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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METAL CONTENTS OF BARBECUE BEEF, FISH AND CHICKEN

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Abstract: *The purpose of this work is to investigate the polycyclic aromatic hydrocarbons (PAHs) and heavy metal contents of barbecue fish, beef and chicken obtained from Minna, Niger state, using standard procedures. Extraction of PAHs was done using sonification method. n-Hexane, dichloromethane (DCM) and mixture of both (1:1) were used as extractants. PAHs content was analysed using GC-MS while the heavy metal analysis was done using AAS. The results obtained from the study showed that grilled chicken had the highest content of total PAHs (214.41µg/kg) while beef had the least (25.71µg/kg). 45 to 98% of the fractions of PAHs observed in the samples were 4 and 5 member rings. Benzo (a) pyrene was detected in all the samples analysed, its concentrations varied from 1.82 to 12.89 µg/kg. The concentrations of PAHs in the grilled samples were generally higher than the control. The efficiency of the solvents from this study were in the order: n-hexane > (n-hexane: DCM) > DCM. With the exception of Pb which had a concentration of 3.17 mg/kg to 4.68 mg/kg, other metals investigated were within safe limit based on international standard. The concentrations of the metals in chicken and beef samples were in the general order: Fe>Pb>Cu>Mn. There is need to continually check the contents of PAHs and heavy metals in barbecue food since they have bio-accumulative tendencies and are deleterious to health.*

Keywords: *barbecue; heavy metals; polycyclic aromatic hydrocarbons*

1. Introduction

The demand for fish, beef and chickens as well as their products is increasing in most countries, especially in view of their significant nutritional values. They are major components of human diets and supply essential amino acids and minerals [1]. However, some of these food substances get contaminated during processing either by heat treatment and other exposure processes. These contaminants such as polycyclic aromatic hydrocarbons (PAHs), polycyclic amines and heavy metals have lethal effects. This has raised an increasing concern about the quality and safety of foods in several parts of the world [2]. Some of the contaminants even when present in trace amount have significant ecological impacts due to their ability to enter the food chain and bio-accumulate in the tissues of living organisms [3]. PAHs are known to constitute one of the prominent classes of hazardous pollutants. Of primary concern is the fact that some of them are carcinogenic, teratogenic and mutagenic [4]. PAHs are group of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. Their formation occurs during processing of coal, crude oil and natural gas, as well as incomplete combustion of coal and other organic substances including food [5]. The presence of these compounds in processed meat and other food substances has become a subject of much concern in the recent years [6]. The concentration of PAHs determined in a food matrix could be affected by processing method, extraction techniques as well as the efficiency of the solvent employed. Various studies have reported the presence of PAHs in processed food substances in different parts of the world [7]; but much has not been done on barbecue fish, beef and chicken obtained from Minna and its environs in Niger State, Nigeria. Thus the aim of this study is to determine the PAHs and heavy metals content of barbecue fish, chicken and meat obtained from Minna in Niger State.

2. Materials and methods

2.1 Sampling method

A total of six (6) samples were used for the purpose of this study. At each of the sampling point, five (5) subsamples were collected and homogenised so as to ensure a representative sampling. The samples collected from each area include beef, chicken (Agric. type) and fish (Catfish-*Clarias gariepinus*) processed using charcoal grilling. Fresh samples were also collected and used as control. The samples were packed in aluminium foil, placed in polyethylene bags and then transported to the laboratory for pre-treatment and subsequent analyses [8].

2.2 Sample pre-treatment

The samples were dried in an oven at low temperature of 105°C for eight hours. They were then homogenised using a mortar and pestle and stored in a refrigerator at 4°C prior to extraction and other analyses.

2.3 Preparation of PAHs standard and calibration curve

A mixture of 16 PAHs reference standards was purchased from Supleco Inc., USA. Five standard solutions each containing 1.0, 2.0, 5.0, 10.0 and 20.0 cm^3 of 500 mg/L of each standard PAH was made up to 100cm^3 with dichloromethane. These were transferred into a capped and sealed vial until ready for analysis. Calibration curve of PAHs was obtained by running standards of 5, 10, 25, 50 and 100 mg/L. A calibration curve was obtained by analysing each of the standard PAHs solutions prepared with GC/MS. The concentration of each PAH in the sample was determined in line with procedure reported by [9].

2.4 Determination of selected physicochemical properties

Some physicochemical parameters (ash, moisture and fat contents) were determined in line with [10].

2.5 Ultrasonic assisted extraction of samples

The ultrasonic extraction was carried out in line with the method reported by [9] with slight modifications. The extract was then filtered and concentrated using a rotary evaporator under controlled vacuum.

2.6 Silica gel column clean-up

The clean-up was performed in line with the method reported by [11] using activated florisil (Magnesium silicate) and anhydrous Na_2SO_4. The eluate was collected into an evaporating flask and rotary evaporated to dryness. The clean-up procedure was repeated using the other solvents (*n*-hexane and DCM) as eluents.

2.7 Determination of heavy metal concentration

The determination of selected heavy metals (*Mn, Zn, Cu, Pb, Cr* and *Cd*) was done using the method reported by [12]. Analysis of variance (ANOVA) was performed on PAH concentration data using SPSS software. The significant statistical level was set at $P<0.05$.

3. Results and discussion

3.1 Physico-chemical properties

Table 1

Physico-chemical properties of analysed Samples

	Fish	Chicken	Beef
Fat content (%)	16.40±0.70	27.30±0.20	20.81±1.01
Moisture content (%)	25.15±1.21	15.30±1.90	29.40±0.99
Ash content (%)	2. 20±0.93	1.62±0.12	1.41±1.20

Results expressed as mean±SD for triplicate determinations.

Table 1 shows the physico-chemical parameters of the samples analysed. The fat contents were 16.40±0.70, 27.30±0.20 and 20.81±1.01% for fish, chicken and beef respectively. Barbecue beef had the highest moisture content (29.40±0.99%) while chicken had the least (15.30±1.90%). Moisture content relates to the freshness and stability of food for long time storage [13]. Food samples with very low moisture content are less prone to microbial attack hence less perishable [14].
The ash content of the samples in this study was generally low (1.41 to 2.20%) which relates to the mineral compositions of the sample. Ash is a measure of the inorganic components in food sample after removal of water and organic matter. Fish had the highest ash content (2.20±0.93%) while beef had the least.
Fat content determination is basic to PAHs analysis, since the dripping of fat into the flame is one of the mechanisms of PAHs formation. Chicken had the highest fat content (27.30±0.20%) while fish had the least (16.40 ±0.70%). The low value of fat observed in some of the samples may be attributed to the fact that the samples were not analysed the same day of collection thereby resulting to the oxidation of fat. The difference in some of these parameters with findings from other studies can be attributed to geographical areas, sex, amongst others [15].

3.2 Polycyclic Aromatic Hydrocarbon Contents of the Samples

Most of the lower members PAHs were not detected in the fish samples (table 2). This observation may be connected with the higher volatility of PAHs of lower molecular weight. The highest amount of benzo (a) pyrene was obtained from *n*-hexane (12.89 µg/kg) and the mixture of the two solvents (12.79 µg/kg). This implies that *n*-hexane is a better extraction solvent for B(a)P using sonification method. *n*-Hexane extract gave a higher total PAHs content for fish sample (99.27µg/kg) than DCM (33.05µg/kg).

The total PAHs obtained from fish using n-hexane was about thrice that of DCM. This shows that *n*-hexane is more efficient for PAHs extraction. The concentration of B(a)P ranged from 0.99±0.31 to 12.89±0.13 µg/kg. The values for *n*-hexane and combined solvent extracts are higher than 0.63±0.57µg/kg reported by [16] for B(a)P in a related study. The total PAHs obtained is higher than a maximum of 14.95mg/kg documented by [17] in charcoal broiled beef burger. The concentration of B(a)P in DCM extract is however lower than 5.0µg/kg based on [17] standard.

Table 2

PAHs Content of Grilled Fish (µg/kg) using Different Extractants

PAHs	DCM	*n*-hexane	DCM: *n*-hexane (1:1)	Control
1. Napthalene	ND	ND	ND	ND
2. Napthalene, 2-methyl	ND	ND	ND	ND
3. Biphenylene	ND	ND	ND	ND
4. Acenapthene	ND	ND	ND	ND
5. Fluorene	ND	16.01±0.13[b]	1.59±0.09[a]	ND
6. Phenanthrene	1.16±0.18[a]	7.01±0.91[b]	7.01±0.10[b]	ND
7. Fluoranthene	1.06±0.12[a]	1.12±0.08[a]	1.17±0.11[a]	ND
8. Pyrene	1.19±0.80[a]	4.18±0.10[b]	4.18±0.14[b]	0.98±0.91[c]
9. Benz [a] anthra	1.51±0.12[a]	11.69±0.16[b]	11.73±0.04[b]	3.10±0.32[a]
10. Triphenylene	1.74±0.10[a]	1.21±0.09[a]	1.21±0.21[a]	2.01±0.19[a]
11. Benz[b] flu.	7.95±0.18[b]	12.39±0.11[c]	12.39±0.09[c]	5.98±0.12[a]
12. Benzo [a] pyrene	1.92±0.13[a]	12.89±0.13[b]	12.79±0.11[b]	0.99±0.31[a]
13. Indeno [1,2,3]pyrene	5.12±1.07[a]	10.11±0.30[b]	10.11±0.08[b]	2.98±0.08[a]
14. Dibenz [a,h] anthrh	6.29±0.10[a]	17.19±0.05[b]	17.11±0.10[b]	ND
15. Benzo [ghi] peryle	5.11±0.11[a]	5.49±0.81[a]	5.49±0.59[a]	ND
Σ PAH	**33.05**	**99.27**	**84.78**	**16.04**

Results are expressed as mean ± SD. Values with same superscript on same row do not differ significantly at $p < 0.05$. ND: Not detected.

Fig.1 presents the sum total fraction of PAHs from the grilled fish. Medium molecular weight (MMW) PAHs (4 and 5 ring members) constitute majority (61.09 to 71.46%) of the total PAHs.

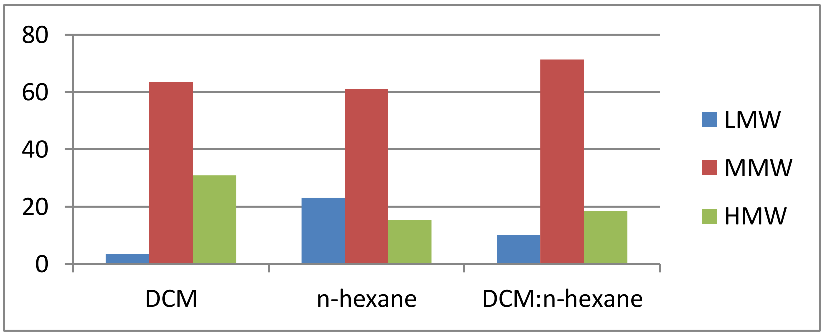

Fig.1 The fractions of total PAHs in grilled fish using different extractants LMW: low molecular weight (2 and 3 rings Pahs); MMW: medium molecular weight (4 and 5 rings PAHs); HMW: high molecular weight (6 rings).

Studies on carcinogenicity of PAHs have shown that medium and higher molecular weight PAHs (4 to 6) rings are more carcinogenic when compared to lower molecular weight members [18]. Also DCM showed better efficiency in the extraction of six ring PAHs (30.95%) when compared to *n*-hexane (15.25%).

Table 3 gives the PAHs content of the barbecue chicken using various extractants. The total PAHs concentration in chicken ranged from 165.63 to 214.41µg/kg. The highest for total PAHs was observed in the extract of the combined solvents. *n*-Hexane gave a higher yield of total PAHs (185.63µg/kg) when compared to DCM (165.63µg/kg). While individual solvents did not extract naphthalene and 2-methylnaphthalene, but with a combination of the extracting solvents, concentrations of 1.08±0.23 to 2.16±0.10 µg/kg were obtained. The least amount was in the control (1.01µg/kg) The PAHs contents obtained here are comparable to the findings of [19] in their study on smoked fish from southern Nigeria. The concentrations of PAHs obtained is lower than 0.12mg/kg reported by [20] for B(a)P in their study on sardine.

Table 3

PAHs Content of Grilled Chicken (µg/kg) using Different Extractants

	PAHs	DCM	*n*-hexane	DCM: *n*-hexane (1:1)	Control
1.	Napthalene	ND	ND	$1.08±0.23^a$	ND
2.	Napthalene, 2-methyl	ND	ND	$2.16±0.10^a$	ND
3.	Biphenylene	$1.26±0.09^a$	ND	$16.11±0.06^b$	ND
4.	Acenapthene	$2.66±0.01^a$	$13.39±0.89^b$	$13.41±0.12^b$	ND
5.	Fluorene	$3.57±0.11^a$	$11.89±1.03^b$	$11.85±0.09^b$	ND
6.	Phenanthrene	$4.94±0.01^a$	$6.91±0.10^a$	$5.89±0.92^a$	ND
7.	Fluoranthene	$1.19±0.08^a$	$3.73±0.04^b$	$3.77±0.10^b$	ND
8.	Pyrene	$50.91±0.01^b$	$76.41±0.33^c$	$76.39±2.07^c$	$1.01±0.01^a$
9.	Benz [a] anthracene	$8.19±0.15^c$	$3.09±0.09^b$	$3.09±0.02^b$	$0.99±0.21^a$
10.	Triphenylene	$1.78±0.91^a$	$4.01±0.02^b$	$6.74±0.03^c$	$0.04±0.03^a$
11.	Benzo [b] fluoranthene	$10.39±0.01^b$	$15.01±0.12^c$	$11.51±0.10^{bc}$	$2.97±0.01^a$
12.	Benzo [a] pyrene	$16.11±0.10^c$	$11.01±0.07^b$	$10.61±0.07^b$	$3.02±0.10^a$
13.	Indeno[1,2,3,-cd] pyre	$9.04±1.09^b$	$8.02±1.01^b$	$11.59±0.11^c$	$4.98±0.01^a$
14.	Dibenz [a,h] anthracene	$24.79±0.09^c$	$19.11±0.10^b$	$28.11±0.11^c$	$7.01±0.10^a$
15.	Benzo [ghi] perylene	$30.81±0.12^c$	$13.11±0.09^b$	$12.11±0.09^b$	$5.99±0.03^a$
	Σ Pah	**165.63**	**185.68**	**214.41**	**26.01**

Results are expressed as mean ± SD. Values with same superscript on same row do not differ significantly at p< 0.05. ND; Not detected

Fig.2 shows the distribution of PAHs extracted from chicken in terms of the number of rings present. *n*-Hexane was more effective in extracting 4- 5 ring PAHs (71.28%) compared to DCM (68.43%), mixture of both solvents gave the least (62.14%). DCM however gave the highest yield for 6 ring members (dibenz(a,h)anthracene and benzo[ghi]perylene).

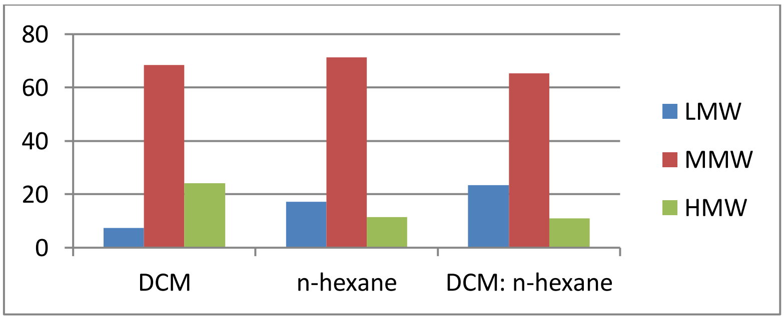

Fig. 2. The fractions of total PAHs in grilled chicken using different extractants

The PAHs content in barbecued beef is presented in table 4. The first five members were not detected in the beef extract. Indeno [1,2,3-cd] a six ring PAH was present in highest concentration among other PAHs (4.88 ±0.06 to 8.79±0.13μg/kg). The concentration of B(a)P in the grilled samples ranged from 1.48±0.11 to 5.62±0.11μg/kg. Benzo (a) pyrene (B(a)P) is considered as a biomarker hence it presence is used in assessing other PAHs in the environment [19]. The concentration of dibenzo(a,h)anthracene and fluorene observed here are higher than 0.0099mg/kg to 0.104 mg/kg reported by [21] in roasted meat. They are however lower when compared to 1.34 to 5.56 μg/kg reported by [22] in a similar study at Port Harcourt. The presence of pyrene and benzo(a) pyrene in all the extract is in agreement with findings of [23].

Table.4

PAHs Content of Grilled Beef (μg/kg) using Different Extractants

PAHs	DCM	n-hexane	DCM: n-hexane (1:1)	Control
1. Napthalene	ND	ND	ND	ND
2. Napthalene, 2-methyl	ND	ND	ND	ND
3. Biphenylene	ND	ND	ND	ND
4. Acenapthene	ND	ND	ND	ND
5. Fluorene	ND	ND	ND	ND
6. Phenanthrene	1.08±0.71[a]	1.11±0.09[a]	ND	0.49±0.01[a]
7. Fluoranthene	ND	1.07±0.55[a]	ND	1.01±0.03[a]
8. Pyrene	7.01±0.011[c]	1.01±0.06[a]	ND	0.01±0.21[b]
9. Benz [a] anthracene	1.29±0.07[a]	2.54±0.10[a]	1.59±0.01[a]	0.98±0.11[a]
10. Triphenylene	2.36±0.02[a]	2.66±0.09[a]	2.49±0.10[a]	1.02±0.15[b]
11. Benzo [b] fluoranthene	1.65±0.06[a]	4.31±0.05[b]	1.88±0.07[a]	1.03±0.31[a]
12. Benzo [a] pyren	1.48±0.11[a]	1.82±0.10[a]	5.62±0.11[b]	1.01±0.23[a]
13. Indeno [1,2,3, -cd] p	4.88±0.06[a]	7.89±0.07[b]	8.79±0.13[b]	ND
14. Dibenz [a,h] anthrace	5.95±0.11[a]	6.96±0.12[a]	6.06±0.09[a]	2.03±0.01[a]
15. Benzo [ghi] perylene	ND	5.49±1.08[a]	ND	ND
Σ PAH	**25.71**	**34.86**	**26.52**	**7.58**

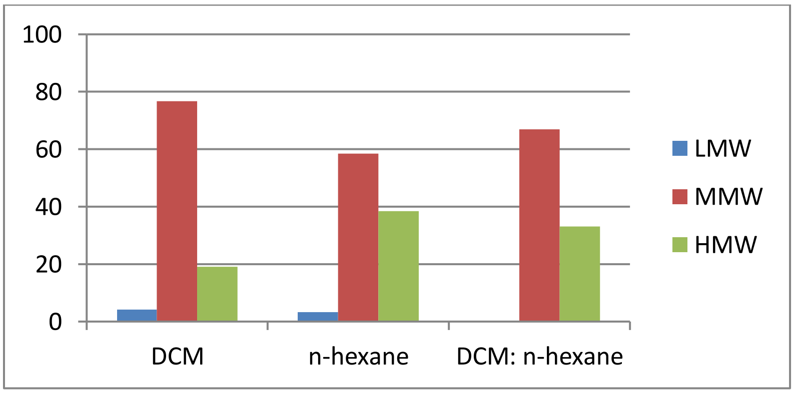

Fig. 3. Fractions of total PAHs in grilled beef using different extractants

The fractions of PAHs extracted from beef in terms of molecular weight and ring number is shown in fig. 3 The fraction of lower molecular weight PAHs in all the beef extracts where insignificant (0.00 to 4.20%). 4 and 5 rings PAHs constituted the major components of the total PAHs. These groups appear to be of more concern due to their carcinogenic risk. DCM gave 76.81% of the 4-5 rings compared to 58.43% from *n*-hexane. Presence of 4-5 member rings above 50% of total PAHs shows dominance of combustion.

3.3 Heavy metal concentration

Table 5 shows the concentrations of the metals in the samples. Chromium was not detected in all the samples analysed. [24] reported the concentration of chromium as 0.43 to 1.22mg/kg in his study on barbecued food. The concentration of Fe ranged from 2.52±0.036 to 3.52±0.100mg/kg. Barbecued beef had the highest amount while the least was in fish. Contamination by Fe and other metals may occur during processing of meat and other foods through contact with processing materials and penetration through surfaces exposed to surrounding environmental contaminants [25]. The concentration of Fe from this study is in consonance with 2.13 to 4.65mg/kg reported by [24] in a similar study. Mn had the least concentration among the elements analysed. The least content was in barbecued chicken. Earlier studies have attributed the presence of some toxic metals to some of the additives and spices which some barbecued meat sellers add prior to processing [26].The highest amount of copper was in beef (2.97±0.35mg/kg), which was significantly higher than those of fish and chicken. The high content of copper may be related to environmental contamination due to exposure in the course of heating alongside background content. [21] in a related study on heavy metals in meat reported a significant increase in the concentration of metals after grilling, which he attributed to the method employed in cooking. [25] in a related study highlighted the content of copper as 0.15 to 0.35mg/kg. Pb had the highest concentration which ranged from 1.17±0.100 to 2.68±0.037mg/kg. Pb content varied significantly among the samples studied. Fish sample had the highest content of Pb. The concentration of Pb in all samples from the present study is higher than the permissible limit of 0.5mg/kg in meat [8]. The value from the present study is also higher than 0.02mg/kg reported by [27] in roasted meat and 0.125 mg/kg documented by [26] in a related study.

Table 5

Heavy Metals Content of analysed Samples (mg/kg)

Metals	Fish	Chicken	Beef	Permissible Limit
Cr	ND	ND	ND	1.0 (USDA, 2006)
Fe	2.52±0.036^{a}	2.58±0.047^{a}	3.52±0.100^{b}	43 (WHO, 2011)
Mn	0.51±0.015^{b}	0.28±0.019^{a}	0.48±0.015^{b}	5.5 (WHO, 2011)
Cu	0.94±0.045^{a}	0.95±0.050^{a}	2.97±0.350^{b}	10 (WHO, 2011)
Pb	2.68±0.037^{c}	1.57±0.011^{b}	1.17±0.100^{a}	0.10(WHO, 2011)

Result expressed as mean ± SD for triplicate determinations. Values on same row with same superscript do not differ significantly at $p<0.05$.

3.4 Inter-elemental correlation coefficient

There was a strong positive association between Fe and Cu (0.99) using Spearman coefficient (table 6). Fe correlated negatively with Pb. A weak positive association (0.35 to 0.38) was observed among most of the other metals considered. The positive association among some of the metals shows their common origin.

Table 6

Interelemental correlation coefficients in the samples

	Cr	Fe	Mn	Cu	Pb
Cr	1	-	-	-	-
Fe	-	1	0.35	0.99**	-0.73
Mn	-	0.35	1	0.38	0.37
Cu	-	0.99**	0.38	1	-0.72
Pb	-	-0.73	0.37	-0.72	1

**. Correlation is significant at the 0.01 level (2-tailed).

4. Conclusion

Findings from this study have reveal that 4 to 5-membered ring PAHs made up major fraction (60 to 80%) of the total PAHs from the barbecued fish, chicken and beef. With a few exceptions, the efficiency of solvents in extraction of total PAHs from the present study could best be arranged in the order: n-hexane> mixture (1:1) >DCM. The mean concentrations of PAHs in the sample were in the following order: chicken>fish>beef. The concentration of B(a)P in some of the samples analysed exceeded the maximum acceptable limit of 5µg/kg (EFSA, 2008). For heavy metal analysis, Fe had the highest concentration in chicken and beef while Mn had the least in all the samples analysed.

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