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Effect of Extraction Methods on the Polycyclic Aromatic Hydrocarbons Content Smoked Catfish Species in Niger State of Nigeria.

Ajai A. Ikechukwu[1]*, Suleiman M.Tanko[1], Dauda B.Emmanuel[1], Sadiku O.Suleiman[2] and Aberuagba, F.[3]

[1]Department of Chemistry, [2]Department of Fishery, Aqua culture and Fresh water Resources, [3]Department of Chemical Engineering, Federal University of Technology Minna, Nigeria

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Abstract

Catfish species collected from three different fishing zones namely; Katcha/Baro, Shiroro/Sarkin-Pawa and Wushishi/Gwarjiko/Zungeru areas of Niger state smoked with the traditional kilns (the drum and the mud-type smoking kilns) were screened for their polycyclic aromatic hydrocarbons (PAHs) content using three different extraction methods namely; accelerated solvent, Soxhlet and solid-liquid and GC/MS. Results from the study showed that the PAHs content in the studied smoked *Clarias gariepinus* (Catfish), ranged between 0.75-2.25, 0.40-2.00 and 0.25-1.75µg/kg for the accelerated solvent Soxhlet and solid-liquid extraction method irrespective of zone, while the index of PAH contamination, benzo(a)pyrene range between 1.28-1.96, 0.4-1.62 and 0.25 -1.54 µg/kg for the accelerated solvent, Soxhlet and solid-liquid extraction method, respectively. Generally, the PAHs content in the studied smoked fish species from the various zones were comparable and ranged between 0.40-2.25µg/kg and were below the European Union's recommended limit of 5µg/kg for carcinogenicity in smoked meat and fish products. The results also show that the accelerated solvent method was more efficient for the extraction of PAHs in the studied fish species than the Soxhlet and solid-liquid extraction method. The findings also revealed that the variation across zones may have been due to the type of smoking kilns used.

k**eywords:** Smoking kilns, smoked catfish, polycyclic aromatic hydrocarbons, carcinogenicity, extraction, Clarias gariepinus.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are formed when complex organic substances are exposed to high temperature or pressure or by the incomplete combustion of woods, coal or oil. They can be found in complex mixtures throughout the environment (Easton *et al*, 2002; Storelli *et al*, 2003; Grova *et al*, 2005; Wretling, *et al* 2010). At ambient temperature, PAH are solids with low volatility. They are relatively insoluble in water and soluble in many organic solvents and are highly lipophilic. They have low vapour pressure, relatively high melting and boiling points due to their high molecular masses. Most PAH can be photo-oxidized and degraded to simpler substances (IPCS, 1998).

Foods can be contaminated by PAHs from environmental sources, industrial food processing and during home food preparation. Industrial food processing represents the major source of human exposure from diet (Zabik *et al*, 1996; Kannapan *et al,* 2000; Wretling *et al*, 2010). As PAHs represent an important class of carcinogens, their presence in foods has been intensively studied. Of the several hundreds of PAHs, sixteen (16) of them have been identified as priority PAHs because they have been considered to be more harmful to man than the others (Andrzej and Zdzislaw, 2005; Chimezie and Hebert, 2006; Wretling *et al*, 2010).

In carrying out analysis of PAHs content in sample matrix, different reagents, extraction methods and instrumental analysis could be used in order to obtain precise information on the extent of contamination in the sample matrix. Extraction is usually the first step in analytical procedures applied to the determination of organic compounds in solid matrices. The use of a convenient type of extraction not only influences the accuracy of results, but also determines the total analysis time and in this way affects sample throughput and analysis costs. Several efficient extraction techniques have been developed and are commonly used for analyte isolation from solid matrices.

Often extraction procedures are non- selective and will extract a broad spectrum of organic samples. The resulting extract has to be purified by the removal of impurities, which may interfere with the analysis of PAHs. The techniques employed for smoked fish vary by the method used to enhance the action of the solvent for the extraction, and ranges from the classic Soxhlet extraction to modern microwave extraction. Different workers have

* Corresponding author: talk2alexajai@gmail.com

reported various methods that could be used for extraction of PAHs from sample matrix (Wretling *et al,* 2010; Khan *et al*, 2005; Juhani *et al*, 2004; Camel, 2001; Bjerkland *et al*, 2000, Hawthorne and Grabanski, 2000; Wang *et al*, 1999; USEPA, 1996; Majors, 1995). Linjinsky and Shubik (1965) first reported the presence of PAHs in smoked meat, since then many studies have confirmed the presence of PAHs in different smoked foods including fish (SCF 2002; EFSA 2008). PAHs concentrations ranging from 0.01-200µg/kg have been reported by different workers in smoked fish and meat products using different extraction and instrumental methods (Wretling *et al.*, 2010; Ajai *et al*., 2010; EFSA 2008; SCF 2002; De Vos *et al*., 1990, Emerole *et al*., 1982, Prinsen and Kennedy, 1977, Steinig and Meyer, 1976).

The PAHs in smoked foods are highly variable. These variations can be attributed in part to the different procedures used to evaluate the PAHs content, but the main reason for such discrepancies is the difference in procedures used for smoking. Such as the type and composition of woods, type of generator, oxygen accessibility, temperature of smoke generation and smoking time (SCF 2002, Vincent *et al*., 2007, Wretling *et al*., 2010) This work is thereof aimed at determining the PAHs content in smoked cat fish species in Niger State using different extraction methods in order to ascertain the best extraction method that will give the highest yield of PAHs from the smoked fish species.

2. Materials and Methods

Agilent gas chromatography (HP 68990GC) manufactured by Agilent Technology, Palo, Alto CA, USA and mass spectrophotometer with flame ionization detector (HP 5973) manufactured by Agilent Technology, Palo, Alto CA, USA. Accelerated solvent extractor ASE 200 by Dionex Corporation, California, USA and Soxhlet extractor B810/428 by Gemini scientific Ltd United Kingdom, were use for this study.

PAH reference standards mixture (500µg/ml) containing the 16 target PAHs obtained from NIST Baltimore, MD, PAH internal standard mixture containing five isotopically labeled PAHS acenaphthene-$_{d10}$, pyrene-$_{d10}$, chrysene-$_{d12,}$ perylene$_{d12}$ and benzo(ghi)perylene-$_{d12}$ obtained from LGC Prochem, Boras, Sweden, dichloromethane, pesticides grade obtained from J. T. Baker, Germany, fluorobenzene-2-fluorobiphenyl, pesticide residue grade obtained from Merck Darmstadt, Germany, silica gel 100/120 mesh obtained from BDH laboratories, and petroleum ether (40-60%), analytical grade obtained from BDH laboratories were used for this study.

Smoked *Clarias gariepinus* species (catfish) were collected from the local fish processors in Katcha and Baro in Katcha, Shiroro in Shiroro, Sarkin Pawa in Munya, Wushishi, Zungeru and Gwarjiko in Wushishi local government areas of Niger state.

2.1. Preparation of standard solutions

Five standard solutions each containing the 16 target compounds were prepared by diluting 1.0, 2.0, 4.0, 10.0 and 20.0 cm^3 of 500µg/mL of each standard PAH with 100 cm^3 of dichloromethane. To all of these solutions were added 0.5µg each of the five internal standards namely acenaphthene-$_{d10}$, pyrene-$_{d10}$, chrysene-$_{d12,}$ Perylene$_{d12}$ and benzo(ghi) perylene-$_{d12}$. The solutions were transferred into capped and sealed vials until ready for analysis.

2.2. Recovery studies

Prior to extraction 0.5µg of each of the five surrogate standards were added to the sample to monitor the recovery of different target compounds. This was used to monitor unusual matrix effect and gross sample processing error. The surrogate standards used include acenaphthene-$_{d10}$, pyrene-$_{d10}$, chrysene-$_{d12}$, perylene$_{d12}$ and benzo(ghi)perylene-$_{d12}$. Those standards serve as surrogates the different sets of target PAHs because they have molecular masses and chemical characteristics close to those of the surrogates. The surrogate standards were subjected to the same extractions procedures as described above. The surrogate percentage recovery was calculated using the expression:

$$\% \text{ Recovery} = \frac{\text{Quantity determined} - \text{Quantity added}}{\text{Quantity added}}$$

2.3. Extraction procedures

In this work three extractions methods, the accelerated solvent, Soxhlet and solid-liquid extraction methods were used to extract the PAHs content in the different fish species studied.

2.3.1. Accelerated solvent extraction method

Prior to extraction, silica gel was activated by oven-drying for 24 hours at 130^{o}C. Concentrated H_2SO_4 acid was then added to the silica gel (1:1v/v) and the mixture shaken vigorously. The mixture was then stored at room temperature prior to use.

The extraction cell was prepared and then tightly packed with 0.5g of sand and 6.5g of activated silica gel. The cap of the extraction cell was temporally removed and 50cm^3 of dichloromethane was passed over the column for conditioning. Then the cell was packed with 5g of dried, ground and well homogenized fish sample followed by 0.5g sand and finally with cellulose filter before capping the cell. The cell was placed into the carousel for extraction. 20cm^3 dichloromethane was then introduced into the extraction cell in the carousel to extract the PAHs in the fish sample. The operating temperature and pressure of the setup was then programmed to 160^{o}C and 2000psi respectively, and the sample heated by direct contact with the oven.

The extraction was achieved by direct contact of the sample with the hot solvent in both static and dynamic modes. The static extraction time used in this study was 5 minutes. Compressed nitrogen gas was finally used to purge the extract into a collector vial, capped and stored in a refrigerator prior to clean up. Same procedure was used for other fish samples.

2.3.2. Soxhlet extraction method

Five gram of the pounded fish sample was weighed and homogenized with 5g of anhydrous sodium sulphate in a laboratory mortar until a complete homogenate was obtained. The extraction was carried out using a Soxhlet extractor apparatus consisting of a 250cm^3 round bottomed flask, condenser and an extractor tube, seated in a

temperature-controlled heating mantle. A Fischer brand rotary evaporator was used to evaporate the extract to the desired concentration. The homogenate was carefully transferred into the extraction thimble placed in the extraction chamber of a Soxhlet extraction unit. The extraction was carried out as recommended by USEPA 3540 method (USEPA, 1994), using 150 cm^3 dichloromethane for 16 hours. The extract was concentrated to 2 cm^3 using a rotary evaporator in a water bath that was pre-set to a temperature of 35^oC and was stored in an amber bottle and kept in a refrigerator to avoid oxidation of the extract prior to clean up. Also, same procedure was used for all fresh fish samples collected.

2.3.3. Solid-liquid extraction method

Five gram of anhydrous Na_2SO_4 and 5 pre-cleaned glass beads were added into a pre-cleaned extraction flask. 5g of well ground homogenized fish sample was placed inside the separatory funnel. 20ml of dichloromethane was then added and the separatory funnel was capped tightly. The flask was shaken vigorously until a slurry was formed. More Na_2SO_4 was added and shaken vigorously to produce free flowing finely divided slurry. The samples were extracted by the use of a centrifuge.

The solvent layer was pipetted into a collecting vial through a small glass funnel containing a layer of anhydrous sodium sulphate (Na_2SO_4) over a plug of glass wool. The extract was then filtered into a 25 cm^3 concentrator flask using a glass funnel packed with plug of glass wool. The sample was extracted twice more using 5 cm^3 of dichloromethane and the extracts combined.

The combined extracts were transferred into a concentrator flask. Boiling chips were added to the concentrator flask and the extract was evaporated in a constant temperature hot water bath until the volume was reduced to approximately 1cm^3, then removed and allowed to cool. The extract was collected, and concentrated using a Kuderna-Danish concentrator. The extract was transferred into a vial fitted with a screw cap and stored in a refrigerator prior to clean up. Same procedure was used for extracting fresh fish samples.

2.4. Sample purification

The extracted samples were purified by passing them through a silica gel column prepared by loading 10g of activated silica gel onto a chromatographic column (1cm internal diameter) to about 5cm. This was topped with 1cm of anhydrous Na_2SO_4. It was then conditioned with dichloromethane. 2 cm^3 of the concentrated extract was loaded and eluted with 20 cm^3 of dichloromethane. This method is able to remove the very polar lipids off the extract. Prior to analysis with GC/MS, the extracts obtained were preserved in an amber bottle to avoid oxidation.

2.5. GC/MS Analysis

An Agilent 6850 gas chromatograph equipped with auto sampler connected to an Agilent FID mass selective detector was used. 1µl of sample solution was injected in the pulsed spilt-less mode onto a 30m x 0.25 mm id DB-12 ms coated fused silica column with a film thickness of 0.15µm. Helium was used as the carrier gas and the column head pressure was maintained at 35 psi to give constant flow 1.1ml/min. Other operating conditions were pre-set, pulse time 0.90mins, purge flow 50 cm^3, purge time 1min, and injection temperatures 300^oC. The column temperature was initially held at 70^oC for 3mins, increased to160^oC at a rate of 20^oC/min, then to 210^oC at a rate of 3^oC/min and to a final temperature of 310^oC at a rate of 5^oC/min and held for 10mins and transfer line of 320^oC. The mass spectrometer (MS) condition was electron impact positive ion mode. The retention time and quantifying ions of PAHs and internal standards are shown in table1. The PAHs identification time was based on retention time since each of the PAHs has its separate retention time in the column. Those with lower retention times were identified first followed by those with longer retention time.

2.6. Calibration

A calibration curve was obtained by analysing each of the standard PAHs solutions prepared with the GC/MS. The target PAH compound/internal standard peak heights were plotted against the PAH concentration to obtain a linear graph Y= mx + b, with an intercept (b) on the y-axis. The concentration of PAH in each sample was calculated using the formula

$$\text{PAH (µg/kg)} = \frac{\text{RP/RIS-b} \times \text{MIS (µg)}}{\text{X (g)} \times 1000}$$

Where

RP = Response PAH peak height

RIS = Response internal standard peak height

b = intercept on the y-axis of the standard calibration curve

MIS = Mass of added internal standard

X = weight of sample used

The limit of detection (LOD) and the limit of quantification (LOQ) for each PAH were calculated from the standard deviations of results obtained from the analysis of the several dilutions of the analyte, table1. The LOD for individual PAH in the fish samples were calculated as 3 times the standard deviation of the mean and the LOQ as 10 times the standard deviation of the mean.

3. Results and Discussion

The results obtained from the study are shown in tables 1-4 with table 1 showing the chromatographic characteristics of the target compounds, table 2 gives the percentage recovery, table 3 shows PAHs profiles in the studied fish species and table 4 summarizes the statistical analysis of the obtained results using one way Anova and Tukey's Multi range comparison test.

Table 1. Chromatographic characteristics of the target compounds

	Retention time (Minutes)	Major Peak ion		Mean conc.	SD	LOD	LOQ	RSD (%)
PAHs	GC	m/z	Internal Standard	µg/kg		µg/kg	µg/kg	
Naphthalene	8.93	128	$Acenaphthene_{d10}$	0.362	0.014	0.04	0.14	3.87
Acenaphthylene	13.03	152	$Acenaphthene_{d10}$	0.830	0.012	0.04	0.12	1.45
Acenaphthene	13.61	154	$Acenaphthene_{d10}$	0.845	0.011	0.03	0.11	1.30
Fluorene	15.03	166	$Acenaphthene_{d10}$	0.125	0.005	0.02	0.05	4.00
Phenathrene	17.55	178	$Pyrene_{d10}$	0.186	0.010	0.03	0.10	5.38
Anthracene	17.72	178	$Pyrene_{d10}$	0.423	0.014	0.04	0.14	3.31
Fluoranthene	20.77	202	$Pyrene_{d10}$	0.537	0.014	0.04	0.14	2.61
Pyrene	21.41	202	$Pyrene_{d10}$	0.412	0.015	0.05	0.15	3.64
Benz(a)anthracene	24.78	228	$Chrysene_{d12}$	0.506	0.020	0.06	0.20	3.95
Chrysene	24.99	228	$Chrysene_{d12}$	0.796	0.021	0.06	0.21	2.64
Benzo(b)fluoranthene	27.64	252	$Perylene_{d12}$	2.320	0.035	0.01	0.35	1.52
Benzo(k)fluoranthene	27.72	252	$Perylene_{d12}$	1.465	0.042	0.01	0.42	2.87
Benzo(a)pyrene	28.33	252	$Perylene_{d12}$	1.180	0.045	0.01	0.45	3.79
Indeno(1,2,3-cd)pyrene	30.92	276	$Benzo(ghi)perylene_{d12}$	1.120	0.062	0.02	0.62	5.54
Dibenz(a,h)anthracene	31.01	276	$Benzo(ghi)perylene_{d12}$	1.968	0.114	0.34	1.14	5.79
Benzo(ghi)perylene	31.49	278	$Benzo(ghi)perylene_{d12}$	1.850	0.155	0.05	1.55	8.38

SD = Standard Deviation LOD = Limit of Detection

LOQ = Limit of Quantification, RSD = Relative Standard Deviation

3.1. Chromatographic characteristics of the target compounds

Table 1 shows the chromatographic characteristics of the target compounds. The retention time increases with increasing m/z ratio of the target compounds. The retention time obtained was within the limit reported by other workers of not more than 32 minutes (Andrzej and Zdzislaw, 2005). The mean PAHs concentration range between 0.125-2.320µg/kg with benzo(b)fluoranthene having the highest of 2.320µg/kg and fluorene the lowest of 0.125 µg/kg. The LOD range between 0.01-0.34 µg/kg, with dibenz(a,h)anthracene having the highest (0.34µg/kg) and benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene the lowest. This signifies that any of these parameters that fall below these values in the course of analysis cannot be detected by the instrument and will therefore fall below non-detectable limit. The LOQ, range between 0.05-1.55µg/kg with benzo(ghi)perylene having the highest of 1.55µg/kg and fluorene the least with 0.05µg/kg respectively. The (RSD) range between 1.30-8.97% with benzo(ghi)perylene having the highest 8.38% and acenaphthene the lowest with 1.30% respectively.

Table 2. Percentage recovery by each extraction method (%)

Compounds	ASE	SOX	SLE	Internal Standard
Naphthalene	87.34	72.30	69.25	$Acenaphthene_{d10}$
Acenaphthylene	87.34	72.30	69.25	$Acenaphthene_{d10}$
Acenaphthene	87.34	72.30	69.25	$Acenaphthene_{d10}$
Fluorene	87.34	72.30	69.25	$Acenaphthene_{d10}$
Phenanthrene	91.58	89.60	64.34	$Pyrene_{d10}$
Anthracene	91.58	89.60	64.34	$Pyrene_{d10}$
Fluoranthene	91.58	89.60	64.34	$Pyrene_{d10}$
Pyrene	91.58	89.60	64.34	$Pyrene_{d10}$
Benz(a)anthracene	71.25	67.24	58.72	$Chrysene_{d12}$
Chrysene	71.25	67.24	58.72	$Chrysene_{d12}$
Benzo(b)fluoranthene	82.43	80.32	55.50	$Perylene_{d12}$
Benzo(k)fluorathene	82.43	80.32	55.50	$Perylene_{d12}$
Benzo(a)pyrene	82.43	80.32	55.50	$Perylene_{d12}$
Benzo(ghi)perylene	75.55	68.26	52.24	$Benzo(ghi)perylene_{d12}$
Indeno(1,2,3-cd)pyrene	75.55	68.26	52.24	$Benzo(ghi)perylene_{d12}$
Dibenz(ah)anthracene	75.55	68.26	52.24	$Benzo(ghi)perylene_{d12}$

ASE=Accelerated solvent extraction, SOX= Soxhlet,
SLE = Solid liquid extraction

3.2. Extraction Efficiency

The result of percentage recovery which measures the efficiency of each extraction method is shown in table 2. According to the European Commission (2005), PAHs recovery of 50-120% is an indication that an analytical procedure adopted for PAHs analysis is an acceptable procedure. The results show that that accelerated solvent extraction method gave the highest extraction efficiency and range between 71.25-91.58 %. The solid-liquid extraction method gave the lowest extraction efficiency of 52.24-69.25% respectively. In using the accelerated solvent extraction method, phenanthrane, anthracene, fluoranthene and pyrene gave the highest percentage recovery of 91.58%, while benz(a)anthracene and chrysene the lowest 71.25% respectively. The Soxhlet extraction also had the highest extraction efficiency of 89.60% in phenanthrane, anthracene, fluoranthene and pyrene, while benz(a)anthracene and chrysene the lowest 67.24%. The solid liquid extraction method gave the least extraction efficiency of all the parameters and had its highest extraction efficiency of 69.25% in naphthalenee, acenaphthylene, acenaphthene and fluorene and the lowest of 52.24% in benzo(ghi)perylene, indeno(1,2,3-cd)pyrene and dibenz(ah)anthracene respectively. It should be noted that groups of PAHs that have similar percentage recovery along column were extracted using same internal standard as indicated on the table.

The PAHs content in *Clarias gariepinus* species from zone A using accelerated solvent method (Table3), ranged between 0.84-2.25µg/kg, the PAHs profiles using Soxhlet extraction method ranged between 0.40-2.00µg/kg.

The solid-liquid extraction method ranged between 0.36-1.85µg/kg. From this zone, accelerated solvent extraction method has the highest cumulative PAHs burden of 24.08µg/kg followed by Soxhlet extraction method (20.41µg/kg) and solid-liquid extraction method (15.90µg/kg) in the studied fish species.

The PAHs profile in samples from zone B table 3 ranged between 0.75-2.02µg/kg using accelerated solvent extraction method, PAHs profiles using Soxhlet extraction method range between 0.40-1.94µg/kg. PAHs profile using solid-liquid extraction method range between 0.25-1.75µg/kg. Accelerated solvent extraction yielded the highest cumulative PAHs burden (21.53µg/kg) followed by Soxhlet (20.33µg/kg) and solid- liquid extraction method with 17.13µg/kg respectively.

The PAHs profiles in *Clarias gariepinus* species from zone C using GC/MS (Table 3), ranged between 0.70-1.94µg/kg using accelerated solvent extraction method, also using Soxhlet extraction method, the PAHs profiles in *Clarias gariepinus* species range between 0.59-1.88µg/kg, the PAHs profiles using solid-liquid extraction method range between 0.30-1.33µg/kg. The Soxhlet extraction method yielded the highest cumulative PAHs burden of 19.86µg/kg followed by accelerated solvent extraction method (19.31µg/kg) and by solid-liquid extraction method (13.55µg/kg). The PAHs content in the studied samples compared favourably with those reported by Karl and Leinamann (1996); Simko, (2000), Ajai *et al*., (2010) and Wretling *et al*., (2010) in smoked and non-smoked fish.

Table 3. PAHs Profiles in smoked *Clarias gariepinus* species using different extraction method (µg/kg).

Zones	A			B			C		
PAHs/Extraction methods	**X**	**Y**	**Z**	**X**	**Y**	**Z**	**X**	**Y**	**Z**
Naphthalene	0.84	0.45	0.50	1.36	1.25	1.03	0.96	0.65	0.30
Acenaphthylene	1.14	1.25	0.86	2.00	0.40	1.20	1.94	1.34	1.30
Acenaphthene	2.14	0.75	1.25	1.00	1.65	1.25	1.46	1.78	0.79
Fluorene	ND	1.15	1.00	1.28	1.30	1.12	1.40	1.30	0.87
Phenathrene	1.75	1.50	ND	1.92	1.83	ND	0.96	0.59	0.67
Anthracene	2.20	1.58	1.20	2.02	1.94	1.75	1.50	1.88	1.25
Fluoranthene	1.88	1.00	0.95	1.29	1.15	0.73	0.80	1.20	1.00
Pyrene	2.05	2.00	ND	1.90	1.50	ND	ND	1.34	0.62
Benz(a)anthracene	1.95	1.60	1.12	1.45	1.69	1.10	0.95	0.85	0.72
Chrysene	1.25	1.10	0.86	1.87	1.55	1.42	1.20	1.62	0.86
Benzo(b)fluoranthene	2.25	2.00	1.60	0.87	ND	0.25	1.25	1.60	1.33
Benzo(k)fluoranthene	ND	0.96	0.84	0.75	0.96	0.64	1.50	0.60	1.04
Benzo(a)pyrene	1.86	0.40	0.36	1.57	1.62	1.54	1.28	1.20	ND
Indeno(1,2,3-cd)pyrene	1.53	1.44	1.20	1.27	0.62	1.10	1.91	1.25	0.94
Dibenz(a,h)anthracene	1.60	1.38	1.26	1.25	1.17	1.35	1.50	1.75	1.23
Benzo(ghi)perylene	1.69	1.75	1.05	1.63	1.30	1.15	0.70	0.91	0.63
Cumulative PAHs	24.08	20.41	15.90	21.53	20.33	17.13	19.31	19.86	13.55

X =Accelerated solvent extraction method, Y = Soxhlet extraction method, Z = solid-liquid extraction method. A,B,C are the different zones. ND= Not derected

The PAHs content in *Clarias gariepinus* species from zone A using accelerated solvent method (Table3), ranged between 0.84-2.25µg/kg, the PAHs profiles using Soxhlet extraction method ranged between 0.40-2.00µg/kg. The solid-liquid extraction method ranged between 0.36-1.85µg/kg. From this zone, accelerated solvent extraction method has the highest cumulative PAHs burden of 24.08µg/kg followed by Soxhlet extraction method (20.41µg/kg) and solid-liquid extraction method (15.90µg/kg) in the studied fish species.

The PAHs profile in samples from zone B table 3 ranged between 0.75-2.02µg/kg using accelerated solvent extraction method, PAHs profiles using Soxhlet extraction method range between 0.40-1.94µg/kg. PAHs profile using solid-liquid extraction method range between 0.25-1.75µg/kg. Accelerated solvent extraction yielded the highest cumulative PAHs burden (21.53µg/kg) followed by Soxhlet (20.33µg/kg) and solid- liquid extraction method with 17.13µg/kg respectively.

The PAHs profiles in *Clarias gariepinus* species from zone C using GC/MS (Table 3), ranged between 0.70-1.94µg/kg using accelerated solvent extraction method, also using Soxhlet extraction method, the PAHs profiles in *Clarias gariepinus* species range between 0.59-1.88µg/kg, the PAHs profiles using solid-liquid extraction method range between 0.30-1.33µg/kg. The Soxhlet extraction method yielded the highest cumulative PAHs burden of 19.86µg/kg followed by accelerated solvent extraction method (19.31µg/kg) and by solid-liquid extraction method (13.55µg/kg). The PAHs content in the studied samples compared favourably with those reported by Karl and Leinamann (1996); Simko, (2000), Ajai *et al*., (2010) and Wretling *et al*., (2010) in smoked and non-smoked fish.

The benzo(a)pyrene irrespective of zones ranged between 1.28-1.86, 0.40- 1.62 and nd-1.54µg/kg for the accelerated solvent, Soxhlet extraction and solid-liquid extraction methods respectively, with the accelerated solvent extraction method having the highest yield and the solid-liquid extraction method the least. These values fell within the limit reported by Karl and Lienemann (1996) and Steinig (1976) for smoked fish and below the maximum of 5.0µg/kg recommended by European Union for smoked fish and meat products respectively.

Table 4. One way Anova of the effect of extraction methods on the PAHs content in the studied smoke SEM) (µg/kg) n=2

Fish Species	A			B			C		
PAHs/Extraction methods	**X**	**Y**	**Z**	**X**	**Y**	**Z**	**X**	**Y**	**Z**
Naphthalene	0.84 ± 0.01^{b}	0.45 ± 0.03^{a}	0.50 ± 0.03^{a}	1.36 ± 0.04^{b}	1.25 ± 0.04^{b}	1.03 ± 0.01^{a}	0.96 ±	0.65 ± 0.01^{b}	0.30 ± 0.03^{a}
Acenaphthylene	1.14 ± 0.10^{b}	1.25 ± 0.01^{b}	0.86 ± 0.01^{a}	2.00 ± 0.01^{c}	0.40 ± 0.03^{a}	1.20 ± 0.01^{b}	1.94 ± 0.06^{b}	1.34 ± 0.03^{a}	1.30 ± 0.04^{a}
Acenaphthene	2.14 ± 0.01^{c}	0.75 ± 0.04^{a}	1.25 ± 0.04^{b}	1.00 ± 0.04^{a}	1.65 ± 0.04^{c}	1.25 ± 0.03^{b}	1.46 ± 0.03^{b}	1.78 ± 0.04^{c}	0.79 ± 0.03^{a}
Fluorene	0^{a}	1.15 ± 0.03^{c}	1.00 ± 0.03^{a}	1.28 ± 0.03^{b}	1.30 ± 0.01^{b}	1.12 ± 0.03^{a}	1.40 ± 0.04^{b}	1.30 ± 0.03^{b}	0.87 ± 0.01^{a}
Phenathrene	1.75 ± 0.04^{c}	1.50 ± 0.03^{b}	0^{a}	1.92 ± 0.03^{b}	1.83 ± 0.04^{b}	0^{a}	0.96 ± 0.01^{b}	0.59 ± 0.04^{a}	0.67 ± 0.03^{a}
Anthracene	2.20 ± 0.04^{c}	1.58 ± 0.01^{b}	1.20 ± 0.03^{a}	2.02 ± 0.03^{b}	1.94 ± 0.06^{b}	1.75 ± 0.01^{a}	1.50 ± 0.02^{b}	1.88 ± 0.01^{c}	1.25 ± 0.03^{a}
Fluoranthene	1.88 ± 0.03^{b}	1.00 ± 0.01^{a}	0.95 ± 0.03^{a}	1.29 ± 0.01^{c}	1.15 ± 0.01^{b}	0.73 ± 0.03^{a}	0.80 ± 0.01^{a}	1.20 ± 0.03^{c}	1.00 ± 0.04^{b}
Pyrene	2.05 ± 0.03^{b}	2.00 ± 0.03^{b}	0^{a}	1.90 ± 0.06^{c}	1.50 ± 0.04^{b}	0^{a}	0^{a}	1.34 ± 0.06^{c}	0.62 ± 0.03^{b}
Benz(a)anthracene	1.95 ± 0.03^{c}	1.60 ± 0.03^{b}	1.12 ± 0.01^{a}	1.45 ± 0.01^{b}	1.69 ± 0.04^{c}	1.10 ± 0.01^{a}	0.95 ± 0.01^{b}	0.85 ± 0.04^{b}	0.72 ± 0.03^{a}
Chrysene	1.25 ± 0.01^{c}	1.10 ± 0.01^{b}	0.86 ± 0.03^{a}	1.87 ± 0.06^{b}	1.55 ± 0.04^{a}	1.42 ± 0.03^{a}	1.20 ± 0.01^{b}	1.62 ± 0.04^{c}	0.86 ± 0.04^{a}
Benzo(b)fluoranthene	2.25 ± 0.01^{c}	2.00 ± 0.03^{b}	1.60 ± 0.01^{a}	0.87 ± 0.06^{c}	0^{a}	0.25 ± 0.03^{b}	1.25 ± 0.01^{a}	1.60 ± 0.03^{b}	1.33 ± 0.04^{a}
Benzo(k)fluoranthene	0^{a}	0.96 ± 0.04^{b}	0.84 ± 0.04^{b}	0.75 ± 0.03^{b}	0.96 ± 0.01^{c}	0.64 ± 0.03^{a}	1.50 ± 0.04^{c}	0.60 ± 0.03^{a}	1.04 ± 0.06^{b}
Benzo(a)pyrene	1.86 ± 0.01^{b}	0.40 ± 0.03^{a}	0.36 ± 0.04^{a}	1.57 ± 0.04^{a}	1.62 ± 0.03^{a}	1.54 ± 0.06^{a}	1.28 ± 0.03^{c}	1.20 ± 0.01^{b}	0^{a}
Indeno(1,2,3-cd)pyrene	1.53 ± 0.03^{b}	1.44 ± 0.06^{b}	1.20 ± 0.03^{a}	1.27 ± 0.01^{c}	0.62 ± 0.03^{a}	1.10 ± 0.01^{b}	1.91 ± 0.01^{c}	1.25 ± 0.03^{b}	0.94 ± 0.06^{a}
Dibenz(a,h)anthracene	1.60 ± 0.02^{b}	1.38 ± 0.06^{b}	1.26 ± 0.01^{a}	1.25 ± 0.03^{b}	1.17 ± 0.04^{a}	1.35 ± 0.04^{b}	1.50 ± 0.04^{b}	1.75 ± 0.03^{c}	1.23 ± 0.04^{a}
Benzo(ghi)perylene	1.69 ± 0.01^{b}	1.75 ± 0.01^{b}	1.05 ± 0.04^{a}	1.63 ± 0.04^{c}	1.30 ± 0.01^{b}	1.15 ± 0.04^{a}	0.70 ± 0.03^{a}	0.91 ± 0.01^{b}	0.63 ± 0.03^{a}
Cumulative PAHs	24.08	20.41	15.90	21.53	20.33	17.13	19.31	19.86	13.55

Those with different superscripts across rows within zones are significantly different from each other (P<0.05), where c>b>a. X =Accelerated solvent extraction method, Y=Soxhlet extraction method, Z= liquid-solid extraction method. A,B,C are the different zones

To validate the extraction methods, the obtained results were subjected to statistical analysis using one way Anova at 95% confidence level (Table 4). The results of statistical analysis show significant differences between the different extraction methods in their extraction of PAHs from the sample matrix as represented by different superscripts letters across rows P<0.05 (Table 4). There are variations in their extraction efficiency of the different PAHs in the smoked fish samples. Of the sixteen PAHs determined, the accelerated solvent extraction method yielded higher amount of PAHs in thirteen of them (indicated by superscript c), indicating better extraction method (c>b>a), while the Soxhlet three (3) of the parameters and solid-liquid extraction method the least. 4.Conclusion

From the study it is obvious that smoked catfish contained some PAHs as contaminants which could be as a result of the deposition of some of the by-products of the pyrolysis of woods on the fish during smoking. Also, based on the different extraction methods used in this study, the accelerated solvent extraction method seems to be more efficient than the Soxhlet and the solid-liquid extraction methods, while the solid-liquid extraction method which was least efficient was also most tedious in its experimental approach.

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