 250^0C, injector temperature 200^0C; ion source temperature 250^0C, splitter: 10:1. The electron energy was 70eV and the emission current, 100mA. The 15N-methionine was used as the internal standard for quantitative determination (AOAC, 2005).

PAHs Analysis

Analysis of both fresh and smoked fish samples, polycyclic aromatic hydrocarbons (PAHs) compounds, using a gas chromatography-tandem mass spectrometry (GC-MS/MS) method accredited according to ISO 17025 as described by Veyrand *et al.* (2013).

Statistical Analysis

Amino acid profiles and PAHs compositions were analysed using Multivariate Analysis in General Linear Model to determine significant differences in amino acid composition and PAHs of fresh and smoked fish samples and among the four sampling locations. Post-hoc Duncan's multiple range test was conducted for comparisons of individual amino acids. SPSS version 27 package was used for the analysis.

RESULTS AND DISCUSSION

Amino Acids Concentration

Results presented in Table 1 indicate that there was a significant difference ($p<0.05$) in both essential and nonessential amino acids of fresh *O. niloticus* caught from four distinct aquatic environments across the study locations, with proline (5.483±0.05g/100g protein and 5.024±0.05g/100g protein) recording the highest mean value in fish caught from Romo and

Wurno respectively, likewise leucine composition (5.320±0.05 and 5.19±0.058g/100g protein) from fish of Shagari reservoir and Wurno water body respectively, while the amino acid with the lowest mean value is phenylalanine and methionine (1.95±0.025 and 1.93±0.05g/100g protein) respectively. This provides a comprehensive profile of both essential and non-essential amino acids in fresh *Oreochromis niloticus* fish species. Their habitat and environmental conditions influence the nutritional value of fish. There is significant variation in amino acid concentrations across the different locations, indicating that their habitat and environmental conditions influence the nutritional value of the fish. This could be due to differences in the availability of nutrients, the composition of the fish diet, or the overall health of the ecosystem. The result of this finding is similar in some amino acids as describe by Omolara *et al*. (2017), but different from the work of Abdulkarim *et al*.; (2017), except in isoleucine (4.13±0.03g/100g) mean value of Shagari reservoir fish species (4.295±0.02g/100g), Amino acids are associated with health issues and their deficiencies lead to a number of diseases. Hence, knowledge of the amino acid composition of food products serves as a basis for establishing their potential nutritive value (Mohanty *et al*., 2014). Amino acids are also important in healing processes and the composition of amino acids in fish is like that in humans. People can obtain essential amino acids in copious amounts and proper balance by consuming fish. The essential amino acids cannot be mass-produced in human bodies but can be obtained from food. The current study indicated that the fresh *O. niloticus* species had all the essential amino acids investigated. Deficiency in the essential amino acids may delay the healing recovery process (Mat jail *et al*., 1994). Leucine promotes the healing of bones, skin, and muscle tissue. Isoleucine is necessary for haemoglobin formation, stabilizing and regulating blood sugar and energy. Glycine, which is one of the major components of human skin collagen, together with other essential amino acids such as alanine, form a polypeptide that will promote regrowth and tissue healing (Witte *et al*., 2002).

Fish from Shagari reservoir and Wurno generally appear to have a higher concentration of most essential amino acids, making them potentially more nutritionally valuable from a protein position. Fish from Romo lake generally have the lowest concentrations of many amino acids, with the notable exceptions of tryptophan and proline, which are highest in this location. This highlights the importance of the specific aquatic environment on the nutritional profile of the fish. This phenomenon is in-line with the report of Steffens (2006), and can be attributed to several factors:

Environmental and Dietary Influences: The primary driver of the observed differences is likely the dietary composition of the fish at each location. The availability and type of food sources (e.g., algae, aquatic insects, detritus) can vary significantly between a dam, a river, and a lake. For instance, fish in Shagari dam, which shows the highest concentrations for several amino acids like Valine, Isoleucine, and Threonine, may have access to a more protein-rich food supply compared to fish in Romo lake. This is in line with the work of Sadiku and Oladimeji (1991). This is a crucial point, as the amino acid composition of fish directly reflects the amino acid composition of their diet (Tiamiyu *et al*., 2018). Romo lake consistently showed the lowest levels for most amino acids, particularly essential ones like Phenylalanine, Valine, and Lysine. This suggests that the lake's ecosystem may be less productive in terms of protein-rich food sources. However, the unusually high levels of Tryptophan and Proline in Romo lake fish could indicate that their diet contains a unique component rich in these specific amino acids, this could be a specific type of plankton or a particular species of plant. Shagari reservoir and Wurno consistently yielded fish with higher

overall amino acid concentrations. This points towards a more robust and nutritionally diverse ecosystem in these locations, offering the fish a richer diet.

Genetic and Physiological Factors: While environmental factors are the most probable cause, it is also important to consider potential genetic variations within the *O. niloticus* population across different water bodies (Fagbenro, 2005). Different strains or populations of the species might have slight genetic differences that influence their metabolism and nutrient accumulation. Moreover, the age and size of the sampled fish, if not standardized, could introduce variability, as amino acid composition can change throughout a fish life cycle. The physiological stress levels, which could be higher in certain environments, might also influence the amino acid profile (Nurnadia, 2011).

Table 1: Mean concentration of amino acid in fresh *O. niloticus* across the selected lending sites in Sokoto state (g/100g)

Amino Acids	Molecular Formular	Wurno fish species	River Rima species	Romo lake species	Shagari dam species
Essential					
Phenylalanine	$C_9H_{11}NO_2$	2.821 ± 0.025^{c}	2.62 ± 0.026^{b}	1.95 ± 0.025^{a}	2.94 ± 0.025^{d}
Valine	$C_6H_{13}NO_2$	3.699 ± 0.038^{b}	4.085 ± 0.039^{c}	3.193 ± 0.038^{a}	4.197 ± 0.038^{d}
Lysine	$C_5H_{11}NO_2$	4.316 ± 0.079^{d}	3.291 ± 0.08^{b}	2.443 ± 0.079^{a}	3.937 ± 0.079^{c}
Arginine	$C_6H_{14}N_4O_2$	2.574 ± 0.083^{b}	2.479 ± 0.085^{b}	2.202 ± 0.083^{a}	2.925 ± 0.083^{c}
Leucine	$C_6H_{14}N_2O_2$	5.195 ± 0.058^{c}	3.99 ± 0.060^{b}	3.769 ± 0.058^{a}	5.320 ± 0.058^{c}
Isoleucine	$C_6H_{13}NO_2$	3.431 ± 0.109^{b}	3.634 ± 0.11^{b}	2.814 ± 0.109^{a}	4.295 ± 0.109^{c}
Threonine	$C_4H_9NO_3$	2.86 ± 0.085^{b}	3.112 ± 0.087^{c}	2.156 ± 0.085^{a}	3.581 ± 0.085^{d}
Histidine	$C_6H_9N_3O_2$	2.264 ± 0.091^{a}	2.88 ± 0.092^{c}	2.646 ± 0.097^{bc}	2.442 ± 0.091^{ab}
Methionine	$C_5H_{11}NO_2$	2.823 ± 0.025^{b}	2.083 ± 0.053^{a}	1.933 ± 0.052^{a}	2.721 ± 0.052^{b}
Tryptophane	$C_{11}H_{12}N_2O_2$	3.240 ± 0.025^{c}	2.392 ± 0.025^{a}	4.568 ± 0.025^{d}	2.454 ± 0.025^{b}
Non-Essential					
Serine	$C_3H_7NO_3$	3.996 ± 0.035^{b}	4.827 ± 0.036^{c}	3.722 ± 0.035^{a}	4.748 ± 0.035^{c}
Alanine	$C_3H_7NO_2$	2.650 ± 0.047^{b}	3.153 ± 0.048^{c}	2.265 ± 0.047^{a}	3.503 ± 0.047^{d}
Glycine	$C_2H_5NO_2$	3.138 ± 0.040^{b}	4.431 ± 0.04^{c}	2.948 ± 0.040^{a}	4.832 ± 0.040^{d}
Aspartic acid	$C_4H_8N_2O_3$	2.91 ± 0.032^{a}	4.64 ± 0.033^{c}	2.97 ± 0.032^{a}	4.46 ± 0.032^{b}
Tyrosine	$C_9H_{11}NO_3$	3.383 ± 0.013^{b}	2.469 ± 0.013^{a}	2.263 ± 0.014^{a}	3.092 ± 0.013^{b}
Proline	$C_5H_9NO_2$	5.024 ± 0.055^{c}	3.043 ± 0.056^{a}	5.489 ± 0.055^{d}	3.579 ± 0.055^{b}

Mean ± standard deviation, mean with the same later of super script along the same raw shows no significant difference($p>0.05$).

The result indicates a wide disparity with significant differences ($p<0.05$) in essential and non-essential amino acid content based on the landing site. Fish from the River Rima have the highest concentrations of phenylalanine, leucine, tryptophan, and histidine, suggesting a rich and diverse protein source from this location. Leucine is an important molecule that can stimulate muscle protein synthesis and has a therapeutic value associated with stress, trauma, and burns (Vijayan *et al*., 2016). Fish sample from Wurno have the highest levels of arginine (4.486±0.029 g/100g) and lysine (4.600±0.021 g/100g), which are crucial for growth and protein synthesis. Lysine plays an important role in supporting circulation and sustaining normal cell growth (Swastawati *et al*., 2023). These amino acids increase after smoking (Table 2). Shagari fish have the highest concentration of methionine (3.065±0.033 g/100g), a key amino acid for metabolism. Romo fish consistently have the lowest concentrations of many essential amino acids, including phenylalanine, methionine, and threonine. However, their levels of valine and tryptophan are notably high. The length

of time of treatment and the smoking method can play a role in changes in the amino acid content of fish (Oluwaniyi *et al*., 2010). A decrease in water content will cause an increase in amino acids (Lopes *et al*., 2015).

Fish sample from Wurno show the highest concentrations of Alanine (4.616±0.026 g/100g), Glycine (4.399±0.059 g/100g), and Aspartic acid (3.712±0.029 g/100g). These are important for energy metabolism and collagen structure. Shagari fish have the highest levels of Serine (3.699±0.026 g/100g) and Tyrosine (3.482±0.021 g/100g), which plays a role in neurotransmitter production. Romo fish have the highest concentration of Proline (5.207±0.023 g/100g). Proline is a major component of collagen, suggesting that fish from this site may have a different muscle or connective tissue composition.

Table 2**:** Mean concentration of amino acid in smoked *O. niloticus* spp across selected landing site in Sokoto state (g/100g)

Amino Acids	Molecular Formular	Wurno fish species	River Rima species	Romo species	Shagari species
Essential					
Phenylalanine	$C_9H_{11}NO_2$	2.532 ± 0.031^{c}	2.912 ± 0.031^{d}	2.207 ± 0.030^{a}	2.414 ± 0.033^{b}
Isoleucine	$C_6H_{13}NO_2$	3.107 ± 0.027^{ab}	3.375 ± 0.027^{c}	3.167 ± 0.027^{b}	3.082 ± 0.028^{a}
Valine	$C_5H_{11}NO_2$	3.137 ± 0.024^{a}	3.812 ± 0.024^{c}	3.759 ± 0.025^{c}	3.567 ± 0.025^{b}
Arginine	$C_6H_{14}N_4O_2$	4.486 ± 0.029^{d}	2.129 ± 0.029^{a}	2.799 ± 0.028^{c}	2.288 ± 0.030^{b}
Lysine	$C_6H_{14}N_2O_2$	4.600 ± 0.021^{d}	2.005 ± 0.021^{a}	3.512 ± 0.020^{b}	3.818 ± 0.022^{c}
Leucine	$C_6H_{13}NO_2$	4.159 ± 0.046^{b}	5.812 ± 0.046^{d}	3.916 ± 0.045^{a}	4.333 ± 0.048^{c}
Threonine	$C_4H_9NO_3$	2.359 ± 0.041^{b}	2.392 ± 0.041^{b}	2.233 ± 0.041^{a}	2.645 ± 0.043^{c}
Histidine	$C_6H_9N_3O_2$	2.244 ± 0.028^{b}	3.173 ± 0.029^{d}	2.607 ± 0.029^{c}	2.120 ± 0.031^{a}
Methionine	$C_5H_{11}NO_2$	2.235 ± 0.031^{b}	2.618 ± 0.031^{c}	2.033 ± 0.031^{a}	3.065 ± 0.033^{d}
Tryptophan	$C_{11}H_{12}N_2O_2$	3.579 ± 0.027^{b}	5.986 ± 0.026^{d}	4.316 ± 0.027^{c}	3.007 ± 0.028^{a}
Non-Essential					
Serine	$C_3H_7NO_3$	2.024 ± 0.025^{a}	2.404 ± 0.024^{b}	3.218 ± 0.025^{c}	3.699 ± 0.026^{d}
Alanine	$C_3H_7NO_2$	4.616 ± 0.026^{d}	3.861 ± 0.025^{c}	3.495 ± 0.026^{b}	2.661 ± 0.027^{a}
Glycine	$C_2H_5NO_2$	4.399 ± 0.059^{d}	3.109 ± 0.058^{b}	3.613 ± 0.057^{c}	2.279 ± 0.061^{a}
Aspartic acid	$C_4H_8N_2O_3$	3.712 ± 0.029^{d}	3.218 ± 0.029^{b}	3.326 ± 0.028^{c}	2.668 ± 0.03^{a}
Tyrosine	$C_9H_{11}NO_3$	2.461 ± 0.020^{b}	3.083 ± 0.020^{c}	2.200 ± 0.020^{a}	3.482 ± 0.021^{d}
Proline	$C_5H_9NO_2$	4.787 ± 0.023^{b}	4.888 ± 0.022^{c}	5.207 ± 0.023^{d}	4.511 ± 0.024^{a}

Mean ± standard deviation, mean with the same later of super script along the same raw shows no significant difference($p>0.05$).

Concentrations of Polycyclic Aromatic Hydrocarbons

The mean concentrations of polycyclic aromatic hydrocarbons (PAHs) in *O. niloticus* fresh fish samples from different landing sites in Sokoto state, Nigeria, show significant differences ($p<0.05$) among the sites and between the different types of PAHs (Table 3). Most abundant PAH: Chrysene (CHRY) consistently shows the highest concentration across all four landing sites, with a particularly high mean concentration at the River Rima, Romo, and Shagari sites (>21 µg/kg). The concentration of Chrysene is significantly ($p<0.05$) lower at the Wurno site (14.673±0.110 µg/kg), indicating a notable site-specific variation. Least abundant PAH is Dibenzo [a, h] anthracene (D [a, h] A) generally has the lowest concentration, with values ranging from 0.160±0.042 µg/kg at River Rima to 0.380±0.290 µg/kg at Wurno. The results reveal distinct patterns for each landing site, Wurno site has significantly ($p<0.05$) higher concentrations of Fluorene (10.927±0.828 µg/kg) and

Pyrene (1.410±0.130 µg/kg) compared to the other sites. It also shows a notably higher concentration of Acenaphthylene and Dibenzo [a, h] anthracene. River Rima, Romo, and Shagari sites show a comparable pattern of PAH concentrations, often having significantly ($p<0.05$) higher levels of Phenanthrene, Fluoranthene, Benzo [a] anthracene, Chrysene, and Benzo [b] fluoranthene than the Wurno site. The Naphthalene (NAP) concentrations are consistent and not significantly different ($p>0.05$) across all sites, ranging from 2.020±0.308 µg/kg to 2.144±0.153 µg/kg.

Acenaphthene (ACE) concentrations are also relatively consistent across all sites, with values between 5.470±0.112 and 5.667±0.420 µg/kg. Acenaphthylene (ACY) in Wurno shows a significantly higher ($p<0.05$) concentration (3.227±0.172 µg/kg) than the other three sites (2.0 µg/kg). This suggests a specific source of this compound in the Wurno area. High molecular weight PAHs (HMW PAHs) are PAHs with 4 or more rings, which are generally more toxic and persistent in the environment.

The elevated levels of these specific PAHs suggest a localized contamination source such as oil spills, industrial runoff, or waste from local activities (Mirja *et al*., 2018). The higher concentration of Acenaphthylene (3.227pm 0.172 µg/kg) and Dibenzo [a, h] anthracene (0.380pm0.290 µg/kg) also points to unique pollution inputs at this location as described by Ding *et al*. (2012) and Qu *et al*. (2017).

River Rima, Romo, and Shagari Sites, show a relatively consistent pattern of higher PAH concentrations, particularly for the high molecular weight (HMW) PAHs. The most notable finding is the exceptionally high concentration of Chrysene (21.776−22.084 µg/kg), which is significantly ($p<0.05$) higher than the Wurno site's concentration (14.673pm0.110 µg/kg). Elevated levels of other HMW PAHs like Benzo [a] anthracene (5.973−6.087 µg/kg) and Benzo [b] fluoranthene (0.953−1.167 µg/kg) similarly suggest a common, widespread source of contamination affecting these areas. The presence of PAHs in fish samples is a matter of concern due to their carcinogenic, mutagenic, and teratogenic properties (ATSDR 1995). Fish are at the top of the aquatic food chain and can accumulate these persistent organic pollutants in their tissues, posing a direct threat to human consumers (Law and Speirs, 2012).

Benzo[a]anthracene and Benzo[b]fluoranthene: These are classified as probable human carcinogens by regulatory bodies, including the U.S. Environmental Protection Agency (EPA, 2017). The result shows significant variation in PAH levels, with much higher concentrations in smoked fish compared to fresh fish (Table 4). The significant variations between sites indicate divergent smoking techniques and/or environmental contamination.

The smoking process significantly ($p<0.05$) increases PAH concentration in the fish samples, with some PAHs presenting much higher levels than others. "Not Detected,"(ND) meaning the concentration of that specific PAH was below the detection limit of the analytical method.

The results further show enormous increase in PAH concentrations in smoked fish compared to the fresh *O. niloticus* fish samples, for example, Naphthalene concentrations, which were around 2 µg/kg in fresh fish, skyrocketed to a maximum of 48.743 µg/kg in smoked *O. niloticus* from the Shagari site which is almost similar with the findings of Nnaji and Ekwe (2018), 39.705 ±0.099 in smoked Tilapia species. This site stands out with the highest concentrations of several PAHs, including Naphthalene (NAP), Acenaphthylene (ACY) and Benzo [a] anthracene (B[a]A), 48.743±0.193 µg/kg, 30.863±0.215 µg/kg and 38.673±0.565 µg/kg respectively. The high levels of these PAHs, along with Dibenzo [a, h] anthracene (9.434±0.80 µg/kg) and Anthracene (22.946±0.410 µg/kg), suggest that the

smoking process at this site is highly inefficient and likely uses materials that release significant amounts of PAHs. This might be similar with some other findings like Ramalhosa *et al*. (2009), Bordajandi *et al*. (2008) and Domingo *et al*. (2007) who stated that the highest PAHs generation during grilling or barbecue through pyrolysis during charcoal broiling of meat products and either deposition and penetration of smoke components into foods and they found a link between that foods and PAHs levels (Ezike and Ohen, 2018). The high levels of these PAHs, some of which are known carcinogens, pose a serious risk to consumers as reported by IARC (2010).

River Rima site has the highest concentrations of some PAHs, but others are not detected. Naphthalene (NAP), Fluorene (FLU), and Phenanthrene (PHEN) are found at very high concentrations. However, Acenaphthylene (ACY), Benzo [b] fluoranthene (B[b]F), Dibenzo [a, h] anthracene (D [a, h] A), and Chrysene (CHRY) are not detected (ND). This is similar with the findings of Ezike and Ohen (2018). This could be possibly due to a different type of wood or a more controlled smoking environment that allows for the volatilization of certain compounds, and it might be due to a more controlled fire or a specific type of wood that does not produce certain HMW PAHs as described by Law and Speirs (2012).

Wurno site shows relatively lower concentrations of most PAHs compared to Shagari and River Rima, but it has the highest concentration of Pyrene (31.863±0.164 µg/kg) and Acenaphthene (23.167±0.933 µg/kg). These compounds are typically formed during the incomplete combustion of organic materials. The high levels of these specific PAHs could be an indication of the type of wood or fuel used for smoking, or the temperature and duration of the process.

Romo site has the lowest concentrations for most of the listed PAHs, except for a few. It has a high concentration of Fluoranthene (22.081±0.101 µg/kg), which is comparable to River Rima. It also has a high concentration of Acenaphthylene (5.999±0.050 µg/kg) and Benzo [b] fluoranthene (5.510±0.040 µg/kg). Chrysene is detected at a very low concentration (0.770±0.035 µg/kg), which is significantly lower than the concentration in fresh fish from the same site. This is a very unusual finding and may suggest an analytical anomaly or a smoking process that somehow reduces or volatilizes Chrysene from the fish. Different types of wood produce varying amounts and types of PAHs. For example, resinous wood tends to produce more PAHs than hardwood. Direct smoking (where fish are in direct contact with smoke) results in much higher PAH levels than indirect smoking (EFSA, 2008). The estimated Dietary Daily Intake (DDI), recommended by the European Union 16 PAHs ranged between 1.18 to 3.78mg/kg according to Nisbet and LaGoy (1992).

Table 3: Mean concentration of PAH in fresh *O. niloticus* fish samples from selected landing sites in Sokoto state

PAHs (µg/kg)	Mol. Formular	Wurno species	River Rima species	Romo species	Shagari species
Naphthalene (NAP)	$C_{10}H_8$	2.120±0.010^a	2.139±0.042^a	2.144±0.153^a	2.020±0.308^a
Acenaphthene (ACE)	$C_{12}H_{10}$	5.470±0.112^a	5.503±0.044^a	5.533±0.252^a	5.667±0.420^a
Acenaphthylene (ACY)	$C_{12}H_8$	3.227±0.172^b	2.080±0.046^a	2.110±0.105^a	2.023±0.255^a
Fluorene (FLU)	$C_{13}H_{10}$	10.927±0.828^b	5.407±0.042^a	5.377±0.202^a	5.543±0.304^a
Phenanthrene (PHEN)	$C_{14}H_{10}$	5.113±0.157^a	5.673±0.061^b	5.640±0.163^b	5.720±0.35^b
Anthracene (ANTH)	$C_{14}H_{10}$	3.940±0.292^a	3.912±0.047^a	3.933±0.107^a	3.844±0.292^a
Fluoranthene (FLTH)	$C_{16}H_{10}$	2.310±0.135^a	3.20±0.037^b	3.177±0.103^b	3.143±0.287^b
Pyrene (PRY)	$C_{18}H_{12}$	1.410±0.130^b	0.524±0.028^a	0.526±0.251^a	0.677±0.420^a
Benzo [a] anthracene (B[a]A)	$C_{18}H_{12}$	5.494±0.804^a	6.000±0.052^b	5.973±0.112^b	6.087±0.229^b
Chrysene (CHRY)	$C_{18}H_{12}$	14.673±0.110^a	22.084±0.099^b	21.776±0.667^b	22.010±0.458^b
Benzo [b] fluoranthene (B [b]F)	$C_{20}H_{12}$	0.731±0.041^a	0.953±0.111^{ab}	1.042±0.127^b	1.167±0.265^b
Dibenzo [a, h] anthracene (D [a. h] A)	$C_{22}H_{14}$	0.380±0.290^b	0.160±0.042^a	0.190±0.061^a	0.223±0.132^{ab}

Mean ± standard deviation, mean with the same later of super script along the same raw shows no significant difference($p>0.05$).

Table 4: Mean concentration of PAH in smoked *O. niloticus* fish samples from selected landing sites in Sokoto state

PAHs (µg/kg)	Mol. Formular	Wurno species	River Rima species	Romo species	Shagari species
Naphthalene (NAP)	$C_{10}H_8$	3.544±0.316^a	28.201±0.558^c	16.208±0.046^b	48.743±0.193^d
Acenaphthylene (ACY)	$C_{12}H_8$	3.249±0.293^b	ND	5.999±0.050^c	30.863±0.215^d
Acenaphthene (ACE)	$C_{12}H_{10}$	23.167±0.933^b	8.873±0.358^a	4.410±0.027^a	18.599±0.761^c
Fluorene (FLU)	$C_{13}H_{10}$	3.723±0.209^a	28.215±0.508^b	4.269±0.045^a	16.72±0.573^c
Phenanthrene (PHEN)	$C_{14}H_{10}$	4.954±0.409^a	29.628±0.802^b	0.993±0.097^a	9.916±0.512^c
Pyrene (PRY)	$C_{16}H_{10}$	31.863±0.164^c	9.882±0.505^b	2.136±0.036^{ab}	ND
Fluoranthene (FLTH)	$C_{16}H_{10}$	5.508±0.710^b	19.890±0.865^c	22.081±0.101^c	ND
Anthracene (ANTH)	$C_{14}H_{10}$	2.998±0.337^b	30.863±0.215^d	0.177±0.042^a	22.946±0.410^c
Benzo [a] anthracene (B[a]A)	$C_{18}H_{12}$	3.637±0.540^a	22.946±0.410^b	2.090±0.040^a	38.673±0.565^c
Benzo [b] fluoranthene (B [b]F)	$C_{20}H_{12}$	0.753±0.113^b	ND	5.510±0.040^c	ND
Dibenzo [a, h] anthracene (D [a. h] A)	$C_{22}H_{14}$	0.650±0.180^a	ND	0.521±0.029^a	9.434±0.80^b
Chrysene (CHRY)	$C_{18}H_{12}$	17.970±0.101^b	ND	0.770±0.035^a	ND

Mean ± standard deviation, mean with the same later of super script along the same raw shows no significant difference($p>0.05$).

CONCLUSION

The variations in amino acid concentrations of *Oreochromis niloticus* across the four landing sites demonstrate the profound impact of habitat and diet on nutritional quality. This result is vital for understanding the dietary benefits of fish from different locations and for guiding nutritional recommendations for consumers in the region. This study strongly suggests that the traditional smoking methods used in these locations are a major source of PAHs contamination and require attention from public health and regulatory bodies.

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