



Evaluation of polycyclic aromatic hydrocarbons in frozen and roasted Shawa Fish (*Clupea harangus*) sold in Sango fish shop, Ilorin, Kwara State, Nigeria

Abubakar, M.I^{1*}, Adeshina, I¹, Abdulraheem, I², Idris, S.U³

¹Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, Ilorin, P.M.B.1515, Nigeria

²Department of Aquaculture and Fisheries, FUNAAB, P. M. B. 2240, Abeokuta, Nigeria

³Department of Biology, School of Sciences, F.C.T., C.O.E., Zuba-Abuja, P. M. B. 61, Abuja, Nigeria

*Corresponding author, Email address: abubakar.im@unilorin.edu.ng

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Abstract: The aim of this study is to determine the presence of polycyclic aromatic hydrocarbons (PAHs) in Frozen and roasted Shawa Fish (*Clupea harangus*) sold in Sango fish shop, Ilorin, Kwara State, Nigeria were screened for the presence of polycyclic aromatic hydrocarbons (PAHs). The amount of PAHs present in each sample was identified using chromatography with flame ionization detector. PAHs in frozen and roasted fish samples were compared using the retention times of the peak with those obtained from standard mixture of PAHs. Quantification of PAHs was done on external curves prepared from the standard solutions. The results showed that frozen fishes had higher PAHs than the roasted fishes. Conclusion were made that roasting process reduced polycyclic aromatic hydrocarbons on the frozen fish sold in Sango, Ilorin, Kwara State, Nigeria. It was recommended that fish sold in Sango Fish Shop should always be roasted to reduce/eliminate the PAHs in them.

Keywords: Sango; Frozen fish Roasted fish; Chromatography; PAHs

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental contaminants that originate from the pyrolysis or incomplete combustion of organic matter (Costes and Druelle, 1997). They are universal contaminants of our environment and of the human food chain (Lacose *et al.*, 2003). Polycyclic aromatic hydrocarbons (PAHs) were described as hydrophobic organic compounds composed of carbon and hydrogen atoms containing two or more aromatic rings (Singh *et al.*, 2016). Hydrophobic organic compounds contain about 200 PAH compounds (Singh *et al.*, 2016). In food, PAHs are formed during processing and food preparation, either industrial or domestic, especially during the processes of smoking, drying and cooking (Moret *et al.*, 1997; Bardolato *et al.*, 2006). PAH

compounds have been evaluated by the Food Scientific Committee (SCF) as well as the Committee of Food Additives Experts (JECFA) and the European Food Safety Authority (EFSA) respectively (Singh *et al.*, 2016). 16 PAH compounds are known to be of a priority (Singh *et al.*, 2016). The 16 PAH compounds are: Naphthalene (Nap), Acenaphthene (Ace), Acenaphthylene (Acy), Fluorene (Fle), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flu), Pyrene (Pyr), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[a]anthracene (BaA), Chrysene (Chr), Benzo[a]pyrene (BaP), Indeno[1,2,3-cd]pyrene (IcdP), Dibenzo[a,h]anthracene (DahA) and Benzo[ghi]perylene (BghiP) (EFSA, 2008; Singh and Agarwal, 2018). In 2005 and again in 2008, the European Commission has established maximum limits for PAHs in different foodstuffs (Regulation (EC) No 1881/2006). Kluska (2003) observed that food that contributes PAHs compounds are very difficult to be determined quantitatively due to their wide varieties in a sample. Experimental data on PAHs in animals proved that some of these compounds induce health effects such hepatic, hematological and immunological effects and the development of arteriosclerosis respectively (Ramesh *et al.*, 2004).

For better understanding of the health risks associated with consuming contaminated foods with PAHs, the European Food Safety Authority (EFSA, 2008) classified PAHs into three groups according to their carcinogenic, mutagenic, and toxic activities: PAH2 (BaP and Chr), PAH4 (BaA, BaP, BbF, and Chr), and PAH8 (BaA, BaP, Chr, BkF, BbF, IcdP, DahA, and BghiP) respectively. In vivo research conducted on experimental animals revealed that PAHs compounds have mutagenic effect on somatic cells (EFSA, 2008). These compounds are also potentially genotoxic and carcinogenic in humans (Domingo and Nadal, 2015; Lee and Shim, 2007; Yoon *et al.*, 2007). Due to its effects on human health, the maximum incidence of BaP and PAH4 compounds in foodstuffs has been determined to control people's exposure to these compounds in many countries (TFC, 2011). In recent years, the importance of PAHs has increased due to their large sources of transmission (TFC, 2011). People's exposure to PAHs compound is majorly through diet (Alomirah *et al.*, 2011). PAHs are transmitted into foods in two ways (Bansal and Kim, 2015) which are the environmental contamination (air, water and soil) and processed and cooked foods (Bansal and Kim, 2015). Food processing such as smoking and drying as well as cooking of food at high temperature (frying, grilling, and roasting) usually lead to the formation of PAHs (Bansal and Kim, 2015; Jiang *et al.*, 2018; Singh and Agarwal, 2018). In foods cooked at temperature higher than 200°C, pyrolysis occurs as a result of oil dripping into the flame and the fumes generated, PAHs infect the food through the fumes (Jiang *et al.*, 2018). In food cooked in charcoal flames, PAHs formed depend on the amount of fat contained in the food, the cooking temperature and the duration (Aydın and Şahan, 2018; Duedahl-Olesen *et al.*, 2015; Farhadian *et al.*, 2010). PAHs in smoked fish that appeared on open heat can damage DNA and increase the risk of cancer (Lee *et al.*, 2016). Cooking methods such as barbecue (direct contact with fire) and barbecue revealed more carcinogenic compounds such as PAH in food (IARC, 2015). Consumer habits such as economic conditions and diversity of processed foods increases consumption of fish and fish products (Agarwal, 2018). Aydın and Şahan, (2018) studied the relationship between the types of foods, their chemical structures and their cooking methods for the determination of PAH compounds. When food particularly fish is smoked, roasted, barbecued, or grilled; PAHs are formed as a result of incomplete combustion or thermal decomposition of the organic materials (WHO, 2006). Pyrolysis of the fats in the fish generates PAH that become deposited on the fish. PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the fish and the proximity of the fish to the heat source (Kazerouni *et al.*, 2001). Several analyses of charcoal roasted/grilled on common food items have proven the presence of PAHs such as benzo[a]pyrene, anthracene, chrysene, benzo[a]anthracene, indeno[1,2,3-c,d]pyrene (Duke and Albert, 2007; Linda *et al.*, 2011; Akpambang

et al., 2009). Most of these PAHs have been found to be carcinogenic while some are not (Duke and Albert, 2007; Alonge, 1988). The main purpose of this study was to evaluate the types and quantities of PAHs compounds in the samples of frozen and roasted Shawa Fish (*Clupea harangus*) sold in Sango fish shop, Ilorin, Kwara State, Nigeria.

2. Methodology

2.1 Collection of fish items

Samples of frozen and roasted Shawa Fish (*Clupea harangus*) used for the study were obtained from Sango fish shop, Ilorin, Kwara State, Nigeria. They were taken to the University of Ilorin Central Research Laboratory in polythene bags for laboratory analysis.

2.2 Extraction of fish samples for PAHs determination

The extraction of fish samples for PAHs determination was done following the method of Amos-Tautua *et al.*, (2013). 2 gram of homogenized fish samples were thoroughly mixed with anhydrous sodium sulphate (Na_2SO_4) salt to absorb moisture which were later extracted with 10ml of dichloromethane (CH_2Cl_2). The dichloromethane extract was subjected to a column packed with anhydrous Na_2SO_4 salt. The resulting extract was concentrated on a rotary evaporator to give an oily residue which was dissolved in 1ml of dichloromethane and 1 μL respectively. This was injected into the gas chromatography (Model- Hewlett Packed 589 0 series II) with flame ionization detector (Model-Hewlett Packard, Wilmington, DE, USA) for analysis. The comparison of the retention times of the peaks (matrix) with those obtained from standard mixture of PAHs (standards supplied by instrument manufacturer) was used for the identification of PAHs while quantification of the PAHs was based on external calibrations curves prepared from the standard solution of each of the PAHs.

2.3 Statistical analysis

Data obtained were analyzed with Microsoft Excel and expressed as a mean \pm SD. Statistical significance of difference among the mean values of the treatments was tested at 95% probability by Tukey's HSD Post-hoc test

3. Results and Discussion

3.1. Polycyclic Aromatic Hydrocarbons (PAHs) in Sample A (Roasted fish)

The names of Polycyclic Aromatic Hydrocarbons in Sample A (Roasted fish) are presented in Table 1.

Table 1: Names of Polycyclic Aromatic Hydrocarbons found in Sample A (Roasted fish)

S/No	Polycyclic Aromatic Hydrocarbons	Names
1	rBv	4-Isothiazolecarbonitrile, 3-(methylthio)-5-phenyl-
2	rvB	Benzo(A)pyrene
3	rvv	3-Hexanoyltetrahydrofuran -2,4-dion
4	rBv2	Acenaphthylene
5	rBv3	Benzo[B]fluoranthene

Table 1 revealed the polycyclic aromatic hydrocarbons (PAHs) in Sample A (Roasted fish)

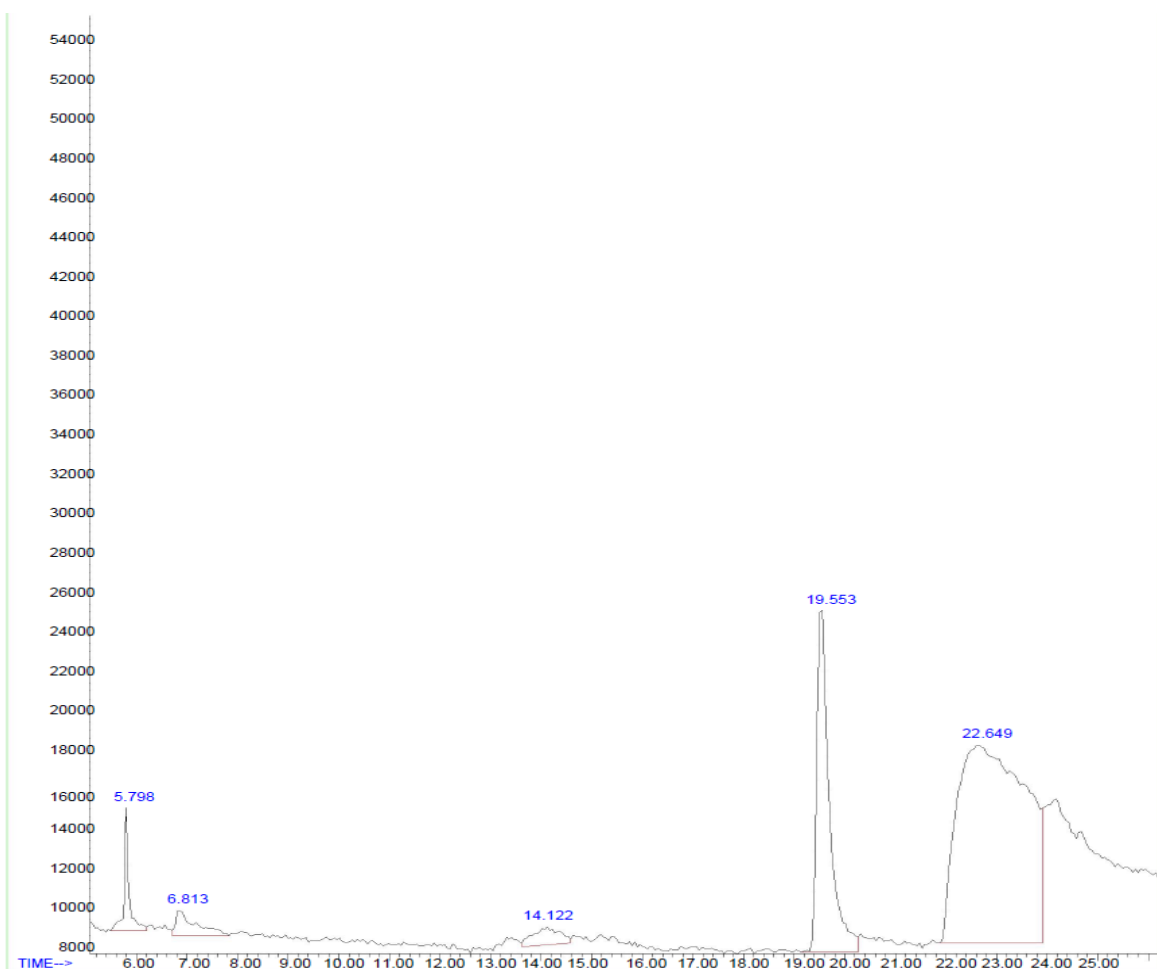


Figure 1: Chromatogram of mix standart of polycyclic aromatic hydrocarbons in Sample A (Roasted fish).

Figure 1 shows the peak and matrices of the polycyclic aromatic hydrocarbons in the Roasted fish with rBv at 5.798, rvB at 6.813, rvv at 14.122, rBv2 at 19.553 and rBv3 at 22.649 respectively. The names of Polycyclic Aromatic Hydrocarbons (PAHs) in Sample B (Frozen fish) are presented in Table 2 below:

Table 2: Names of Polycyclic Aromatic Hydrocarbons found in Sample B (Frozen fish)

S/No	Polycyclic Aromatic Hydrocarbons	Names
1	rvv	2,4-bis(1,1- dimethylethyl) Phenol,
2	rBv2	Naphthalene, 1,4-dihydro-1,4-bis(cyamino)- 8-
3	rBv	2-Butanol, 1,2-diphenyl-4-(methylamino)
4	rvB4	3-methyl-, propionate
5	rVB	1H-Phenalen-1-one
6	rBvB	
7	rBv	
8	rBv	4(1H)-Pteridinone, 5,6,7,8-tetrahydro-6-methyl-
9	rBv	5-Cyano-4-oxo-6-phenyl-2-thioxohexahydropyrimidine
10	RvB3	Phenol, 2,5-bis(1,1-dimethylethyl)

Table 2 revealed the polycyclic aromatic hydrocarbons (PAHs) in Sample B (Frozen fish)

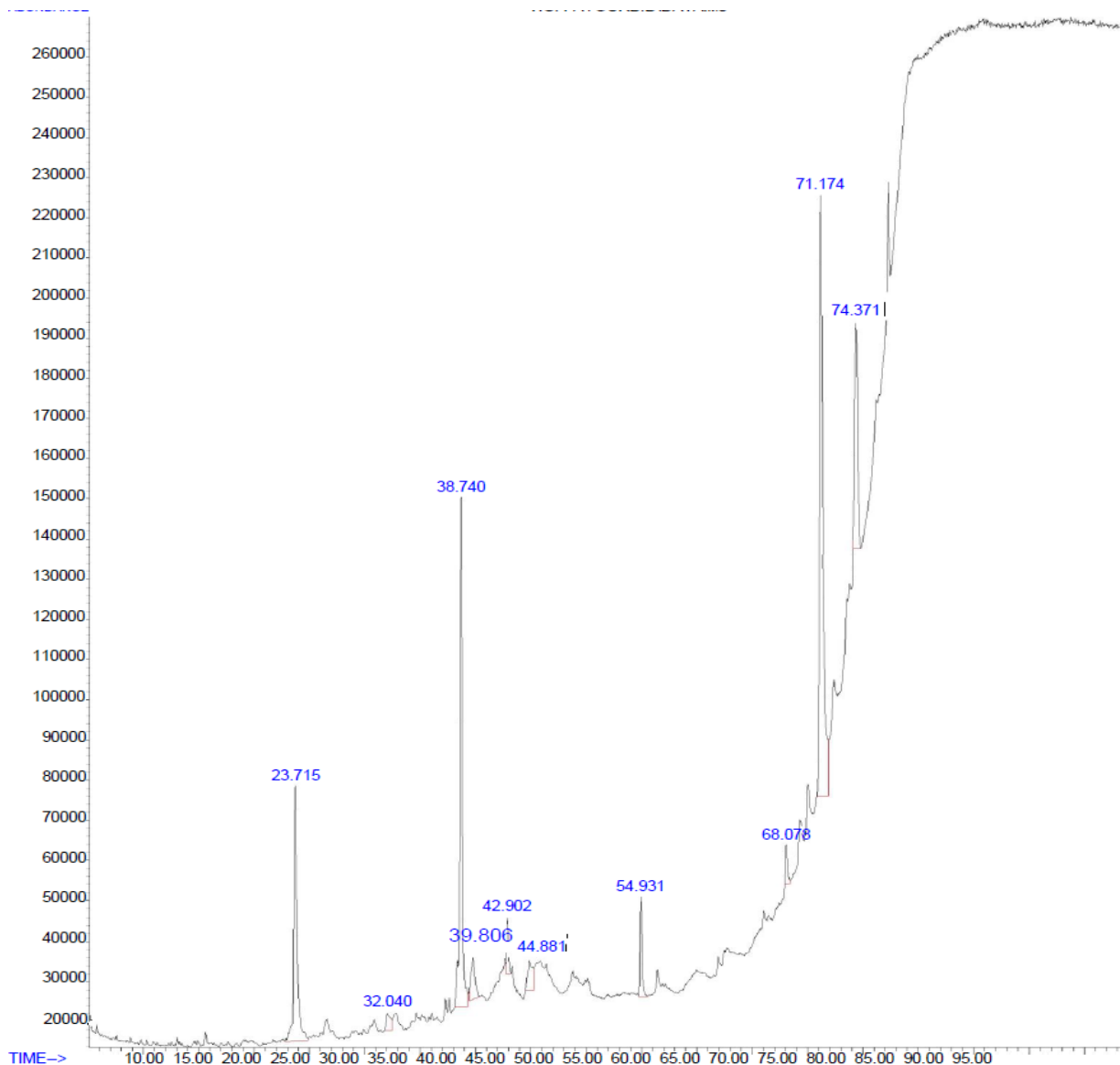


Figure 2: Chromatogram of mix standart of polycyclic aromatic hydrocarbons in Sample B (Frozen fish).

Figure 2 shows the peak and matrices of the polycyclic aromatic hydrocarbons in the frozen fish with rvv at 23.715, rBv2 at 32.040, rBv at 38.740, rvB4 at 39.806, rvB at 42.902, rBvB at 44.881, rvB at 54.931, rBv at 68.078, rBv at 71.174 and rvB3 at 74.371 respectively.

Table 3: Mean of PAHs levels in Sample A (Roasted fish)

S/No	PAHs	Roasted fish($\mu\text{g}/\text{kg}$)
1	rBv	2.88 ± 0.002
2	rvB	2.53 ± 0.004
3	rvv	2.44 ± 0.003
4	rBv2	22.39 ± 0.002
5	rBv3	69.75 ± 0.001

Table 3 indicates the mean for each of the polycyclic aromatic hydrocarbons contained in the roasted fish: rBv has 2.88 ± 0.002 , rvB has 2.53 ± 0.004 , rvv has 2.44 ± 0.003 , rBv2 has 22.39 ± 0.002 and rBv3 with 69.75 ± 0.001 respectively.

Table 4: Mean of PAHs levels in Sample B (Frozen fish)

S/No	PAHs	Frozen fish($\mu\text{g}/\text{kg}$)
1	rvv	15.35 ± 0.003
2	rBv2	1.37 ± 0.003
3	rBv	21.72 ± 0.004
4	rvB4	3.07 ± 0.003
5	rvB	1.66 ± 0.006
6	rBvB	2.55 ± 0.002
7	rvB	3.28 ± 0.003
8	rBv	2.63 ± 0.001
9	rBV	32.51 ± 0.007
10	rvB3	11.84 ± 0.002

Table 4 signifies the mean for each of the polycyclic aromatic hydrocarbons contained in the frozen fish: rvv has 15.35 ± 0.003 , rBv2 has 1.37 ± 0.003 , rBv has 21.72 ± 0.004 , rvB4 has 3.07 ± 0.003 , rvB has 1.66 ± 0.006 , rBvB has 2.55 ± 0.002 , rvB has 3.28 ± 0.003 , rBv has 2.63 ± 0.001 , rBV has 32.51 ± 0.007 and rvB3 with 11.84 ± 0.002 respectively.

Table 5: Mean Comparisons of PAHs ($\mu\text{g}/\text{kg}$) in Frozen and Roasted fishes

Nature	Sample B-Frozen fish($\mu\text{g}/\text{kg}$)	Sample-A-Roasted fish($\mu\text{g}/\text{kg}$)
rvv	15.35 ± 0.003^a	2.44 ± 0.003^b
rBv2	1.37 ± 0.003^a	22.39 ± 0.002^b
rBv	21.72 ± 0.004^a	2.88 ± 0.002^b
rvB4	3.07 ± 0.003^d	ND
rvB	1.66 ± 0.006^c	2.53 ± 0.004^a
rBvB	2.55 ± 0.002^a	ND
rvB	3.28 ± 0.003^a	ND
rBv	2.63 ± 0.001^b	ND
rBv	32.51 ± 0.007^a	ND
rvB3	11.84 ± 0.002^a	ND

Means of parameters along the rows are significantly different at $p < 0.05$

ND: Below detection limit

Table 5 shows the means comparisons between the Frozen and Roasted fishes. It revealed that there is a big significant different among the means of polycyclic aromatic hydrocarbons found in the frozen and roasted fishes. The means of the rvv in the frozen fish (15.35 ± 0.003^a) is greater than the mean in the roasted fish (2.44 ± 0.003^b). The means of rBv2 in the frozen fish (1.37 ± 0.003^a) is less than the mean of the roasted fish (22.39 ± 0.002^b). The means of rBv in frozen fish (21.72 ± 0.004^a) is greater than the mean in the roasted fish (2.88 ± 0.002^b). The mean of rvB4 in the frozen fish had 3.07 ± 0.003^d while the mean in the roasted fish is below detection limit (ND). The means of rvB in the frozen fish

(1.66 ± 0.006^c) is less than the mean in the roasted fish (2.53 ± 0.004^a). rBvB in frozen fish had mean of 2.55 ± 0.002^a while the mean in the roasted fish had mean below detection limit (ND). rvBin frozen fish had mean of 3.28 ± 0.003^a while roasted fish had mean below detection limit (ND). rBvin frozen fish had mean of 2.63 ± 0.001^b while roasted fish had mean below detection limit (ND). rBvin frozen fish had mean of 32.51 ± 0.007^a while roasted fish had mean below detection limit (ND). rvB3 in frozen fish had mean of 11.84 ± 0.002^a while roasted fish had mean below detection limit (ND) respectively.

Table 6: Comparisons of PAHs ($\mu\text{g/kg}$) in the frozen and roasted fishes based on the nature of their matrices

Nature	Sample B-Frozen fish($\mu\text{g/kg}$)	Sample-A-Roasted fish($\mu\text{g/kg}$)
rvv	23.715	14.122
rBv2	32.040	19.553
rBv	38.740	5.798
rvB4	39.806	ND
rvB	42.902	6.813
rBvB	44.881	ND
rvB	54.931	ND
rBv	68.078	ND
rBv	71.174	ND
rvB3	74.371	ND
rBv3	ND	69.754

ND: Below detection limit.

Table 6 shows the comparisons of PAHs ($\mu\text{g/kg}$) in the frozen and roasted fishes based on the nature of their matrices. It signifies that based on the nature of the matrix of each of the PAH in the frozen and roasted fishes; Matrix of rvv in frozen fish (23.75) is greater than the matrix of roasted fish (14.122). Matrix of rBv2 in frozen fish (32.040) is greater than the matrix of roasted fish (19.553). Matrix of rBvrvv in frozen fish (38.740) is greater than the matrix of roasted fish (5.798). rvB4 matrix in frozen fish was 39.806 while roasted fish had matrix of below detection limit (ND). Matrix of rvB in frozen fish (42.902) is greater than the matrix of roasted fish (6.813). rBvB matrix in frozen fish was 44.881 while roasted fish had matrix of below detection limit (ND). rvB matrix in frozen fish was 54.931 while roasted fish had matrix of below detection limit (ND). rBv matrix in frozen fish was 68.078 while roasted fish had matrix of below detection limit (ND). rBv matrix in frozen fish was 71.174 while roasted fish had matrix of below detection limit (ND). rvB3 matrix in frozen fish was 74.371 while roasted fish had matrix of below detection limit (ND). rBv3 matrix in roasted fish 69.754 while frozen fish had matrix of below detection limit (ND).

Discussion

In this study, the means of rvv polycyclic aromatic hydrocarbon in the frozen fish (15.35 ± 0.003^a) is greater than the mean in the roasted fish (2.44 ± 0.003^b). This shows that rvv-2, 4 bis (1, 1-dimethylethyl) phenol in the frozen fish was reduced as a result of the effect of roasting process. This agreed with the report of [Akpambang et al., \(2009\)](#) who reported BaP at levels ranging from 2.4 to 31.2 $\mu\text{g/kg}$ wet weights smoked fish meat samples. Roasting process also reduced the matrix of the PAH in the frozen fish (23.715) to 14.122 in the roasted fish. This is in agreement with report of [Collin et al., \(1998\)](#) who

reported that ratios of fluoran to phenol are often used to verify the sources of PAH detected in a sample. The mean of rBv2 (PAH) in frozen fish (1.37 ± 0.003) is less than the mean in the roasted fish (22.39 ± 0.002). This shows that the PAH- rBv2 (1H- Pyrazole-4-chloro-1-phenyl) in the frozen fish was spiked up in the roasted fish as results of the roasting procedure. The roasting procedure also reduced the matrix of the frozen fish (32.040) to 19.553 in the roasted fish. This is in line with the observation of [Samuel \(2016\)](#) who reported acenaphthylene, anthracene and benzol in grilled fish. The mean of rBv(PAH) in the frozen fish (21.72 ± 0.004) is greater than the mean of in the roasted fish (2.88 ± 0.002). This shows that (rBv: Naphthalene, 1, 4-dihydro-1, 4-bis (cyamino)-8) in the frozen fish was reduced down tremendously as a result of the effect of the roasting process. The roasting process also reduced the matrix of PAH in frozen fish (38.740) to 5.798 in the roasted fish. This is in agreement with [EFSA \(2008\)](#) who stated that if MOE value was less than 10.00, it could be a potential concern for human health. The mean of rvB4 (PAH) in the frozen fish (3.07 ± 0.003) is greater than the mean in the roasted fish with below detection limits (ND). This revealed that rvB4 (2-Butanol, 1, 2-diphenyl-4-(methylamine) in the frozen fish has disappeared in the roasted fish as a result of the effect of roasting procedure. This is in disagreement with the report of [Olabemiwo *et al.*, \(2011\)](#) who observed that raw foods usually have lower levels of PAHs but they are usually spike up during processing like roasting, smoking or frying. The roasting process also reduced the matrix of PAH in frozen fish (39.806) to below detection limits (ND) in the roasted fish. This is in agreement with [Benlachen, *et al.*, \(1997\)](#) who reported that ratio of phenanthrene to anthracene with less than ten indicates combustion source and Ph/An<10 is attributed to petrogenic source. The mean of rvB (PAH) in the frozen fish (1.66 ± 0.006) is less than the mean in the roasted fish (2.53 ± 0.004). This signifies that (rvB: 3-methyl, propionate) in the roasted fish was triggered up as a result of the effects of roasting process. The roasting process also reduced the matrix of PAH in the frozen fish (42.902) to 6.813 in the roasted fish. This is also in line with report of [Collin, *et al.*, \(1998\)](#) who reported that ratios of fluoranthene to pyrene and phenan are often used to verify the sources of PAH detected in a sample. The mean of rBvB (PAH) in the frozen fish (2.55 ± 0.002) is greater than the mean in the roasted fish with below detection limits (ND). This indicate that the (rBvB: 1H-Phenalen-1-one) in the frozen fish was reduced as a result of the effect of roasting process. This also shows that the PAHs in the frozen fish have disappeared as result of effect of the roasting procedure. The roasting process also reduced the matrix PAH in the frozen fish (44.881) to below detection limits (ND) in the roasted fish. This is in disagreement with [Duedahl-Olesen *et al.*, \(2015\)](#) who reported a health concern in 7.080 and 8.450 matrices PAHs in contaminated barbecue. The mean rvB (PAH) in the frozen fish (3.28 ± 0.003) is greater than the mean in the roasted fish with below detection limits (ND). This indicate that the (rvB: 3-furan-2-yl-5-imino-methyl) in the frozen fish disappeared as a result of the effect of the roasting procedure. This is in disagreement with the report of [Olabemiwo *et al.*, \(2011\)](#) who observed that raw foods usually have lower levels of PAHs but they are usually spike up during processing like roasting, smoking or frying. The roasting effects also reduced the matrix of PAH in the frozen fish (54.931) to below detection limits (ND) in the roasted fish. The mean of rBv (PAH) in the frozen fish (2.63 ± 0.001) is greater than the mean in the roasted fish with below detection unit (ND). This shows that (rBv-nepthalen-2(3H)-one) in the frozen fish disappeared in the roasted fish as a result of the effect of roasting process. The roasting process also reduced the matrix of PAH in the frozen fish (68.078) to below detection limits (ND) in the roasted fish. The mean of rBv (PAH) in the frozen fish (32.5 ± 20.007) is greater than the mean in the roasted fish with below detection limits (ND). This shows that (rBv-silacyclopenta-2,4-diene) in the frozen fish disappeared in the roasted fish as a result of the effect of roasting procedure. The roasting process also reduced the matrix of PAH in the frozen fish (71.371) to below detection limits (ND) in the roasted

fish. The mean of rvB3 (PAH) in the frozen fish (11.84 ± 0.002) is greater than the mean in the roasted fish with below detection limits (ND). This shows that (rvB3: phenol, 2, 5-bis (1, 1-dimethylethyl) in the frozen fish disappeared in the roasted fish as a result of the effect of roasting process. The roasting process also reduced the matrix of PAH in the frozen fish (74.371) to below detection limit (ND) in the roasted fish. The roasting process spiked the matrix of rBv3 (PAH) in the roasted fish from below detection limits from the frozen fish to 69.754 in roasted fish. This shows that (rBv3: Benzo (B) fluoranthene) in the frozen fish spiked up PAHs matrix in the roasted fish as a result of the effect of roasting process. This is in line with reports of some researchers that raw foods usually have lower levels of PAHs but they are usually spike up during processing like roasting, smoking or frying (Kayali *et al* 1999 and Olabemiwo *et al.*, 2011).

Conclusion

Based on the report of this study, frozen fish sold in Sango Fish shop were found to be contaminated with marked polycyclic aromatic hydrocarbons, it was concluded that roasting process were found to reduce the polycyclic aromatic hydrocarbons in the roasted fish sold in Sango Fish Shop. Roasting process also has reduction effects on the matrices of the roasted fish respectively.

Recommendation

To reduce some of the polycyclic aromatic hydrocarbons found on frozen fish sold in Sango Fish Shop and its surroundings, it is recommended that the fishes should always be roasted. Considering the potential carcinogenicity of PAH contamination, NAFDAC should not relent in their regulations and surveillance on Nigeria's Fish and Fish processes.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: The experimental protocol and procedures used in this study were approved by Department of Aquaculture and Fisheries, University of Ilorin, Ilorin, Nigeria: Ethical Review Committee (Protocol Identification Code: DERC/AQF/199; DERC Approved Number: DERC/AQF/2221/2291) and conform with the "Guide to the care and use of Animals in Research and Teaching (Ethical Principles for Medical Research. Declaration of Helsinki).

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