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EFFECTS OF WOOD TYPE USED FOR SMOKING ON THE QUALITY OF SMOKED *Clarias gariepinus*

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ABSTRACT

Effects of wood type used for smoking on the quality of smoked *Clarias gariepinus* was investigated. Forty-five (45) C*larias gariepinus* (average weight 324±43g) were collected, smoked in three groups using *Azadiracta indica, Anogeissus leiocarpae and Terminalia glaucensens*. Proximate, organoleptic and Polycyclic Aromatic Hydrocarbons (PAH) were done on the samples. Data obtained was subjected to appropriate statistical analysis using SPSS 16.0 version. Crude protein makes up the largest percentage of smoked *C. gariepinus* (40.46±0.10% to 44.84±0.10%), Wood species used significantly impacted on the odour ($\chi^2 = 9.17$ $p \leq 0.01$) and flavor ($\chi^2 = 6.37$. $p \leq 0.05$) of the smoked fish, *Terminalia glaucencens* is the most significantly preferred in terms of odour (mean value 37.70) and flavor (mean value 37.40) while *Anogeissus leiocarpae* smoked fish is the least preferred in terms of odour and flavor. Twelve (12) polycyclic aromatic hydrocarbons (PAHs) were recorded in smoked *C. gariepinus* and there concentrations were within tolerable limit. The order of total PAHs in smoked *C. gariepinus* based on the wood type is *Terminalia glaucescens* > *Anogeisus leicarpus* > *Azadiracta indica*. It is recommended that the outer layers of smoked fish should always be discarded in order to minimize PAH concentration in them.

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Keywords: Wood type, smoking, smoked fish, *Clarias gariepinus*

INTRODUCTION

Smoking of fish with different wood species has been used in fish preservation for many centuries (Kwaghvihi *et al.,* 2020; Huong, 2014). Smoking impacts flavouring, cooking and preservation on fish by exposing it to smoke from burning or smouldering material; usually wood; it (smoking) gives special flavor, texture and colour to the fish (Sigurgisladottir *et al.*, 2001; Alcicek and Atar, 2010) and extends its shelf-life via the effects of dehydration, anti-microbial and anti-oxidant of the smoke compounds (Eyo, 2001; Pagu *et al.*, 2013;). Visciano *et al.* (2008) and McGee (2004) also stated that smoking as a form of chemical, thermal, diffusive and biochemical processes usually impact special taste, colour and aroma to food, it also enhances preservation due to the dehydrating, bactericidal and antioxidant properties of smoke depending on the type of wood used; meaning that the nature of smoke generated is dependent on the type of wood used for smoking, this partly explains the reason why Huong (2014) stated that the quality of smoked fish is affected by composition of smoke, smoking method, smoke agents, raw material and fish storage conditions. Smoking impacts taste and aroma as well as longer shelf life on fish because of the combined effects of dehydration, antimicrobial and antioxidant activities of several smoke constituents mainly such as formaldehyde, carboxylic acids and phenols (Arvanitoyannis *et al*., 2012). Depending on the source of smoke, smoking is done to inhibit the formation of toxins that may result from mold growth, reduce bacteria growth due to lower water activity, smoking in combination with salting and drying also creates a physical surface barrier to bacteria, mold and other insect vermin (Rorvik, 2000; Swastawati *et al.,* 2000). The way smoke gets into food products can be used to categorize smoke into the following categories: the traditional technique ;where the smoke is formed directly by burning chips or sawdust in the oven (Stołyhwo and Sikorski, 2005; Visciano *et al.*, 2008); the second technique which is a new technique;where an electric field acts on the ionized smoke particles, which quickens the smoke deposition while the third technique is the use of commercial liquid smoke; where commercial liquid smoke is used for flavourings (Duffes, 1999; Martinez *et al.*, 2007). Woods used for smoking are generally composed of cellulose, hemicellulose and lignin, the concentration of these organic compounds varies between different wood species, the pyrolysis of wood by heat treatment would yield different products depending on the type of wood and the method used for burning the wood e.g burning cellulose in excess of air gives CO_2, H_2O and while pyrolyzing wood at atmospheric pressure yields compounds like acid, phenols and carbonyls (Eyo, 2001), hemicellulose content of hardwood are higher than that of soft wood which yields more acids when burnt making it more preferable for fish smoking than soft wood while compounds that are responsible for smoke flavouring (such as phenols and phenolic esters typified by guaiacol and syringol) are usually obtained by pyrolysis of the lignin fraction of wood (McGee, 2004). The fact that woods can be acquired at little or no cost which will make the smoking processes cheaper as compared to modern smoking methods make many local fish processors in Africa adopt wood smoking as a method of elongating fish shelf life (Adeyeye, 2016). The need to evaluate the wood that will yield the best smoked fish quality among the common wood species used in this study area therefore justifies the need for this research while *Clarias gariepinus* is the fish that was smoked in this study due to its availability and acceptability among consumers in the study area.

MATERIALS AND METHODS

Sample Collection

45 samples of C*larias gariepinus* (average weight 324±43g) were purchased from a reputable farm within Ilorin metropolis and transferred to the Department of Aquaculture and Fisheries, University of Ilorin, kwara State Nigeria within $1^1/_2$ hours. Three commonly used wood species for fish smoking in the study area including *Azadiracta indica, Anogeissus leiocarpae and Terminalia glaucensens* were also purchased from a local wood seller within Ilorin metropolis.

Fish Processing and Smoking

Fish samples were euthanized following the method described by Ayeloja (2019) by adding 20 g table salt/kg fish in plastic container for 10 min, after which the fish were gutted, eviscerated, washed in portable water, immersed in 15% brine for 5 min, then divided into three groups comprising 45 pieces each. Each group was smoked using each of the wood species (*Azadiracta indica, Anogeissus leiocarpae and Terminalia glaucensens*) after which proximate analysis, organoleptic assessment and Estimation of Polycyclic Aromatic Hydrocarbons (PAH) analysis were carried out on the smoked fish.

The experimental was completely randomized design with treatment being the three different wood species used for the fish smoking

(1) *Azadiracta indica* (2) *Anogeissus leiocarpae* (3) *Terminalia glaucensens*

Proximate compositions of fish were determined by conventional method of (AOAC, 2000).

Organoleptic assessment

The sensory quality attributes evaluated were based on a 5-point hedonic scale, modified from the methods of Ayeloja (2019). The parameters examined were odor, flavor, and texture; and the following grades were allotted: 8.1 ⩽ 10 = excellent, 6.1 ⩽ 8 = very good, 4.1 ⩽ 6 = good, 2.1 ⩽ 4 = bad, and ⩽2 = very bad. Twelve semi-trained panelists from the Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin were used for the assessment.

Estimation of Polycyclic Aromatic Hydrocarbons (PAHs)

Samples of the smoked dried fish samples were minced into smaller pieces and homogenized using a mortar and pestle. 10g of the ground fish sample was weighed and grinded with 5g of anhydrous sodium sulphate to make homogenate mixture, the mixture was packed into a thimble and loaded into the extracting chamber (Soxhlet apparatus), the samples were extracted with dichloromethane at 45°C boiling point (USEPA 3540 method). The extracted samples were taken to water bath for evaporation. The extracts were then loaded into a Gas chromatography-mass spectrometry (GC-MS) system for PAH analysis.

A standard mixture of sixteen (16) priority PAHs; Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno (1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene was obtained and used for the analysis. 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.

Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene were the assessed non-carcinogenic PAHs, while Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno(1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene) were the assessed carcinogenic PAHs.

Statistical analysis

Data collected on descriptive organoleptic assessment using a hedonic scale were subjected to a nonparametric test (Kruskal-Wallis test) using SPSS 16.0 version, while other data on the effect of wood type on smoked fish were subjected to One-way Analysis of variance (ANOVA) using F-test to determine the treatment level of significance. Means of the significantly different treatments were separated using the Duncan multiple range test at 95% confidence level.

RESULTS

PROXIMATE COMPOSITION OF SMOKED *Clarias gariepinus*

The result of the proximate composition of smoked *Clarias gariepinus* (Table 1) indicates that the percentage moisture content of *Clarias gariepinus* range between 10.40±0.28% to 24.70± 0.42% and there was a significant difference ($p \leq 0.05$) in the moisture content of smoked fish when the various wood species (*AzadirachtaAzadirachta indica, Anogeissus leiocarpae,* and *Terminalia glaucescens*) was used for smoking. Percentage crude protein content ranged from 40.46±0.10 to 44.84±0.10 was also observed and significant differences ($p \leq 0.05$) were also observed in the protein content of the fish smoked with *Azadiracta indica, Anogeissus leiocarpae,* and *Terminalia glaucencens*. The highest crude protein of 44.84±0.10% was recorded in *Anogeissus leiocarpae* smoked fish while the lowest crude protein of 40.46±0.10% was recorded in fish smoked with *Terminalia glaucencens*. This result also indicate that crude protein constitutes the highest percentage in the proximate composition of smoked *Clarias gariepinus.* The highest percentage crude lipid (26.22±0.16%) was recorded in *Anogeissus leiocarpae* smoked catfish and the lowest was recorded in *Azadiracta indica* (17.65±0.58%) smoked fish. Significant difference

($p \leq 0.05$) was also observed in the crude lipid content of the fish when different wood species (*Azadiracta indica, Anogeissus leiocarpae,* and *Terminalia glaucencens)* were used for smoking. The ash content of the smoked fish ranged from 10.62±0.70% to 15.34±0.16% and significant difference ($p \leq 0.05$) was also observed in the ash content of the fish smoked with *Azadiracta indica, Anogeissus leiocarpae,* and *Terminalia glaucencens.* The percentage carbohydrate content of *Clarias gariepinus* is very negligible ranging between 0.13±0.09% to 3.82±0.24%. There was no significant difference ($p \geq 0.05$) in the carbohydrate content of the fish smoked with *Anogeissus leiocarpae,* and *Terminalia glaucencens*, However, there was significant difference ($p \leq 0.05$) in the carbohydrate content of fish smoked with *Azadiracta indica*.

Table 1: Proximate composition of Smoked Clarias gariepinus.

WOOD TYPE	***Azadiracta indica***	***Anogegeissus leiocarpae***	***Terminalia glaucescens***
MOISTURE (%)	24.70 ± 0.42^{a}	10.40 ± 0.28^{c}	20.99 ± 0.55^{b}
CRUDE PROTEIN (%)	43.27 ± 0.10^{b}	44.84 ± 0.10^{a}	40.46 ± 0.10^{c}
CRUDE LIPID (%)	17.65 ± 0.58^{c}	26.22 ± 0.16^{a}	24.11 ± 0.47^{b}
ASH (%)	14.25 ± 0.70^{b}	15.34 ± 0.16^{a}	10.62 ± 0.16^{c}
CHO (%)	0.13 ± 0.09^{b}	3.21 ± 0.57^{a}	3.82 ± 0.24^{a}

*Mean with different superscript in the row indicates significant difference at $p \leq 0.05$

SENSORY ASSESMENT OF SMOKED *Clarias gariepinus*

The sensory assessment result as presented on the Table 2 indicate that the type of wood used for smoking *C. gariepinus* have significant impact on the odour ($\chi^2 = 9.17$ $p\leq0.01$) and flavor ($\chi^2 = 6.37$. $p\leq0.05$) of the end product as consumers preferred the odour of *Terminalia glaucencens* smoked *C. gariepinus* (with mean value 37.70 ± 0.63) followed by that smoked by *Azadiracta indica* (with mean value 32.25±0.39) while *Anogeissus leiocarpae* smoked *C. gariepinus* (with mean value 21.55 ± 0.28) have the least preferred odour. Similarly, the flavour of *Terminalia glaucencens* smoked *C. gariepinus* (with mean value 37.40±0.84) is considered to be the best followed by the flavor of that smoked by *Azadiracta indica* (with mean value 30.40±1.03) while *Anogeissus leiocarpae* smoked *C. gariepinus* (with mean value 23.70±0.77) have the least preferred flavour. However, the type of wood used for smoking *C. gariepinus* have no significant ($\chi^2 = 0.41$ $p\geq0.05$) effect on texture of the fish.

Table 2: Sensory assessment of Smoked *Clarias gariepinus*

WOOD TYPE	***Azadiracta indica***	***Anogeissus leiocarpae***	***Terminalia glaucencens***	χ^2	P-value
ODOUR	32.25 ± 0.39^{b}	21.55 ± 0.28^{c}	37.70 ± 0.63^{a}	9.17	*0.01***
FLAVOUR	$30.40^{b} \pm 1.03$	23.70 ± 0.77^{c}	37.40 ± 0.84^{a}	6.37	*0.04**
TEXTURE	$33.38^{a} \pm 0.36$	$26.92^{a} \pm 0.41$	$31.20^{a} \pm 0.53$	0.41	*0.48*

Kruskal Wallis test (χ^2) is significant along the row $p\leq0.05$.

The effect of wood typed used for smoking on polycyclic aromatic hydrocarbon (PAH) level of smoked *Clarias gariepinus*

The result on Fig 1 which show the effect of wood typed used for smoking on polycyclic aromatic hydrocarbon (PAH) level of smoked *Clarias gariepinus* revealed that a mixture of twelve (12) priority polycyclic aromatic hydrocarbons (PAHs) including Fluorene, Phenanthrene, Anthracene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno (1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene were present in smoked *C. gariepinus* irrespective of the wood type used for smoking. The PAH concentrations obtained from smoked *C. gariepinus* in this study (Fig. 1) revealed that fish samples smoked using *Terminalia glaucescens* recorded the highest levels ranging between 0.1 Mg/ml to 0.6 Mg/ml followed by the fish samples smoked using *Anogeisus leicarpus* varying between 0.1 Mg/ml to 0.4 Mg/ml while the fish samples smoked using *Azadiracta indica* recorded the lowest total PAHs ranging from 0.0 Mg/ml to 0.15 Mg/ml. The concentrations of PAHs in the smoked African catfish varied with the smoke source. The trend of the concentrations of the total PAHs of the fishes based on the various woods revealed the following order: *Terminalia glaucescens* > *Anogeisus leicarpus* > *Azadiracta indica*. The results on figures 2, 3 and 4 show the chromatogram of *C. gariepinus* smoked with *Terminalia glaucescens, Anogeisus leicarpus* and *Azadiracta indica* respectively.

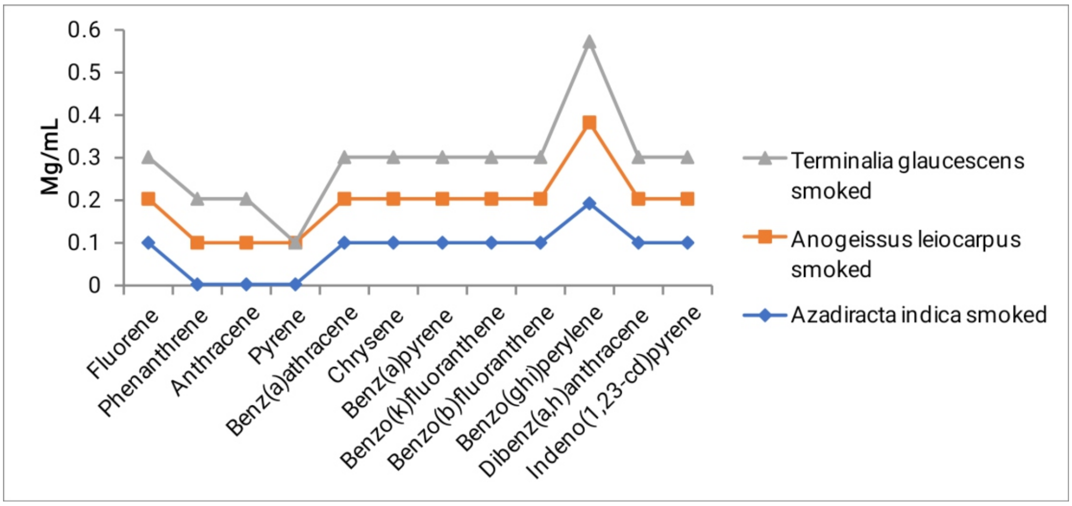

Figure 1. Line chart showing polycyclic aromatic hydrocarbons (PAHs) contents (Mg/ml) of smoked *Clarias gariepinus*

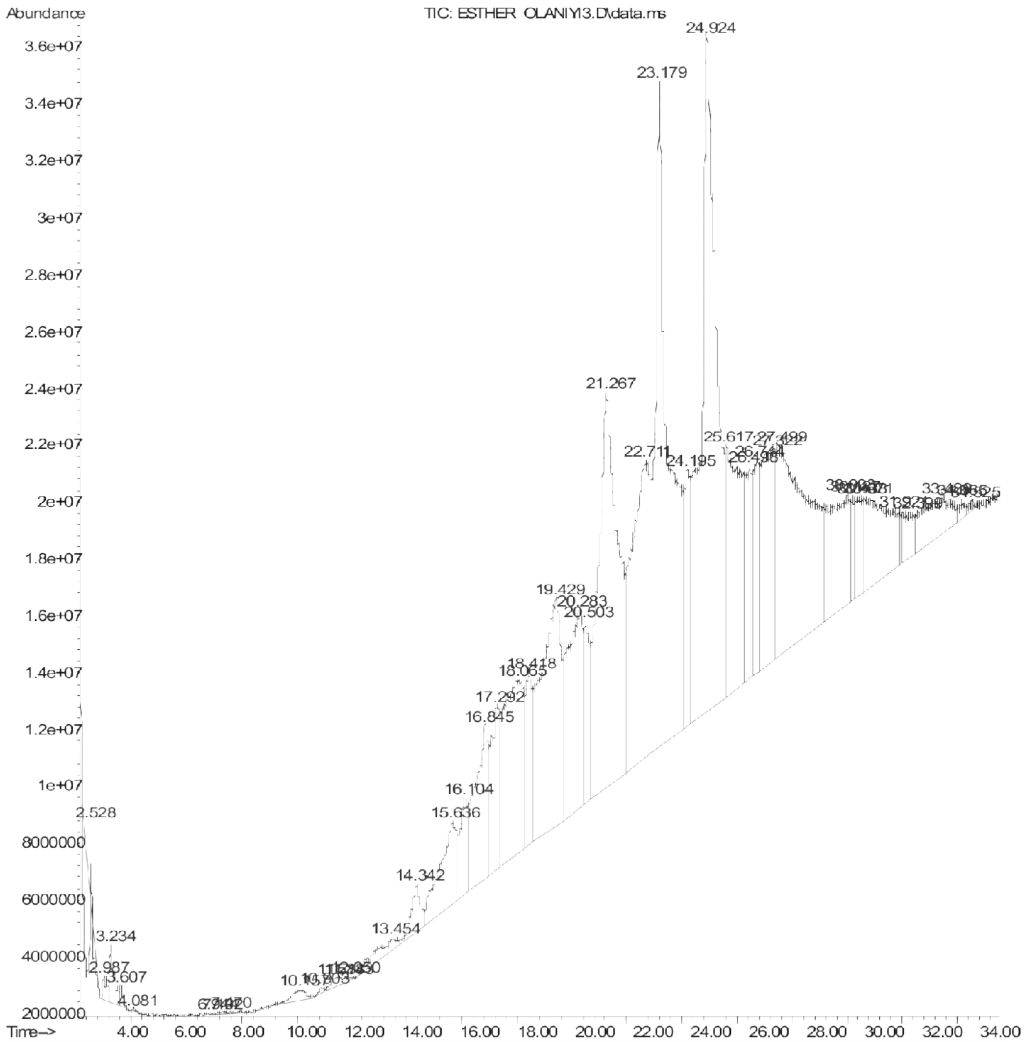

Figure 2. Chromatogram of polycyclic aromatic hydrocarbons (PAHs) compounds in *Terminalia glaucescens*

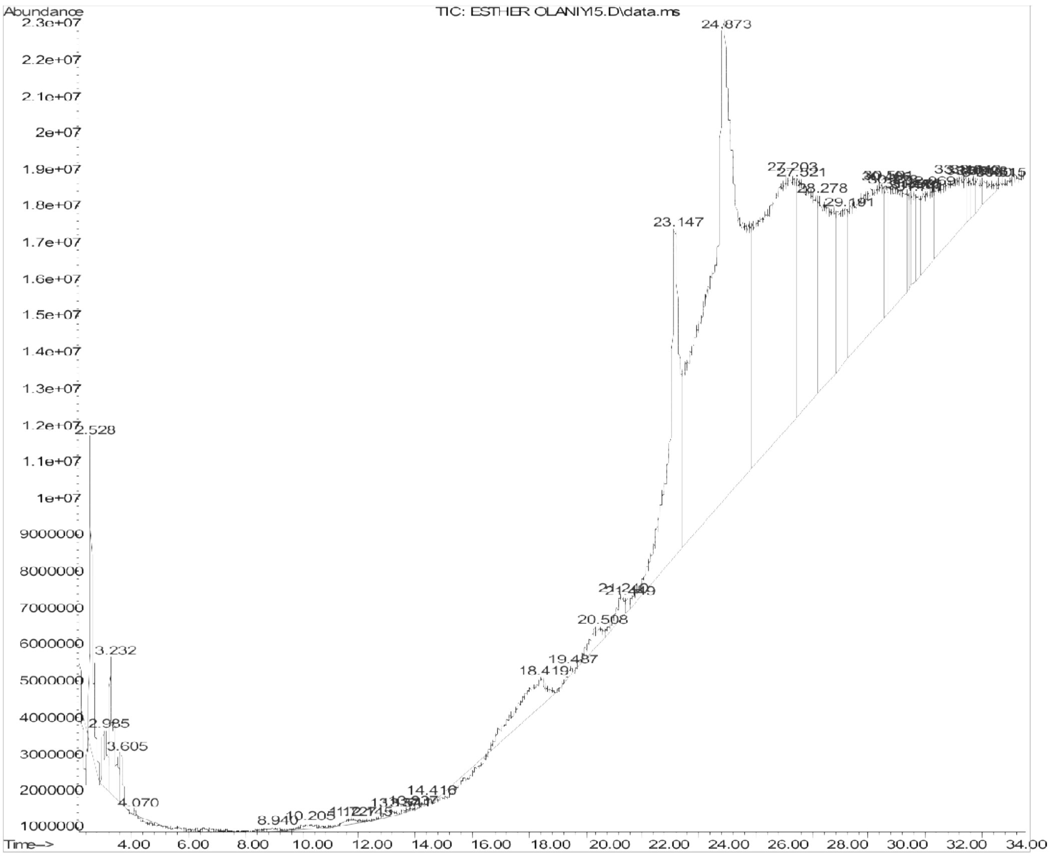

Figure 3. Chromatogram of polycyclic aromatic hydrocarbons (PAHs) compounds in *Anogeisus leicarpus*

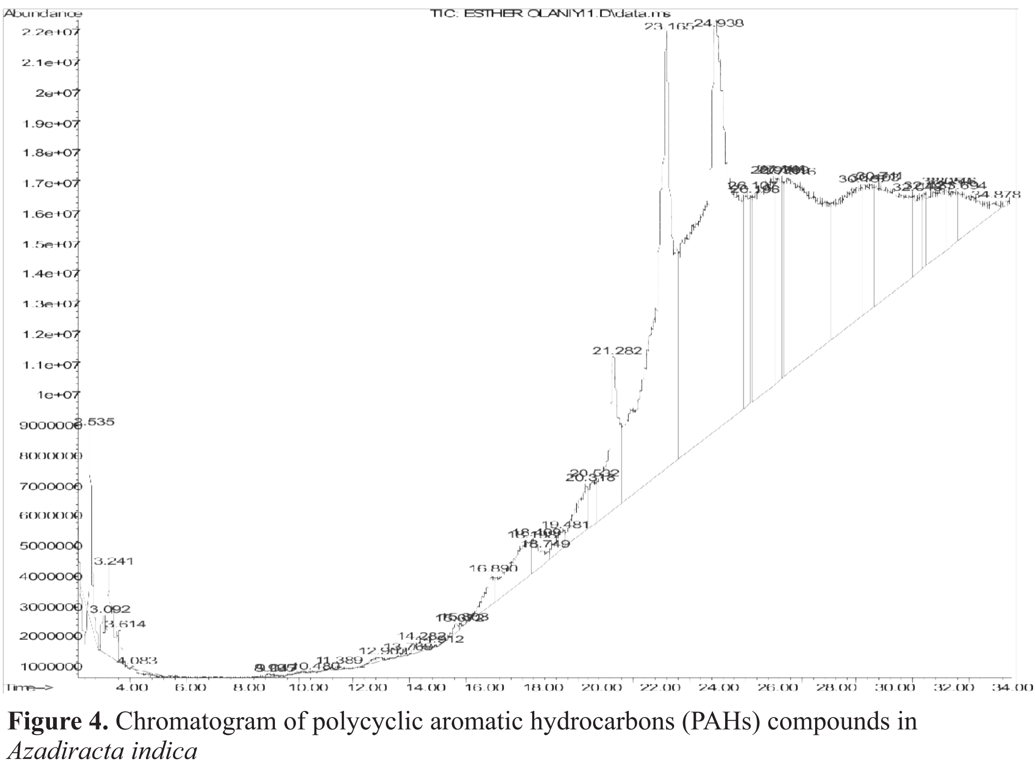

Figure 4. Chromatogram of polycyclic aromatic hydrocarbons (PAHs) compounds in *Azadiracta indica*

DISCUSSION

The percentage moisture content of *Clarias gariepinus* which ranged between 10.40 ± 0.28% to 24.70± 0.42% (Table 1) is similar to that reported by Ayeloja *et al.* (2011) when *Anogeissus leiocarpae* was used to smoke *Clarias gariepinus*. The percentage crude protein content recorded in this study (ranging between 40.46 ± 0.10 to 44.84 ± 0.10) is similar to that reported by other scientists such as Rodrigues, *et al.* (2020) in their study on nutritional indices of freshwater fish species from Serrasalmidae family. This result (Table 1) also indicates that there exists a significant difference (p ≤ 0.05) in the protein content fish smoked with *Azadiracta indica, Anogeissus leiocarpae,* and *Terminalia glaucencens*. The highest crude protein of 44.84 ± 0.10% was recorded in *Anogeissus leiocarpae* while the lowest crude protein of 40.46 ± 0.10% was recorded in *Terminalia glaucencens*. This result also indicate that crude protein constitutes the highest percentage in the proximate composition of smoked *Clarias gariepinus* followed by the percentage lipid of the fish, Ayeloja *et al.* (2019) reported similar result. The highest percentage crude lipid (26.22 ± 0.16%) was recorded in *Anogeissus leiocarpae* smoked catfish and the lowest was recorded in *Azadiracta indica* (17.65 ± 0.58%) smoked fish. However, there was significant difference (p ≤ 0.05) in the crude lipid content of the wood species (*Azadiracta indica, Anogeissus leiocarpae,* and *Terminalia glaucencens).* Significant difference (p ≤ 0.05) was observed in the ash content of the fish smoked with *Azadiracta indica, Anogeissus leiocarpae,* and *Terminalia glaucencens* smoked *C. gariepinus* The sensory assessment result presented on Table 2 indicate that the type of wood used for smoking *C. gariepinus* have significant impact on the odour ($\chi^2 = 9.17$ p≤0.01) and flavor ($\chi^2 = 6.37$. p≤0.05) of the end product as consumers preferred the odour of *Terminalia glaucencens* smoked *C. gariepinus* (with mean value 37.70 ± 0.63) followed by that smoked by *Azadiracta indica* (with mean value 32.25 ± 0.39) while *Anogeissus leiocarpae* smoked *C. gariepinus* (with mean value 21.55 ± 0.28) have the least preferred odour. Similarly, the flavour of *Terminalia glaucencens* smoked *C. gariepinus* (with mean value 37.40 ± 0.84) is considered to be the best followed by the flavor of that smoked by *Azadiracta indica* (with mean value 30.40 ± 1.03) while *Anogeissus leiocarpae* smoked *C. gariepinus* (with mean value 23.70 ± 0.77) have the least preferred flavour. However, the type of wood used for smoking *C. gariepinus* have no significant ($\chi^2 = 0.41$ p≥0.05) effect on texture of the fish. The observed variation in the sensory quality of smoked *C. gariepinus* with wood species is similar to the observation of others scientists such as Rinto *et al.* (2023) in their study of the effect of the use of type of wood on the appearance and flavor of smoked skipjack where it was reported that using different types of firewood affects the appearance and taste of skipjack smoked fish. Atanda *et al.* (2015) also observed variation in the organoleptic qualities of smoked Atlantic Herring (*Clupea harengus*) using different wood type, they observed that a particular wood species improved the smoked fish taste, and aroma, it also gave smoked fish golden brownish colour which gave the appearance alluring outlook. The result on Fig 1 which show the effect of wood typed used for smoking on polycyclic aromatic hydrocarbon (PAH) level of smoked *Clarias gariepinus* revealed that a mixture of twelve (12) priority polycyclic aromatic hydrocarbons (PAHs) including Fluorene, Phenanthrene, Anthracene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno (1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene were present in smoked *C. gariepinus* irrespective of the wood type used for smoking, these PAHs were impacted on smoked *C. gariepinus* from the smoke generated from incomplete combustion of the wood and a multitude of partially oxidized organic chemicals generated in wood smoke (Kwaghvihi *et al.,* 2020). Some of these PAHs have been implicated to have adverse health implication on man especially benzo(a)pyrene and nitrosamines (Muyela *et al.,* 2012). Muyela *et al.* (2012) reported that benzo(a)pyrene and nitrosamines are known to be carcinogens. However, the level of all the PAHs recorded in this study are within the tolerable threshold recommended by 2 µg/kg for benzo(a)pyrene and from 12 to 30 µg/kg for other PAHs (Kwaghvihi *et al.,* 2020) as the PAH concentrations obtained from smoked *C. gariepinus* in this study (Fig. 1) revealed that fish samples smoked using *Terminalia glaucescens* recorded the highest levels ranging between 0.1 Mg/ml to 0.6 Mg/ml followed by the fish samples smoked using *Anogeisus leicarpus* varying between 0.1 Mg/ml to 0.4 Mg/ml while the fish samples smoked using *Azadiracta indica* recorded the lowest total PAHs ranging from 0.0 Mg/ml to 0.15 Mg/ml. The concentrations of PAHs in the smoked African catfish varied with the smoke source. The trend of the concentrations of the total PAHs of the fishes based on the various woods revealed the following order: *Terminalia glaucescens* > *Anogeisus leicarpus* > *Azadiracta indica*. However, it will be proper to discard the outer layers of the smoked fish product as suggested by Eyo (2001), this will minimize PAHs in them since polycyclic aromatic hydrocarbons are concentrated on the surface layer of the product. The results on figures 2, 3 and 4 show the chromatogram of *C. gariepinus* smoked with *Terminalia glaucescens, Anogeisus leicarpus* and *Azadiracta indica* respectively. The result obtained in this study is in line with the result reported by other scientists such as Naeher *et al.* (2007) who stated that PAH concentration in smoked fish is influenced by the kind of wood used for smoking as well as smoking temperature, the method used for developing the smoke and the water content in the wood, Kwaghvihi *et al.* (2020) also stated that source of smoking fuel influences the PAH concentration and organoleptic properties smoked fish.

CONCLUSION

The results obtained in this study indicate that crude protein makes up the largest percentage of smoked *C. gariepinus*, wood type used for smoking impacted significantly (p ≤ 0.05) on the crude protein content of the fish. The sensory results from this study indicate that wood species used for smoking had significant impact on the odour and flavor of *C. gariepinus* with the fish smoked with *Terminalia glaucencens* being the most significantly preferred in terms of odour and flavor followed by the fish smoked using *Azadiracta indica* while the least preferred odour and flavor is the fish smoked with *Anogeissus leiocarpae*. Twelve (12) priority polycyclic aromatic hydrocarbons (PAHs) including Fluorene, Phenanthrene, Anthracene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno (1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene were recorded in smoked *C. gariepinus* irrespective of the wood type used for smoking and there concentrations were within the tolerable limit of 2 µg/kg for benzo(a)pyrene and 12-30 µg/kg for other PAHs indicating that any of the wood could be used to smoke fish meant for human consumption; the trend of the concentrations of the total PAHs of the fishes based on the various woods used for smoking revealed the following order: *Terminalia glaucescens* > *Anogeisus leicarpus* > *Azadiracta indica*. However, it will be proper to discard the outer layers of the smoked fish product as this will minimize PAHs in them since polycyclic aromatic hydrocarbons are concentrated on the surface layer of the product.

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