International Journal of Research
Available at https://edupediapublications.org/journals
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 05 Issue 07
March 2018

Determination of Polycyclic Aromatic Hydrocarbons (Pahs) Residue in Smoked Fish from Selected Fish Markets in Benue State, Nigeria

*Kwaghvihi, O. B., Akombo, P.M. And Makeri, V. A.

[1]Department Of Biological Sciences, College Of Science, Centre For Food Technology And Research Benue State University, Makurdi

[2]Department Of Fisheries Technology, Akperan Orshi College Of Agriculture, Yandev.

kwaghvihiob@gmail.com
+2347032626081

ABSTRACT

Wood smoke contributes to fish preservation by acting as an effective antioxidant, bacteriostatic and bactericidal agent. However, smoking process contaminates fish with polycyclic aromatic hydrocarbons (PAHs), which are found with traces of mutagenicity and carcinogenicity. This study was carried out to determine the PAHs residues in smoked fish sold in some selected fish markets in Benue State. Clarias gariepinus, Synodontis spp and Mormyrus spp were collected from each of Agatu, Abinsi, Gbajimba and Wadata Markets. The samples were analyzed using gas chromatography-mass spectrometry (GC-MS). The PAHs detected in the fish samples were Naphthalene, Benzo[a]anthracene, Chrysene, 5-Methylchrysene, acenaphthylene, anthracene, Acenapthene, phenanthrene, Cyclopenta[c,d]pyrene and 2-Methylnaphthalene. The analyzed samples showed PAH levels ranging from Zero (0) levels to 18.43µg/kg of smoked fish. Nine of the compounds detected in the samples were low molecular weight (LMW) PAHs except for Cyclopenta[c,d]pyrene which was a high molecular weight (HMW) compound. The interaction effects of PAH concentration indicated that there was significant difference ($P < 0.05$) across the fish species and Markets. Clarias gariepinus was within the EU safe PAH limits of 2µg/kg and 12µg/kg for benzo[a]pyrene and sum of PAH4 for muscle meat of smoked fish in Agatu, Abinsi and Gbajimba Markets. While Mormyrus spp was within safe PAH concentration limits in Abinsi, Gbajimba and Wadata Markets. And Synodontis spp was within safe PAH concentration limits in Agatu and Abinsi Markets. From the Markets, it was observed that Abinsi Market was free of PAH contamination in all the samples, while Agatu Market was contaminated with BaA and CHR in Mormyrus spp (14.73µg/kg). Gbajimba Market was contaminated with CPP in Synodontis spp (18.43µg/kg), while Wadata Market was contaminated with CCP and MNP in Clarias gariepinus (14.83µg/kg) and Synodontis spp (14.56µg/kg). The results obtained showed that Abinsi Market was the only Market with safe product within the EU standard for muscle meat of smoked fish. Therefore, there is need for implementation and necessary enforcement of limits for PAHs in fish (foods) in Nigeria and as such regular monitoring of these components in the smoked fish sold in these markets so as to minimize the potential health hazard posed to humans.

KEY WORDS: *SMOKE, POLYCYCLIC AROMATIC HYDROCARBONS, FISH, BENUE STATE*

1.0 INTRODUCTION

Smoke contributes to fish preservation by acting as an effective antioxidant, bacteriostatic and bactericidal agent as well as by providing a protective film on the surface of smoked fish. However, evidence suggests that smoked fish may contain carcinogens. The smoking process contaminates fish with polycyclic aromatic hydrocarbons (PAHs) (Muyela *et al.*, 2012), which are a large group of molecules containing two or more aromatic rings produced by natural and anthropogenic processes. These chemicals are considered as carcinogenic and environmental contaminants (Varlet, *et al.*, 2011; Bourgeois *et al.*, 2014), which can be found in fish (Muyela *et al.*, 2012) and exposure to PAHs is a major concern for human health. This contamination occurs during smoking and intense thermal processing (Chen and Chen, 2001). During intense thermal processing, the contamination occurs by direct pyrolysis of fish nutrients (Orecchio and Papuzza, 2008). The PAHs are also deposited from smoke produced through incomplete combustion of different thermal agents (Muyela *et al.*, 2012).

Nigeria has been a country that consumes much of smoked fish. And there has been few publications on PAH residue in Nigeria in relation to health as prescribed by the Codex Alimenterius Commission/EU and Nigerian Institute of Standard. Therefore, this study is aimed at identifying the PAHs, as well as determines the level of their residue present in each market. Also to carry out a comparative analysis of the residue levels and the standard by CAC/ EU and NIS in relation to health risk in Benue state; where traditional smoking has been the major means of fish preservation.

3.0 METHODOLOGY

3.1 Study Area

The study took place in some selected fish markets within Benue State which are Gbajimba, Wadata, Abinsi, and Obagaji (Agatu) markets. Benue State is located in the middle belt region of Nigeria. Its geographic coordinates are longitude 7º47` and 10º0` East; Latitude 6º25` and 8º8` North, and shares boundaries with five other states namely; Nassarawa to the North, Taraba to the East, Cross-River to the South, Enugu to the South-West and Kogi to the West respectively. It occupies a land mass of 32, 518 square kilometers. The River Benue flows all year round, though the water volume fluctuates with season. The river overflows its banks during the rainy season (May-October), but decreases drastically in volume leaving tiny island in the middle of the river during the dry season (November-April). The river contains several species of freshwater fishes of different families such as *Clariidae*, *Mormyridae*, and *Synodontidae* etc (Obande *et al.*, 2010; Ayuba *et al.*, 2016).

3.2 Sample Collection

Fish samples were purchased from the major fish markets in Benue state; which are Gbajimba, Wadata, Abinsi, and Obagaji (Agatu) markets. Three fish dealers were identified randomly from each market. And from the selected fish dealers, samples of smoked *Clarias gariepinus, Synodontis spp, and Mormyrus spp* each weighing 500g were sourced. The samples were collected and packaged separately using foil paper and polythene bags. Thereafter, there were taken to National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State for laboratory analysis.

3.3 Extraction and analysis for PAH

The extraction process was carried out using the method described by Varlet *et al.*, (2007) with little modifications.

3.3.1 Solid-Liquid Extraction of PAH

2g of milled fish spiked with a mixture of 20 ^{13}C-PAHs at 1µg/kg, was homogenized in 40mL of cyclohexane / ethyl acetate (50:50; v/v) and shaken for 30 min, then centrifuged at 5000g for 30min at 0°C. The liquid part was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was then dissolved in 6mL of cyclohexane. Each PAH quantification was the result of the mean of triplicate measurements carried out on the individual sample.

3.3.2 SPE Clean-up Procedure

Solid-phase extraction cartridges placed on a Vac Elut system will be conditioned with 5mL of water, then 5mL of methanol and finally with 5mL of cyclohexane. 6mL of sample in cyclohexane was introduced into the cartridge and washed with 3mL of cyclohexane in order to remove the fat. The PAHs were eluted with 12mL of a mixture of cyclohexane and ethyl acetate (50:50; v/v) then evaporated to dryness under a nitrogen stream. Finally, the residue was dissolved in 40µL of toluene.

3.3.3 GC-MS Analysis of PAH

Shimadzu GCMS-QP2010Plus Gas Chromatograph coupled to a Hewlett Packard 5972Mass Selective Detector (Hewlett Packard L.P., Palo Alto, CA) was used to separate the compounds.

GC CONDITIONS
Instrument: SHIMADZU GCMS-QP2010
Column: HP5MS 30m x 0.25µm x 0.25mm id
Column Flow: 1.58ml/Min
Injection temp.:250^{o}c
Injection Mode: Split
Pressure: 108kPa
Total flow: 6.2ml/min
Linear Velocity: 46.3cm/sec

MS CONDITIONS
Acquisition mode: Scan
Ion source temp.: 230^{o}c
Interface temp.: 250^{o}c
Solvent cut time: 2.50min
Scan speed : 1250m/s^2
Start : 40.0 m/z
End : 600 m/z

3.4 Identification and Quantification of PAHs

PAHs were identified based on the match in the retention times of the compounds in the samples against those of the PAH standards. A retention time match of ± 1% was considered for confirmation (Ongwech *et al.*, 2013). Once the elution times were identified, the PAHs were confirmed with comparison of mass-to-charge (m/z) ratios to library database values.
Monitored ions were those of PAHs from flu orine to benzo(g,h,i)perylene listed amongst the US EPA 16 priority pollutants with in addition, cyclopenta(c,d)pyren, 5-methylchrysene, benzo(j)fluoranthene and di benzo(a,l)pyrene, dibenzo(a,e)pyrene, dibenzo (a,i) pyrene, dibenzo (a,h) pyrene.

3.5 Statistical Analysis

Analysis of Variance (ANOVA) was performed on PAH concentrations using GenStat (Discovery Version). The means were separated using Fisher's Least Significant Difference (LSD) at $P < 0.05$.

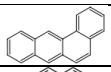
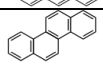
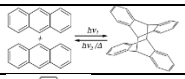
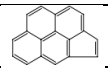
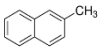
4.0 RESULTS

4.1 PAH Identification

Table 1 below shows the list, structure and quantifying ions of polycyclic aromatic hydrocarbons (PAHs) identified in Agatu, A binsi, Gbajimba and Wadata Markets in Ben ue State
. The PAHs identified from these markets w ere Naphthalene(NAP), Benz[*a*]anthrasene(

BaA), Chrysene (CHR), 5-methylchrysene(MCH), Acenephthylene(ACPL), Acenephth ene(ACP), Anthracene (ANT), Phenanthren e (PHE), Cyclopenta[*cd*]pyrene (CPP), and 2-Methyl naphthalene (MNP). The PAH compounds detected were of low molecular weight (LMW, 2 to 4 ringed) except for Cyclopenta[c,d]pyrene which was a high molecular weight compound (HMW, 5 ringed).

Table 1: List, Structure and Quantifying ions of Polycyclic Aromatic Hydrocarbons Identified in all the Markets

S/no	Name	Acronym	Structure	Quantifying Ions (m/z)
1	Naphthalene	NAP		128
2	Benz[*a*]anthracene	BaA		228
3	Chrysene	CHR		228
4	5-methylchrysene	MCH		242
5	Acenephthylene	ACPL		152
6	Acenephthene	ACP		154
7	Anthracene	ANT		178
8	Phenanthrene	PHE		178
9	Cyclopenta[*cd*]pyrene	CPP		226
10	2-Methyl naphthalene	MNP		142

4.2 PAH Distribution of Fish in Obagaji (Agatu), Abinsi, Gbajimba and Wadata Markets

Figure 2 below represents PAH distribution of fish in Agatu Market. Benzo[a]anthracene and Chrysene were found to occur in all the fish samples while Naphthalene was found in *Clarias gariepinus* and *Synodontis spp*. Acenapthale ne was found in *Synodontis spp* and *Mormyr us spp* while 5-Methyl-chrysene, Acenapthe ne, Anthracene and Phenanthrene were prese nt in *Synodontis spp.* Cyclopenta[c,d]pyrene occurred in *Mormyrus spp* and 2-methylnaphthalene was absent in the fish samples. The concentration of Benzo[a]anthracene and Chrysene was similar by species and 14.73µg/kg in *Mormyrus spp* was found to exceed the EU, (2011) recommended safety limits of 12µg/kg. The rest of the values ranging from zero (0.00µg/kg) to 10.77µg/kg were within the EU safety limits.

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 05 Issue 07
March 2018

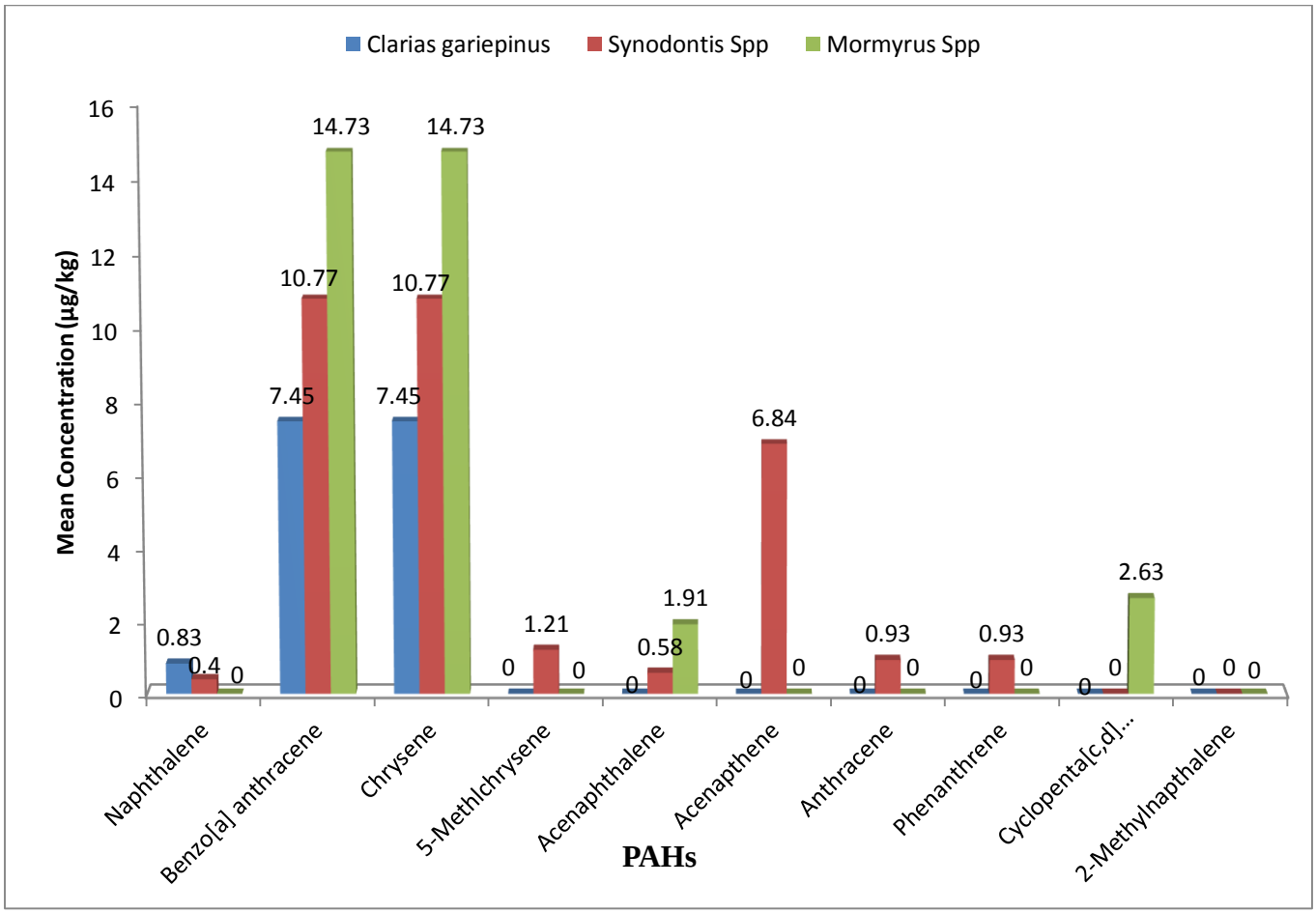


Figure 2: PAH Concentration of Fish in Obagaji (Agatu) Market

Figure 3 below shows PAHs distribution of fish in Abinsi Market. Benzo[a]anthracene and Chrysene were found to occur in *Clarias gariepinus, Synodontis spp* and *Mormyrus spp* with similar concentration per species of 8.45µg/kg, 10.26µg/kg and 8.94µg/kg respectively. Naphthalene was found in *Synodontis spp* and *Mormyrus spp* at concentrations of 0.56µg/kg and 2.16µg/kg. 5-Methylchrysene was present in *Clarias gariepinus* and *Synodontis spp* at concentration of 0.81µg/kg and 1.98µg/kg. Acenaphthalene occurred in *Synodontis spp,* Acenapthene in *Clarias gariepinus* and 2-methylnapthalene occurred in *Mormyrus spp* at concentration of 0.28µg/kg, 0.88µg/kg and 0.52µg/kg respectively. Anthracene, Phenanthrene and Cyclopenta[c,d]pyrene were not in traceable limits. Therefore all the fish samples in this Market were within the EU safety limits.

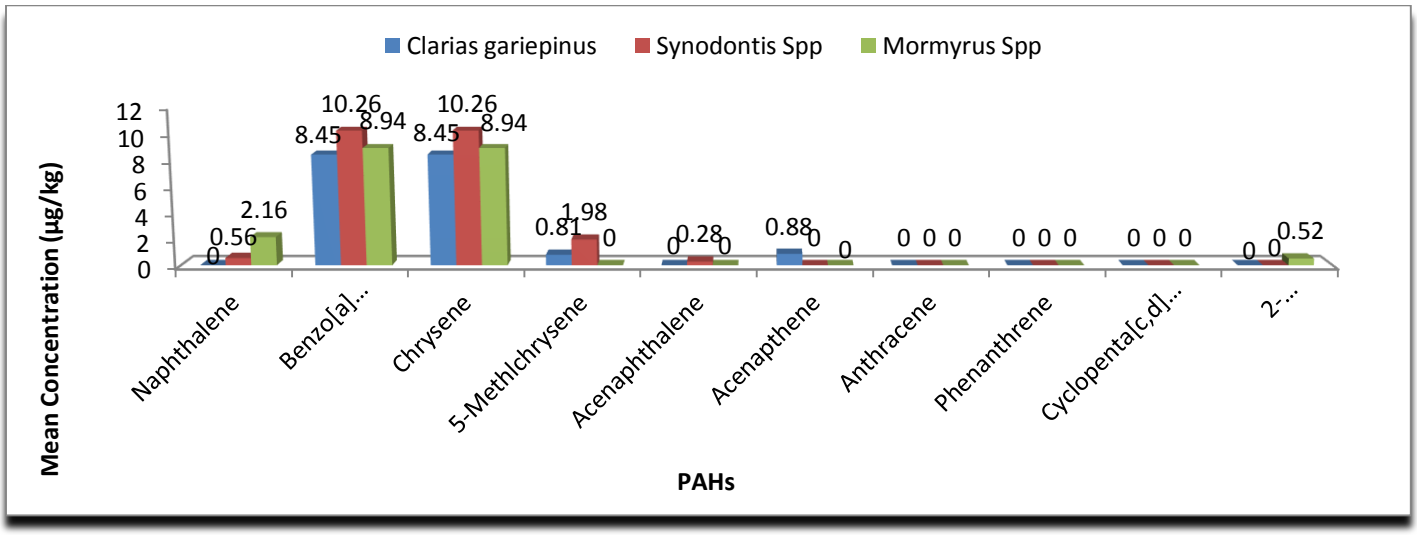


Figure 3: PAH Concentration of Fish in Abinsi Market

Figure 4 below depicts PAHs distribution of fish in Gbajimba Market. It was observed that Acenahthalene, Acenapthene, Anthracene and Phenanthrene were absent (not detected) in all the fish species. Naphthalene was found to be present in *Clarias gariepinus, Synodontis spp* and *Mormyrus spp* at 3.71µg/kg, 0.86µg/kg and 0.67µg/kg respectively. Benzo[a]anthracene and Chrysene were found to occur in *Synodontis spp* and *Mormyrus spp* with similar concentration per species of 5.50µg/kg and 3.68µg/kg. Cyclopenta[c,d]pyrene occurred in *Clarias gariepinus* at 4.14µg/kg in safe limit but occurred beyond safe limits in *Synodontis spp* (18.44µg/kg). 5-methylchrysene was present in *Clarias gariepinus* at 2.48µg/kg and 2-methylnaphthalene occurred in *Mormyrus spp* at 0.49µg/kg, all within the safe limits of EU.

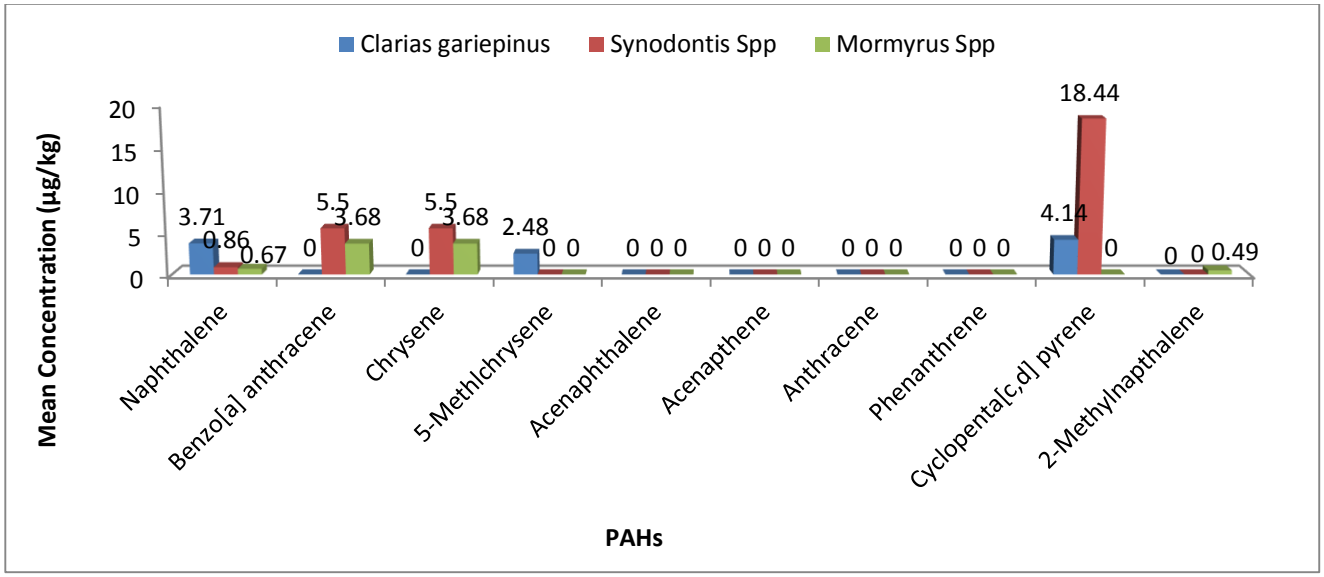


Figure 4: PAH Concentration of Fish in Gbajimba Market

Figure 5 shows PAHs distribution of fish in Wadata Market. It was observed that *Mormyrus spp* recorded none of the PAHs identified. Naphthalene was recorded in *Clarias gariepinus* at 0.72µg/kg, Benzo[a]anthracene and Chrysene were found to occur in *Clarias gariepinus*, and *Synodontis spp* with similar concentration per species of 10.25µg/kg and 5.22µg/kg. 5-methylchrysene had concentration of 1.62µg/kg while Cyclopenta[c,d]pyrene was present in *Clarias gariepinus* (14.83) and 2-methylnaphthalene was present in *Synodontis spp* (14.57), both exceeding the recommended safety limits by EU. Acenahthalene, Acenapthene, Anthracene and Phenanthrene were absent in all the fish species.

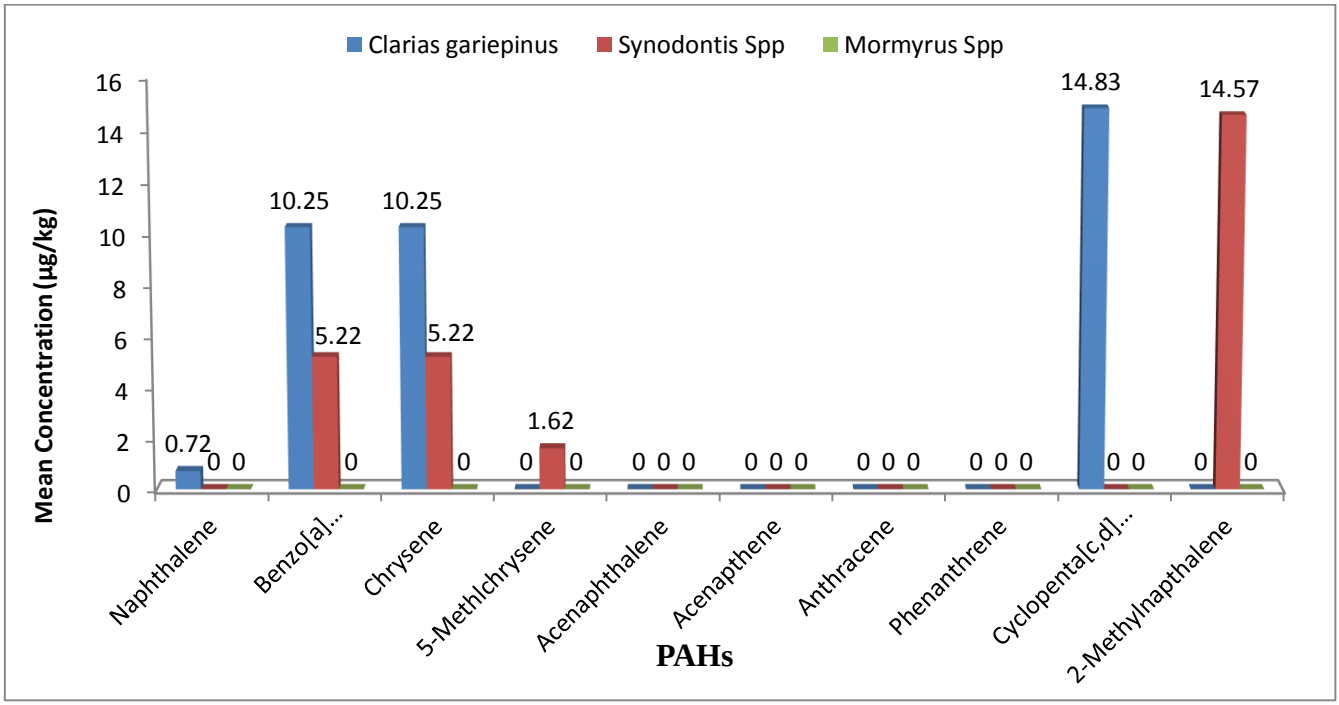


Figure 5: PAH Concentration of Fish in Wadata Market

4.3 PAH Concentration of Fish by Species and Markets

PAH concentration of fish by species and markets indicated that there was significant difference ($P < 0.05$) across the fish species and Markets.

From the fish species evaluated, *Synodontis spp* gave the highest concentration of PAHs while *Clarias gariepinus* gave the lowest concentration. And among the markets, Obagaji recorded highest concentration of PAHs followed by Abinsi, Wadata and Gbajimba respectively (Table 2).

The interaction effects of PAH concentration on fish species and markets indicated that there was significant difference ($P < 0.05$) across the fish species and Markets.

Clarias gariepinus recorded low concentration of PAHs within the EU safety limits of 12µg/kg in Agatu, Abinsi and Gbajimba Markets. While Wadata Market recorded high value of 14.83µg/kg in Cyclopenta[c,d]pyrene (CPP) but the

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 05 Issue 07
March 2018

remaining PAH concentrations were within the EU tolerant limit of safety (Table 3). *Mormyrus spp* recorded lower values of PAH concentration in Abinsi, Gbajimba and Wadata Markets.While Agatu Market recorded high concentrations of 14.73µg/kg in Benzo[a]anthracene (BaA) and Chrysene (CHR) beyond tolerable limits though the remaining PAHs alongside with were within the tolerable safety limits (Table 3). *Synodontis spp* recorded lower levels of PAH concentrations within the EU safety limits in Agatu and Abinsi Markets while Gbajimba was found to exceed in Cyclopenta[c,d]pyrene (18.43µg/kg) and Wadata Market was found to exceed in 2-Methylnaphthalene (14.56µg/kg) beyond tolerable limits while the remaining PAHs were within the recommended EU safety limits (Table 3).

From the Markets, it was observed that Abinsi Market was free of PAH contamination in all the samples, while Agatu Market was contaminated with BaA and CHR in *Mormyrus spp.* Gbajimba Market was contaminated with CPP in *Synodontis spp,* while Wadata Market was contaminated
with CCP and MNP in *Clarias gariepinus* a nd *Synodontis spp* (Table 3).

International Journal of Research
Available at https://edupediapublications.org/journals
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 05 Issue 07
March 2018

TABLE 2: PAH CONCENTRATION OF FISH (µg/kg) BY SPECIES AND MARKETS

TREAETMENT	NAP	BaA	CHR	MCH	ACPL	ACP	ANT	PHE	CPP	MNP
Fish Species										
Clarias gariepinus	1.32	6.53	6.54	0.82	0.00	0.22	0.00	0.00	4.74	0.00
Mormyrus spp	0.71	6.84	6.84	0.00	0.48	0.00	0.00	0.00	0.66	0.25
Synodontis spp	0.45	7.94	7.94	1.20	0.21	1.71	0.23	0.23	4.61	3.64
LSD(0.05)	0.006	0.007	0.008	0.005	0.004	0.002	0.002	0.001	0.003	0.003
Markets										
Obagaji Market	0.41	10.98	10.98	0.40	0.83	2.28	0.31	0.00	0.87	0.00
Abinsi Market	0.90	9.21	9.22	0.93	0.09	0.29	0.00	0.31	0.00	0.17
Gbajimba Market	1.75	3.06	3.06	0.83	0.00	0.00	0.00	0.00	7.52	0.16
Wadata Market	0.24	5.16	5.16	0.54	0.00	0.00	0.00	0.00	4.14	4.85
LSD(0.05)	0.007	0.008	0.009	0.006	0.005	0.003	0.003	0.002	0.004	0.004

Significant difference occurs at points where the difference between the individual PAHs is higher than the LSD value.

International Journal of Research
Available at https://edupediapublications.org/journals
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 05 Issue 07
March 2018

TABLE 3: INTERACTION EFFECTS OF PAH CONCENTRATION OF FISH (µg/kg) BY SPECIES AND MARKETS

Fish Species	Markets	NAP	BaA	CHR	MCH	ACPL	ACP	ANT	PHE	CPP	MNP
Clarias gariepinus	Obagaji Market	0.83	7.44	7.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Abinsi Market	0.00	8.45	8.45	0.81	0.00	0.88	0.00	0.00	0.00	0.00
	Gbajimba Market	3.71	0.00	0.00	2.48	0.00	0.00	0.00	0.00	4.14	0.00
	Wadata Market	0.72	10.25	10.25	0.00	0.00	0.00	0.00	0.00	14.83	0.00
Mormyrus spp	Obagaji Market	0.00	14.73	14.73	0.00	1.91	0.00	0.00	0.00	2.62	0.00
	Abinsi Market	2.16	8.94	8.94	0.00	0.00	0.00	0.00	0.00	0.00	0.52
	Gbajimba Market	0.67	3.68	3.67	0.00	0.00	0.00	0.00	0.00	0.00	0.49
	Wadata Market	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Synodontis spp	Obagaji Market	0.40	10.76	10.77	1.21	0.28	6.84	0.93	0.93	0.00	0.00
	Abinsi Market	0.55	10.26	10.26	1.98	0.58	0.00	0.00	0.00	0.00	0.00
	Gbajimba Market	0.86	5.50	5.49	0.00	0.00	0.00	0.00	0.00	18.43	0.00
	Wadata Market	0.00	5.23	5.24	1.62	0.00	0.00	0.00	0.00	0.00	14.56
LSD (0.05)		0.012	0.014	0.015	0.010	0.008	0.005	0.005	0.003	0.006	0.006

Significant difference occurs at points where the difference between the individual PAHs is higher than the LSD value.

5.1 DISCUSSION

Polycyclic Aromatic Hydrocarbons (PAHs) were determined from the major fish Markets namely Obagaji (Agatu), Abinsi, Gbajimba and Wadata Markets in Benue State. *Clarias gariepinus, Synodontis spp,* and *Mormyrus spp* were the fish species used in this experiment.

Clarias gariepinus, Synodontis spp, and *Mormyrus spp* were procured from each of the four fish Markets bringing to a total number of twelve (12) samples altogether analyzed. The PAHs identified included Nap hthalene (NAP), Benzo[a]anthracene (BaA) , Chrysene (CHR), 5-Methylchrysene (MCH), Acenaphthalene (ACPL), Acenapthene (ACP), Anthracene (ANT), Phenanthrene (P HE), Cyclopenta[c,d]pyrene (CPP) and 2-Methylnaphthalene (MNP). These may be regarded as potentially genotoxic and carcinogenic to humans and, therefore, represent a priority group in the assessment of the risk of long-term adverse health effects following dietary intake of PAHs (Ozcan *et al.*, 2011). Yurchenko and Molder (2005) in their study to determine the point of fish contamination with PAH between fresh and smoked fish samples in Estonian, detected the presence of PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[ghi] perylene and indo[123-cd]pyrene) in smoked fish while there was none detected in fresh fish. Ongwech *et al.*, (2013) detected acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[b]fluoranthene and indeno [1,2,3-cd]pyrene in smoked *Lates niloticus* from selected markets, Gulu District, Uganda. Abdallah, (2013) detected naphthalene, 2-methylnaphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene in 12 bush meat samples obtained from six local producers within the Kumasi Metropolis. The selected three fish species in the four markets from Benue State were found to contain Benzo[a]pyrene (BaP) at undetectable levels; as in none of the analyzed samples was detected. This is in agreement with Ongwech *et al.,* (2013) who reported that, BaP the marker for the occurrence and carcinogenicity of PAHs was undetectable in any of the fish samples analysed in the markets from Gulu District. Similarly, a study by Olabemiwo *et al.*. (2011), reported that BaP was conspicuously absent in both the control and smoked fish samples. However, Anyakora and Coker (2007) reported BaP levels as high as 2.32µg/kg in *Clarias garieppinus* which they attributed to the fact that the fish were caught in highly polluted rivers of the Niger Delta region. Mihalca *et al.*, (2011) also reported high BaP values of 8.4µg/kg in traditional smoked Rainbow trout samples which they attributed to burning log fire which may produce large amounts of PAH and, when used as the source of heat in the grilling of food, very high levels of PAH and BaP could be found in the grilled product. According to Muyela *et al.*, (2012) Benzo [a]pyrene is the only polycyclic aromatic hydrocarbon with sufficient toxicological evidence to allow the setting of a guideline. Its level was lowered from 5 to 2µg/kg while the others were lowered from 30 to12µg/kg (European Union (EU), 2011). The PAH compounds detected were of low molecular weight (LMW, 2 to 4 ringed) except for Cyclopenta[c,d]pyrene which was a high molecular weight compound (HMW, 5 ringed). This result has agreed with the work of Ongwech *et al.*, (2013) who detected high percentage (77.8%) of low molecular weight (LMW) compounds and

22.2% of high molecular weight (HMW) compounds in *Lates niloticus* from three markets in Gulu district, northern Uganda. This high percentage of LMW compounds could possibly be traced to the type of wood used during the smoking process. Pagliuca *et al.,* (2003) reviewed that smoke produced by woods of deciduous trees (hard woods) show high concentrations of low molecular weight PAHs. The presence of Cyclopenta[c,d]pyrene (HMW compound) could be explained from the mechanism of formation of the PAHs. Pagliuca *et al.* (2003) noted that vendors sometimes re-smoke the fish in order to increase their shelf life. During re-smoking, it is possible that the pyrolytic products from the wood combustion add to the intact PAH molecules forming HMW PAHs. Similarly, during prolonged smoking, chances are that the LMW PAHs formed are subsequently converted to the HMW compounds through addition of the pyrolytic products from the continued wood combustion. (Palm *et al.,* 2011; Ongwech *et al.,* 2013).

PAH distribution in Obagaji Market (Figure 2) was found to be within the tolerable limits in C*larias gariepinus* and *Synodontis spp.* While *Mormyrus spp* was found to exceed in Benzo[a]anthracene and Chrysene (14.73µg/kg) beyond the acceptable limits. According to regulation 1881/2006/EC of the European Union (EU), (2011) BaA and CHR were among the 4PAHs of interest. Quantification of these 4PAHs results in the "PAH4 Value" corresponding to the sum of concentration for benzo[a]pyrene, Chrysene, benzo[b]fluoranthene and benzo[a]anthracene. These values were lowered from 5 to 2µg/kg for benzo[a]pyrene and from 30 to 12µg/kg for PAH4. So, recorded levels for BaA and CHR have rendered the product unfit for human consumption. Kartalovic *et al.,* (2015) reported Chrysene as the dominant PAH compound in smoked ham from southwestern Serbia and Vojvodina while Amos-Tautua *et al.,* (2013) detected benzo(a)anthrancene to have the maximum concentration of 7.23µg/g in suya from Amassoma, Niger Delta, Nigeria.

PAH distribution in Abinsi Market as presented in Figure 3 compares favourably with EU standards. This has agreed with the findings of Abdullah, (2013) who determined PAHs in smoked bush meat purchased from three different market centres in Kumasi including Asafo, Central market, Atwemonom (Kejetia) from commercial local meat vendors and concluded that they were all between safety limits. Similarly, Ongwech *et al.,* (2013) also recorded safe PAH values within the Maximum tolerable risk limits (30µg/kg) from all the samples of smoked *Lates niloticus* from selected markets, Gulu District, Uganda. Though this result of Ongwech *et al.,* would have recorded higher limits of Indeno[1,2,3-cd]pyrene (for all the samples) beyond EU standard limits if it was from 2014 to the present day. This is because the values were been changed by the EU in 2014 from 5 to 2µg/kg for benzo[a]pyrene and 30 to 12µg/kg for the 4PAH value.

PAHs distribution in Gbajimba Market was within the safe limits in C*larias gariepinus* and *Mormyrus spp w*hile *Synodontis spp* exceeded limits for CPP (18.44µg/kg). This high value of CPP has agreed with that of Mihaica *et al.,* (2011) who obtained high limits of CPP (19.00µg/kg) in Rainbow trout 1, smoke house 1 in his studies on Polycyclic aromatic hydrocarbons (PAHs) in smoked fish from three smoke-houses in Brasov county, Romania. This could possibly be as a result of re-smoking to

increase shelf-life or prolonged duration of smoking in the area.

PAHs distribution in Wadata Market was within the safe limits in *Mormyrus spp* while Cyclopenta[c,d]pyrene was present in *Clarias gariepinus* (14.83µg/kg) and 2-methylnaphthalene was present in *Synodontis spp* (14.57µg/kg), both exceeding the recommended safety limits by EU. Re-smoking by vendors in order to increase shelf life of fish has possibility of adding to the intact PAH molecules forming HMW PAHs. Similarly, during prolonged smoking, chances are that the LMW PAHs formed are subsequently converted to the HMW compounds through addition of the pyrolytic products from the continued wood combustion (Pagliuca *et al.,* 2003; Palm *et al.,* 2011).

The main effect of PAH concentration on fish species and markets (Table 2) as well as the interaction effects indicated that there was significant difference ($P < 0.05$) across the fish species and Markets (Table 3).

Clarias gariepinus was within the EU safe PAH limits of 12µg/kg for muscle meat of smoked fish in Obagaji (Agatu), Abinsi and Gbajimba Markets. While *Mormyrus spp* was within safe PAH concentration limits in Abinsi, Gbajimba and Wadata Markets. *Synodontis spp* was within safe PAH concentration limits in Agatu and Abinsi Markets. Mahalca *et al.* (2011) noted that PAHs occurred in curing smoke and that they can deposit on the surface of, and migrate into, the fish (food item) being smoked. A number of factors in the smoking process influence the composition of the curing smoke and the uptake of PAHs in the fish being smoked. The combustion temperature during the generation of smoke seems particularly critical and the formation of PAHs in the smoke increases linearly with increasing combustion temperature in the temperature range of 400-1000^{0}C.

From the Markets, it was observed that Abinsi Market was free of PAH contamination in all the samples, while Agatu Market was contaminated with BaA and CHR in *Mormyrus spp.*Gbajimba Market was contaminated with CPP in *Synodontis spp*, while Wadata Market was contaminated with CPP and MNP in *Clarias gariepinus* and *Synodontis spp* (Table 3). The variation of PAH levels in the smoked fish samples in Benue State Markets could be as a result of the environment of the raw materials, smoking temperature, composition of smoke, type of kiln, source of fuel (wood type), duration of smoking and as such re-smoking (Pagliuca *et al.,* 2003; Palm *et al.,* 2011). Similarly, Muyila *et al.*(2012) observed this variation in the levels of PAH among smoked fish from the varying markets to be attributed to the different processing, differences in the type of wood used for smoking or even differences in construction of smoking kilns. They added that Concentrations of PAHs in home prepared meat dishes are dependent on the method of thermal treatment, the type of heat source, cooking time and even fat contents in the meat. It is also important to note that the commercial samples used in the study were smoked and handled differently and that could result in varying PAH load. There is therefore need to monitor the levels of PAHs components in the smoked fish sold in these markets so as to minimize the formation of PAHs during processing of fish thereby reducing the potential health hazard posed to humans.

5.2 CONCLUSION

Polycyclic aromatic hydrocarbons (PAHs) residue were determined in smoked *Clarias*

gariepinus, Synodontis spp and *Mormyrus spp* from Ubagaji (Agatu), Abinsi, Gbajimba and Wadata Markets in Benue State. The results of this study showed the presence of PAHs in the smoked fish in Benue State.

Clarias gariepinus was found to be within safe limits in Obagaji, Abinsi, and Gbajimba Markets. *Mormyrus spp* was found to be within safe limits in Abinsi, Gbajimba and Wadata Markets. While *Synodontis spp* was found within safe limits in Obagaji (Agatu) and Abinsi Markets.

The PAH residue levels were found to exceed the recommended limits in Obagaji, Wadata and Gbajimba Markets, thereby rendering the product unfit for human health.

Abinsi Market was found to be the only Market with safe product within the EU standard for muscle meat of smoked fish.

The interaction effect of PAH concentration across the fish species and markets showed that there exists a significant difference at LSD (0.05) level. Therefore, there is a need to bring out proper strategies on how to minimize the PAH levels on the smoked fish sold in these markets so as to reduce the potential health hazards posed to humans.

REFERENCES

[1] Abdallah, A. (2013). Determination of Polycyclic Aromatic Hydrocarbons in Smoked Bush Meat. Kwame Nkrumah University of Science and Technology (Knust), Kumasi. A Thesis Submitted To The School Of Graduate Studies, Kwame Nkrumah University Of Science And Technology, Kumasi, In Partial Fulfillment of the Requirements for the Award of Degree of Master of Philosophy in Analytical Chemistry.

[2] Amos-Tautua B.M.W., Inengite A. K., Abasi C.Y. and Amirize G.C. (2013). Evaluation of Polycyclic Aromatic Hydrocarbons and Some Heavy Metals in Roasted Food Snacks in Amassoma, Niger Delta, Nigeria. *African Journal of Environmental Science and Technology.* 7(10): 961-966.

[3] Anyakora, C. And Coker, H. (2007). Assessment of Polynuclear Aromatic Hydrocarbon Content In Four Species of Fish in the Niger Delta By Gas Chromatography/Mass Spectrometry. *African Journal of Biotechnology.* 6(6):737-743.

[4] Ayuba, V.O., Omeji, S.O., and Kwaghvihi, O. B. (2016). Parasites of *Heterotis niloticus* From The Lower River Benue At Makurdi. *Octa Journal of Bioscie nces,* 4(2):75- 77.

[5] Bourgeois, M., Veyrand, B., Marchand, P., Monteau, F. and Bizec, L. B. (2014). Quantification of PAHs below Regulatory Limits in Food using the EVOQ GC-TQ LUNAM Université, ONIRIS, Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes-Atlantique, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Atlanpôle-La Chantrerie, BP 50707, Nantes F-44307, France. https://www.bruker.com/fileadmin/user_upload/8PDF Docs/Separations_Mass Spectrometry/Literature/ApplicationNotes/GCMS 05_PAHs_EVOQ_12 e book.pdf

[6] Chen, B.H., Chen, Y.C. (2001). Formation of Polycyclic Aromatic Hydrocarbons in the Smoke from Heated Model Lipids and Food Lipids. *Journal of Agriculture and Food Chemistry.* 49(11):5238-5243.

[7] Codex Alimentarius Commission, (2008). Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods. The Hague, Netherlands, proposed Draft Code of Practice for The Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) From Smoking and Direct Drying Processes 31 March - 4 April.

[8] European Commission (EC), (2005). Commission Recommendation of 4 February 2005 (2005/108/EC) on the Further Investigation into the Levels of Polycyclic Aromatic Hydrocarbons in Certain Foods. *Official Journal of the European Union.* 34: 43–45.

[9] European Food Safety Authority (2008). Polycyclic Aromatic Hydrocarbons in Food Scientific Opinion of the Panel on Contaminants in the Food Chain. *The EFSA Journal*. 724: 1-114

[10] European Union (2011). Commission Regulation (Eu) No. 835/2011 of 19 August 2011 Amending Regulation (Ec) No. 1881/2006 As Regards Maximum Levels for Polycyclic Aromatic Hydrocarbons In Foodstuffs. *Official J. Eur. Union.*

[11] Kartalovic, B., Okanovic, D., Babic, J. Djordjevic, V. Jankovic, S. and Cirkovic, M. (2015). Polycyclic aromatic hydrocarbons in smoked ham. International 58th Meat Industry Conference "Meat Safety and Quality: Where it goes?" *Procedia Food Science* 5: 144-147

[12] Mihalca, G.L., Tița, O., Mihaela, T and Mihalca A. (2011). Polycyclic Aromatic Hydrocarbons (PAHs). In Smoked Fish from Three Smoke-Houses in Brasov County *Journal of Agroalimentary Processes and Technologies*. *17*(4): 392-397

[13] Muyela, B., Shitandi, A. and Ngure, R. (2012). Determination of benzo[a]pyrene levels in smoked and oil fried *Lates niloticus. International Food Research Journal*. 19(4): 1595-1600.

[14] Obande, R.A., Omeji, S. and Nyam S.K., (2010). Checklist and Assessment of Efficiency of Some Traditional Gears and Crafts Used on River Benue. *Journal of Research in Forestry, Wildlife and Environment.* 2 (2).

[15] Olabemiwo OM, Alade AO, Tella AC, Adediran GO (2011). Assessment of polycyclic aromatic hydrocarbons content in smoked *C. gariepinnus* and *T. guineensis* fish species available in Western Nigeria. Int. J. Basic Appl. Sci. 11(2):135-150.

[16] Ongwech, A., Nyakairu, G. W., Mbabazi, J., Kwetegyeka, J. and Masette, M. (2013). Polycyclic Aromatic Hydrocarbons in Smoked *Lates niloticus* from Selected Markets, Gulu District, Uganda. *African Journal of Pure and Applied Chemistry*. 7(4):164-172.

[17] Orecchio, S. and Papuzza, V. (2008). Levels, Fingerprint and Daily Intake of Polycyclic Aromatic Hydrocarbons in Bread Baked using Wood as Fuel. *Journal of Hazardous Materials.* 164: 876–883.

[18] Ozcan, T., Akpinar-Bayizit, A., Sahin, O. I. and Yilmaz-Ersan, L. (2011). The Formation of Polycyclic Hydrocarbons During Smoking Process of Cheese. *Mljekarstvo* 61 (3): 193-198.

[19] Palm, L.M.N., Carboo D, Yeboah OP, Quasie JW, Gorleku OM, Darko A (2011). Characterization of Polycyclic Aromatic Hydrocarbons (PAHs) Present in Smoked Fish from Ghana. *Adv. J. Sci. Technol.* 3(5):332-338.

[20] Pagu, I., Nistor, C., Măgdici , E., Hoha, G., Albu, A., and Păsărin, B. (2013). Research Regarding the Influenc e of Age and Corporal Weight on Efficiency at S moking and Chemical Composition of Smoked Rainbow Trout. *University of Agricultural Sciences and Veterinary Medicine Iasi,* 216-220.

[21] Pagliuca G, Gazzotti T, Zironi E, Serrazanetti GP, Mollica D and Rosmini R (2003). Determination of High Molecular Mass Polycyclic Aromatic Hydrocarbons in a Typical Italian Smoked Cheese by HPLC-FL. *Journal of Agriculture and Food Chemistry*. 51(17):5111-5115.

[22] Varlet V., Serot T., Knockaert C., Cornet J., Cardinal M., Monteau F., Bizec B.L., and Prost C. (2007). Organoleptic Characterization and PAH Content of Salmon (*Salmo salar*) Fillets Smoked According to Four Industrial Smoking Techniques: *Journal of the Science of Food and Agriculture*. 87(5): 847-854

[23] Varlet, V., Serot, T., Monteau, F., Le Bizec, B. and Prost, C. (2011). Determination of PAH Pro_Les by Gc-Ms/Ms In Salmon Muscle Meat Processed With Four Cold Smoking Techniques. *Food Additives and Contaminants* 24 (07): 744-757.

[24] Yurchenko, S and Molder, U. (2005). The Determination of Polycyclic Aromatic Hydrocarbons in Smoked Fish by Gas Chromatography Mass Spectrometry with Positive-ion Chemical I onization. https://www.researchgate.net/publication/223938367.