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POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METALS IN SMOKED BEEF: EFFECT OF SOLVENTS AND EXTRACTION METHODS

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Abstract: *The presence of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in smoked food in appreciable amount is an issue of concern in recent times. The present study focuses on the PAHs and heavy metal contents of smoked beef. The effect of three solvent combinations (n-hexane, dichloromethane and n-hexane:DCM) and two extraction methods (Soxhlet and sonication) on PAHs contents were also assessed. GC-MS was used for the quantification of PAHs while heavy metal contents were analyzed using atomic absorption spectrometer. The concentrations of metals were in the general order: Fe > Zn> Mn > Pb > Cu > Cd. The content of metals was generally lower than the permissible limits based on international standard. Sonication method gave the highest yield of total PAHs (45.15µg/kg) while n-hexane registered the highest extraction efficiency amongst solvents. The concentration of PAHs ranged between 0.09 - 9.90 µg/kg. Benzo[a] pyrene exceeded the 5.0µg/kg maximum limit in some cases. It is necessary to continually check the concentrations of PAHs and heavy metals in food.*

Keywords: *smoking, extraction, heavy metals, beef, polycyclic aromatic hydrocarbons*

1. Introduction

Meat and other food substances have been preserved through smoking for so long. Originally the purpose of heat treatment and smoking was to preserve food, partly by reducing the moisture content and also through the transfer of anti-microbiological components from smoke to food. Currently smoking is primarily used to achieve the characteristic taste and appearance of smoked food, with preservation playing a minor role [1]. PAHs are present in smoked food as they are formed during the smoking process [2]. The process of grilling meat, fish or other food with intense heat over a direct flame results to fat dripping on the hot fire and yielding flame containing a number of PAHs. These chemicals adhere to the surface of the food. The more intense the heat the more the PAHs formed [3]. The main source of human exposure to PAHs is food, and this accounts for more than 90% of total exposure. PAHs account for 4 of the top 10 most hazardous substances on the 2010 Agency of Toxic Substances and Disease Registry Priority List of Hazardous Substances. The pivotal adverse effect resulting from exposure to PAH is carcinogenicity. Increased incidences of lung, skin, and bladder cancers are associated with occupational exposure to PAHs [4]. Several polycyclic aromatic hydrocarbons (PAHs) are among the most potent carcinogens known to exist, producing tumours in some organisms through single exposures to microgram quantities. Levels as high as 200 µg/kg have been found for individual PAHs in

smoked fish and meat samples. For instance, in barbecued meat, 130 μg/kg has been reported whereas the average background values are usually in the range of 0.01 to 1 μg/kg in uncooked foods [5]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a comprehensive risk assessment of PAH in 2005 [2]. JECFA recommended keeping the content of PAHs in food low. The presence of PAHs in the environment and the potential for these contaminants to be present in food has resulted in the need for their safety concern in human health. Extraction methods and solvents have been reported to affect significantly the yield of PAHs in environmental matrices hence give different results on extent of contamination by PAHs [6]. There are very limited studies on PAHs and heavy metal content barbecue beef, chicken and fish obtained from Minna, Niger State, Nigeria. The presence of heavy metals in processed meat, fish and other grilled foods have also been reported. Some of these metals are present in concentrations beyond permissible limit in some available studies [7]. Heavy metal constitutes one of the major groups of environmental contaminants. Heavy metals when released in the environment are toxic to humans and animals. However, some heavy metals are beneficial to the body. The largest man-made sources of these metals include combustion of fossil fuels and industrial processes [3].

Some of the anthropogenic sources of PAHs are mobile while others are stationary [4]. The properties of the individual PAHs depend on the number of hydrocarbon rings. PAHs are generally lipophilic, which means they dissolve poorly in water but well in fats and oils [4]. 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 [8]. PAH typically occur as mixtures in food, with variation in the toxicity (or potency) of individual compounds; some compounds are genotoxic and/or carcinogenic or neither [9]. PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, the time of cooking and the temperature [4]. Heavy metals are naturally-occurring elements that have high atomic numbers and densities higher than the density of water by at least five times. The term "heavy metal" refers to any metallic element that has relatively high density and is poisonous or toxic even at low concentration [9]. "Heavy metal" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than $4g/cm^3$. However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals include Cadmium (Cd), Zinc (Zn), Mercury (Hg), Arsenic (As), Silver (Ag), Lead (Pb) Chromium (Cr), Copper (Cu), Iron (Fe) and Platinum group elements [10].Heavy metals are not biodegradable and are widely distributed in the environment [11]. Heavy metals are naturally present in the environment, however, metals such as arsenic, nickel, mercury, cadmium, and lead do not have any known or reported biochemical importance and their appreciable concentration could constitute significant lethal hazards [12].
Considering the need to provide an additive input on existing data base on PAHs and heavy metals in barbecue beef, it is paramount to assess the effect of various methods of extraction and extraction efficiency of solvents on the PAHs content of smoked beef and also investigate the heavy metal contents.

2. Materials and methods

Sampling method
Raw beefs were collected from twelve different selling points (n=12) within Minna in Niger State from Chanchaga, Bosso, Tunga and Maitubi. They were split into two and a part smoked in a local kiln while the other was kept to check for background concentrations.

Smoking Parameters and Sample pre-treatment
Samples collected were cut into smaller sizes and smoked using charcoal. The smoking temperature was 200ºC. Smoking was done for 2 hours. Smoking temperature was measured using a non-contact infra-red thermometer. The samples were packed in aluminum foil, placed in polyethylene bags and then transported to the laboratory in line with EC directives [13]. The samples were kept in the oven and dried for at 40ºC for two days. The dried samples were grounded using mortar and pestle.

Preparation of Standard Solutions and quantification of PAHs and Metals
Quantification of PAHs was done using external standard method. A calibration mixture containing soxhlet extraction and the 16 polycyclic aromatic hydrocarbons reference standard was used. Solutions of concentrations 5, 10, 25, 50 and 100 µg/mL were used to make a five point calibration curve in line with [14].

Quality control
Quality assurance procedures and precautions were carried out. All materials used for processing were screened with nitric acid and acetone. Computation of Limit of detection (LOD) and limit of quantification (LOQ) were done using linear regression method in line with [15]. Recovery studies were carried out by spiking samples with standard concentrations of mixed PAHs and re-analyzing them at two concentration levels in line with [4].

Determination of Physicochemical parameters
Determination of physicochemical parameters (Moisture, ash and fat contents) was done in line with [16]. Moisture content was determined in oven at 105ºC.

Extraction of Samples and Clean-up
Extractions were done using three solvent combinations (n-hexane, DCM and mixture of both). Soxhlet extraction and ultrasonic extraction were carried out in line with the method reported by [17] with slight modifications. The clean-up was performed using activated florisil (Magnesiumsilicate) and anhydrous Na_2SO_4.

Determination of heavy metal concentration
Digestion of the ground beef (fresh and smoke) was done using nitric acid (HNO_3) and perchloric acid ($HClO_3$) in line with [18]. Dilution of the solution was done to reach the mark of 50ml and heavy metal quantification was carried out using atomic absorption spectrophotometer (AAS).

Statistical analysis
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
Table 1 presents the qualitative and quantitative parameter of the PAHs investigated. The LOD, LOQ and RSD values are also shown for the sixteen PAHs.

Table 1

Qualitative and quantitative parameters of PAHs analyzed

Compound	R^2	RSD	LOD(µg/kg)	LOQ(µg/kg)
Naphthalene	0.99	1.92	0.01	0.02
Naphthalene, 2 methyl	0.99	0.98	0.09	0.10
Biphenylene	0.97	1.34	0.01	0.02
Acenapthene	0.99	1.90	0.09	0.13
Fluorene	0.99	2.10	0.05	0.10
Phenanthrene	0.96	1.45	0.08	0.12
Anthracene	0.99	2.11	0.07	0.09
Fluoranthene	0.97	1.89	0.08	0.10
Pyrene	0.98	1.80	0.05	0.08
Benz [a] anthracene	0.99	0.88	0.09	0.11
Chrysene	0.96	1.23	0.11	0.16
Benzo [b] fluoranthene	0.98	1.87	0.09	0.10
Benzo [a] pyrene	0.99	0.99	0.09	0.11
Indeno [1,2,3, - cd] pyrene	0.08	1.47	0.08	0.10
Dibenz [a,h] anthracene	0.99	1.89	0.02	0.09
Benzo [g,h,i] perylene	0.97	2.10	0.08	0.10

Table 2 gives the result for the percentage recoveries of PAHs using the two different extraction methods and solvent combinations. The percentage recoveries ranged from 76.60 to 99.90%. Sonication method using n-hexane as extractant showed the highest percentage recovery.

Table 2

PAHs recoveries using various extraction methods and extractants

PAHs	Soxhlet extraction			Sonication extraction		
	n-hexane	DCM	Hexane:DCM	n-hexane	DCM	Hexane:DCM
Napthalene	92.30	76.90	80.95	98.10	88.90	93.40
Naphthalene,2-methyl	90.65	81.90	77.30	97.00	87.50	95.60
Biphenylene	87.11	79.80	80.60	89.20	87.70	90.10
Acenaphthene	80.40	80.40	89.40	96.50	90.60	89.90
Fluorene	90.17	77.60	91.30	97.00	88.90	94.67
Phenanthrene	88.20	87.90	90.40	95.30	87.40	92.30
Anthracene	90.40	90.10	88.20	96.50	90.80	93.80
Fluoranthene	79.86	78.98	90.11	96.50	92.10	89.89
Pyrene	90.10	81.20	79.30	98.10	89.80	91.20
Benz[a] anthracene	91.10	89.80	90.20	99.90	95.30	96.10
Chrysene	80.40	79.87	88.70	89.90	90.20	91.80
Benzo[b]fluoranthene	78.86	80.10	83.20	89.80	87.60	80.89
Benzo [a] pyrene	89.89	90.20	91.80	97.80	89.80	90.96
Indeno [1,2,3 – cd]pyrene	90.60	76.80	90.10	96.60	91.20	93.80
Dibenz[a,h]anthracene	91.15	89.90	90.80	97.90	90.70	94.90
Benzo[ghi]perylene	98.70	79.80	91.70	98.70	93.20	93.80

The results of some physico-chemical parameters, polycyclic aromatic hydrocarbons and heavy metal contents of barbecue beef, chicken and fish from Minna in Niger State are reported and discussed below.

Table 3 presents the ash, moisture and fat contents of the raw and smoke beef. Smoke beef had a lower moisture content (9.10%) which could be attributed to the loss of water during smoking. The implication of this is that smoking decreases moisture in food thereby increasing their shelf life. Higher fat content was observed in fresh beef sample. The dripping of fat during smoking could be responsible for the lower fat content of the smoke beef. The ash content where 2.47 and 3.20% for fresh and smoke beef respectively.

Table 3

Physico-chemical properties of samples analyzed (%)

	fresh	smoke
Fat content	13.90±0.30	10.80
Moisture content	11.20±0.23	9.10
Ash content	2.47±0.20	3.20

Results are expressed as mean±SD for triplicate determination

Table 4 shows the PAHs content of fresh beef using soxhlet extraction method. The total PAHs obtained were 9.60, 7.53 and 8.09 µg/kg respectively for n-hexane, DCM and n-hexane: DCM respectively. The concentration of B[a] P ranged from 0.51 to1.03 µg/kg. This shows that the content of B[a] P, commonly described as the marker PAH was generally lower than the permissible limit of 5.0µg/kg based on international standards. The significant difference at $p<0.05$ observed in most of the compounds above shows that the various extractants differ appreciably in their extraction potentials. This may be related to the inherent chemical and physical properties of the solvents such as polarity and volatility [19].

Table 4

PAHs content of fresh beef using soxhlet extraction method

PAHs	n-hexane	DCM	hexane: DCM	ML
Napthalene	ND	ND	0.37±0.14	5.00 (EC, 2014)
Naphthalene, 2-methyl	$0.28±0.09^{b}$	$0.27±0.14^{ab}$	$0.25±0.23^{a}$	
Biphenylene	ND	ND	ND	
Acenaphthene	ND	ND	ND	
Fluorene	$0.19±0.11^{a}$	$0.43±0.01^{b}$	$0.52±0.13^{c}$	
Phenanthrene	$0.37±0.05^{b}$	$0.37±0.15^{b}$	$0.32±0.09^{a}$	
Anthracene	ND	$0.27±0.11^{b}$	$0.13±0.12^{a}$	
Fluoranthene	$0.88±0.13^{a}$	$0.98±0.01^{a}$	$1.24±0.09^{b}$	
Pyrene	$2.53±0.23^{b}$	$2.61±0.12^{b}$	$1.69±0.12^{a}$	
Benz[a] anthracene	$2.19±0.14^{b}$	$1.36±0.09^{a}$	$1.32±0.23^{a}$	
Chrysene	0.13±0.08	ND	ND	
Benzo[b]fluoranthene	ND	ND	ND	
Benzo [a] pyrene	ND	$1.03±0.12^{b}$	$0.51±0.03^{a}$	
Indeno [1,2,3 –cd]pyrene	$1.28±0.09^{c}$	$0.67±0.04^{a}$	$1.14±0.07^{b}$	
Dibenz[a,h]anthracene	$1.75±0.09^{b}$	ND	$0.61±0.01^{a}$	
Benzo[ghi]perylene	ND	ND	ND	
PAHS16	9.60	7.53	8.09	

Results are expressed as M±SD for triplicate determinations. Values with same superscript letters on same row do not differ significantly at $p< 0.05$.

The PAHs content of fresh beef using sonication method is depicted in table 5. With the exception of naphthalene which was detected in the combined mixture of the two solvents, the first four PAHs were not detected in the sample analyzed. The concentration of the PAHs ranged from 0.06 to 3.09 µg/kg. The highest content was pyrene in n-hexane solvent, while the least was anthracene (0.06 ug/kg). The content of the total PAHs in fresh samples using sonication method of extraction range from 6.6 to 9.98 µg/kg. The concentrations of PAHs in fresh beef samples were generally low. This is in line with findings from other researchers that the formation of these compounds occurs during processing of this meat as the fats drip into the flame [8].

Table 5

PAHs content of fresh beef using sonication method (µg/kg)

PAHs	n-hexane	DCM	n-hexane: DCM	ML
Napthalene	ND	ND	0.56±0.21	5.00(EC, 2014)
Naphthalene, 2-methyl	ND	ND	ND	
Biphenylene	ND	ND	ND	
Acenaphthene	ND	ND	ND	
Fluorene	$1.68±0.03^{c}$	$0.48±0.13^{a}$	$0.66±0.12^{b}$	
Phenanthrene	$1.51±0.01^{c}$	$0.62±0.07^{b}$	$0.42±0.23^{a}$	
Anthracene	$0.06±0.02^{a}$	$0.09±0.01^{ab}$	$0.12±0.09^{b}$	
Fluoranthene	$0.95±0.01^{b}$	$1.18±0.21^{c}$	$0.29±0.01^{a}$	
Pyrene	$2.49±0.08^{a}$	$3.09±0.09^{b}$	$2.57±0.26^{a}$	
Benz[a] anthracene	ND	ND	0.88±0.01	
Chrysene	ND	0.14±0.12	ND	
Benzo[b]fluoranthene	$0.20±0.01^{a}$	$0.99±0.16^{b}$	ND	
Benzo [a] pyrene	1.27±0.13b	$1.13±0.09^{a}$	ND	
Indeno [1,2,3 –cd]pyrene	ND	$0.67±0.11^{b}$	$0.49±0.10^{a}$	
Dibenz[a,h]anthracene	$1.82±0.09^{c}$	$1.14±0.09^{b}$	$0.61±0.09^{a}$	
Benzo[ghi]perylene	ND	0.39±0.01	ND	
PAH16	9.98	9.92	6.6	

Results are expressed as M±SD for triplicate determinations. Values with same superscript letters on same row do not differ significantly at $p< 0.05$.

PAHs content in smoked beef using soxhlet extraction method is presented in table 6. There were significant differences in the concentrations of the PAHs obtained from the smoked samples in the various solvents of interest. The concentration of B[a]P ranged from 1.01 to 1.70 µg/kg. When compared to the fresh samples, the values obtained for the different extractants are relatively higher. The significant differences observed amongst most of the values reflect the different extraction efficiencies of the various extractants employed. These are higher than the maximum limit of 5.0µg/kg. In a related study [20] reported 0.015, 0.013 and 0.056 as the concentration of pyrene, fluorene and naphthalene respectively. In a study on PAHs content of grilled meat and chicken [21] reported that Benzo(a)Pyrene concentration in charcoal grilled chicken ranged from 0.49-7.20µg/kg and 2.01 µg/kg mean concentration.

Table 5

PAHs content of smoked beef using soxhlet method (µg/kg)

PAHs	n-hexane	DCM	Hexane: DCM	ML
Napthalene	2.53±0.17^{c}	0.60±0.01^{a}	2.05±0.09^{b}	5.00 (EC, 2014)
Naphthalene, 2-methyl	0.58±0.03^{b}	2.23±0.24^{c}	0.35±0.12^{a}	
Biphenylene	0.22±0.01^{a}	0.29±0.01^{ab}	0.34±0.01^{b}	
Acenaphthene	0.46±0.01^{b}	0.27±0.06^{a}	0.89±0.12^{c}	
Fluorene	9.83±0.26^{b}	7.10±0.56^{c}	6.01±0.18^{a}	
Phenanthrene	9.42±0.29^{b}	8.20±0.11^{b}	4.50±0.24^{a}	
Anthracene	0.20±0.01^{ab}	0.17±0.01^{a}	0.23±0.01^{b}	
Fluoranthene	1.65±0.19^{b}	1.31±0.16^{a}	2.65±0.11^{c}	
Pyrene	7.17±0.24^{c}	4.80±0.03^{a}	5.48±0.28^{b}	
Benz[a] anthracene	3.94±0.14^{b}	2.14±0.08^{a}	3.99±0.03^{b}	
Chrysene	0.36±0.01^{b}	0.19±0.12^{a}	0.15±0.01^{a}	
Benzo[b]fluoranthene	1.32±0.08^{b}	1.37±0.08^{b}	1.20±0.12^{a}	
Benzo [a] pyrene	1.01±0.12^{a}	ND	1.70±0.03^{b}	
Indeno [1,2,3 –cd]pyren	2.96±0.09^{b}	1.86±0.14^{a}	3.33±0.12^{c}	
Dibenz[a,h]anthracene	2.44±0.17^{a}	2.71±0.67^{ab}	2.85±0.14^{b}	
Benzo[ghi]perylene	0.71±0.01	ND	ND	
PAH16	42.80	33.04	35.72	

Results are expressed as M±SD for triplicate determinations. Values with same superscript letters on same row do not differ significantly at $p< 0.05$.

Table 6 shows the content of PAHs in smoked beef using sonication method of extraction. The content of the PAHs were observed to have increased appreciably in the beef samples on smoking. This confirms the findings from other studies [22].

Table 6

PAHs content of smoked beef using sonication method (µg/kg)

PAHs	n-hexane	DCM	Hexane: DCM	ML
Napthalene	3.73±0.89b	1.38±0.10^{a}	1.30±0.07^{a}	5.00 (EC, 2014)
Naphthalene, 2-methyl	0.51±0.01^{a}	0.49±0.01^{a}	0.53±0.03^{a}	
Biphenylene	0.29±0.03^{a}	0.59±0.06^{b}	ND	
Acenaphthene	0.46±0.12^{a}	0.54±0.01^{b}	0.46±0.16^{a}	
Fluorene	8.70±0.09^{c}	8.03±0.12^{b}	5.48±0.12^{a}	
Phenanthrene	9.50±0.13^{b}	9.90±0.09^{b}	8.10±0.18^{a}	
Anthracene	0.22±0.06^{a}	0.20±0.01^{a}	0.37±0.04^{b}	
Fluoranthene	2.06±0.06^{a}	2.77±0.10^{c}	2.10±0.09^{b}	
Pyrene	6.50±0.19^{a}	7.64±0.23^{b}	6.71±0.06^{a}	
Benz[a] anthracene	2.76±0.11^{c}	2.28±0.13^{a}	2.98±0.11^{b}	
Chrysene	0.21±0.08^{b}	0.34±0.02^{c}	0.13±0.03^{a}	
Benzo[b]fluoranthene	1.63±0.06^{c}	1.01±0.05^{b}	0.72±0.01^{a}	
Benzo [a] pyrene	1.75±0.01^{a}	3.82±0.23^{b}	1.54±0.14^{a}	
Indeno [1,2,3 –cd]pyrene	3.90±0.19^{c}	2.25±0.05^{a}	2.96±0.02^{b}	
Dibenz[a,h]anthracene	1.63±0.07^{a}	2.97±0.23^{b}	1.84±0.01^{a}	
Benzo[ghi]perylene	1.19±0.01^{a}	0.85±0.01^{a}	0.93±0.01^{a}	
PAH16	45.04	45.15	36.15	

Results are expressed as M±SD for triplicate determinations. Values with same superscript letters on same row do not differ significantly at $p< 0.05$.

It was also reported by [3] that the formation of PAHs in food is favored by high temperature cooking processes. These concentrations were slightly above the limit of 5.0ug/kg and their continuous consumptions is unsafe for humans. The concentrations of PAHs obtained is lower than 0.12mg/kg reported by [20] for B(a)P in their study on sardine. Sonication method was observed to be more efficient in the yield of PAHs when compared to the conventional soxhlet extraction method. The combined solvents had the least total PAHs of 36.15µg/kg.

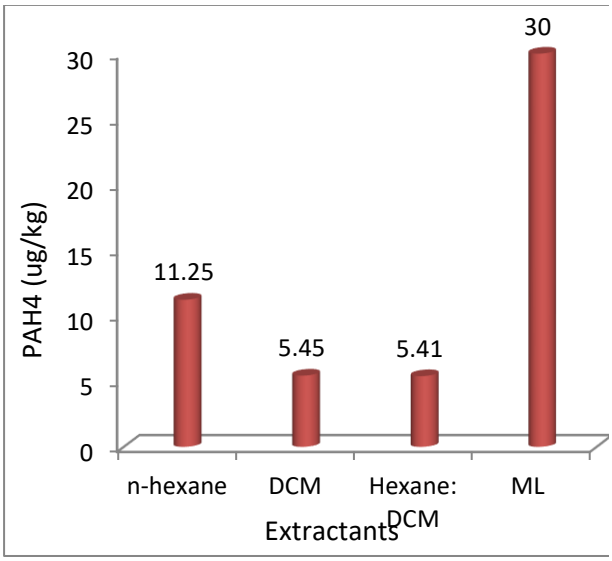

Fig. 1: PAH4 content in smoked beef using sonication extraction (µg/kg)

Figure 1 gives the PAH4 content in various extractants in fresh beef using sonication method of extraction. n-hexane had the highest (11.25 µg/kg) while DCM had the least (5.45 µg/kg). The content of PAH4 in all the extractants were lower than the maximum limit of 30 µg/kg specified by international standards. The result shows that the PAH4 content increased on smoking while for fresh sample the content varied from 0.88 to 2.93 µg/kg. A concentration range of 3.38 to 20.06 µg/kg was obtained by [23] for the concentration of PAH4 in a related study on smoked meat.

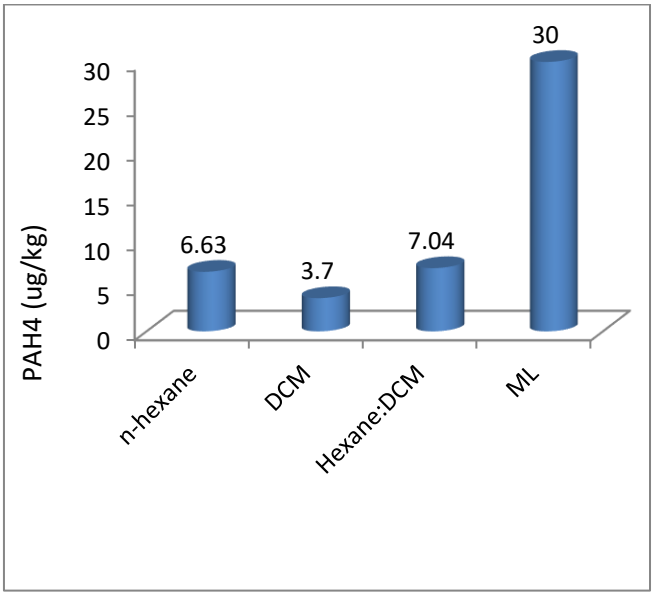

Fig. 2: PAH4 content in smoked beef using soxhlet extraction (µg/kg)

The content of PAH4 in smoked beef using soxhlet extraction method is shown in figure 2. The concentrations were 6.63, 3.7, 7.04 and 30 µg/kg for n-hexane, DCM, n-hexane and maximum limit respectively. The values obtained where generally higher when compared to the PAH4 content in the fresh beef sample. This observed difference can be attributed to the formation of PAHs during smoking.

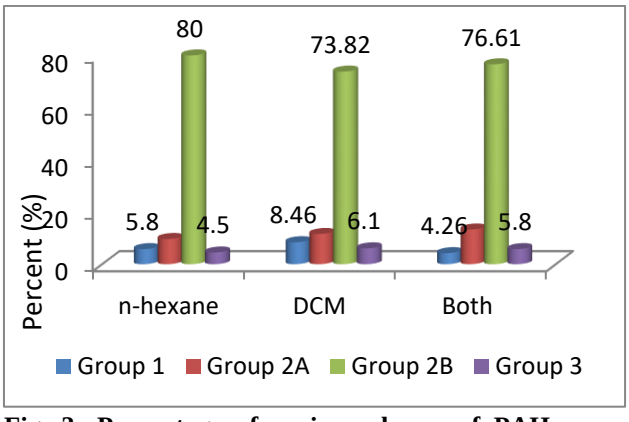

Fig. 3: Percentage of various classes of PAHs based on carcinogenicity present in smoked beef

The percentage of various classes of PAHs based on carcinogenicity present in

smoked beef is presented in figure 3 above. Group 2A PAHs were mostly present in all the extractants. Their values ranged from 73.82 to 80%. n-hexane had the highest while DCM had the least. Group 1 PAHs had the least percentage composition. Group 1 PAHs are those known with established evidences of carcinogenity. This is no doubt the most dreaded. The content in the smoked beef was the lowest (4.26 to 5.80%) of the total PAHs. Benzo [a] pyrene is the only PAH that fits into this class hence was long used as a reference for assessing the presence of other PAHs. The highest class of PAH were those considered to be possibly carcinogenic.

Table 7

Heavy metal contents in beef (mg/kg)

Metal	Fresh	Smoked	ML	Source
Fe	110.56±1.91	118.11±1.01	300	WHO, 2014
Mn	6.98±0.01	6.20±0.15	15.00	WHO, 2008
Zn	17.64.±0.19	19.07±0.17	40.00	FAO, 1978
Cu	0.75±0.24	0.81±0.51	5.00	EC, 2006
Cd	0.24±0.03	0.27±0.01	0.50	WHO, 2014
Pb	0.91±0.37	1.17±0.34	2.00	WHO, 2014

Results are expressed as mean±SD.

Table 7 shows the heavy metal content in the fresh and smoked beef. The concentration of Cd ranged from 0.24 to 0.27 mg/kg for the fresh and smoked beef respectively. There was increase in the concentration of all the metals except manganese on smoking. The concentrations of the metals were generally lower than the maximum limits based on international standards. The concentration of Fe was observed to be affected most by the smoking process. The higher values of iron in smoke beef may be related to the additives added or the interaction with the metal gauze. Iron plays a vital role in animal and plants physiology [24].

The concentration of Mn did not differ significantly between raw and smoke. The slight decrease in the concentration of Mn was from 6.98 to 6.82 on grilling. Manganese is recognized as an essential trace element. Exposure to high concentration is however toxic to health. The detrimental effect of manganese to health is more targeted to the central nervous system and the brain [25].The concentrations of Mn were safe for humans when compared for to the maximum limit of 15.00mg/kg reported by [26]. Manganese is an essential trace element that is normally present in all mammalian tissues in concentrations ranging from 0.3 to 2.9 μg/g [27]. The content from this study was however lower than 3.06 mg/kg and maximum 6.141 mg/kg reported by [28] in a related study.

Slight drop in the content of Zn was also observed in the beef and fish samples on smoking. The content of Zn in the fish and beef sample was however considered safe with respects to the maximum limit of 40.00 mg/kg by FAO, 1978. In a related study, [24] obtained 4.95–48.23 $mg.kg^{-1}$ for Zn in some fresh chicken and turkey meat. A mean levels of 28.53± 3.39 $mg.kg^{-1}$ Zn in lean meat of poultry was reported by [29] in Lahore.

The change in concentrations of copper was not significant in the smoked fish and beef. These values were however lower than the permissible limit of 5.0mg/kg for copper in smoked food as reported by [13]. [30], in his study also reported that the

heavy metals concentration in the fresh and smoked beef samples did not differ significantly (p> 0.05) from each other. Cows and other animals also feed directly on these crops with their pesticide residues. In a related study, [24] reported 0.01–5.15 $mg.kg^{-1}$ as the content of Cu in fresh turkey.

Cd is one of the most toxic heavy metal and has no known function in biological system. Cd did not differ significantly between the fresh and smoked samples. A slight increase by 0.04 was observed in smoked beef. [24] obtained 0.01–5.68 $mg.kg^{-1}$ as the range of Cd in selected fresh meat. The presence of Cd in raw beef could be from contaminated feed, water sources etc. Also the relatively higher content of Cd in smoked sample could result loss of water, exposure to contaminants during grilling and presence of additives. Much of the cadmium which enters the body by ingestion comes from terrestrial foods [27].

The concentration of Pb was observed to increase on smoking. Lead is a very toxic metal and has the potential of binding with enzymes and other vital cellular components thereby causing damage to vital body organs like brain and kidney [29]. Like cadmium, lead is highly toxic and has the tendency to accumulate in tissues of organisms as they are consumed [28]. Pb is dispersed over long distances through air and water usually in areas with high industrial plants [27]. In a related study, [30] reported a rise in the concentration of Pb from 0.07 in fresh beef to 2.24mg/kg on smoking.

Table 8

Inter-elemental Correlation Coefficient of metal in smoked beef

	Cu	Mn	Zn	Fe	Cd	Pb
Cu	1	0.55	0.52	0.78*	0.55	0.79*
Mn		1	0.90**	0.49	0.91**	0.73
Zn			1	0.72*	0.67	0.89**
Fe				1	0.79*	0.61
Cd					1	0.92**
Pb						1

*Correlation is significant at the 0.05 level (2 tailed)
** Correlation is significant at the 0.01 level (2 tailed)

Table 8 shows the inter-elemental correlation coefficient among the heavy metals studied. There was strong positive association among Cu with Fe and Pb, Mn with Zn and Cd, and Cd with Pb. Strong positive association may suggest a close source between such metals.

4. Conclusion

The study shows that the concentration of PAHs in smoked beef was significantly higher than the raw beef which confirms that PAHs formation occurs due to the smoking process. Sonication method was observed to be more efficient in the recovery of PAHs. *n*-hexane was found to be better than DCM and the mixture of both solvents in PAHs extraction.

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