

COMPARISON OF THE HEALTH RISK OF PAHs FROM WHEAT (*Triticum specie*) BAMBARA NUT (*Vigna subterranea*) and PIGEON PEAS (*Cajanus cajanifolia*) CONSUMED IN NIGERIA

Abstract

Polycyclic aromatic hydrocarbons, PAHs are carcinogenic and genotoxic in nature and have been of worldwide concern. This study aimed at determining the PAH contamination levels in types of wheat (*Triticum specie*), bambara groundnut (*Vigna subterranea*) and pigeon peas (*Cajanus cajanifolia*) commonly consumed in the eastern part of Nigeria and assess the health risk associated with their consumption. The grain samples were analyzed of sixteen priority PAHs using gas chromatography coupled with flame ionization detector (GC-FID) after extraction by sonication. Estimation of daily intakes were carried out using adult male and female consumers while margin of exposure was used to assess the health risk applying benchmark dose levels for the indicators-BaP, PAH2, PAH4 and PAH8. The sixteen PAHs were detected in all the analyzed grains. The \sum 16 PAHs concentrations ($\times 10^{-2}\mu\text{g}/\text{kg}$) detected ranged from 25.004 ± 20.553 in white pigeon peas to 36.493 ± 20.305 in red pigeon peas. The eight probable carcinogenic PAHs (\sum PAH8) detected ranged from 10.913 ± 4.295 to 17.444 ± 7.023 also in white and red pigeon peas respectively. From the estimation of daily intake calculated, the total dietary exposure of male ($41.42 \mu\text{g}/\text{kg bw}/\text{day}$) was less than that of female ($48.24 \mu\text{g}/\text{kg bw}/\text{day}$) implying that adult female are more exposed. The MOE for adult male individual ranged from 49,893 in pure white bambara groundnut to 392,943 in pigeon peas. While for adult female individual, the values of MOE ranged from 48,110 in bambara groundnut to 336,770 in pigeon peas. The values of margin of MOE obtained for all the indicators were much higher than 10000 which according to EFSA indicate low concern for human health and considered low priority for risk management actions. The PAHs values detected were all below $1.0 \mu\text{g}/\text{kg}$ which is the permissible limit established by EFSA. Based on these facts, these grains are safe for consumption.

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Keywords: Polycyclic aromatic hydrocarbons, Bambara groundnut, wheat, Estimated daily intake, Margin of exposure, Gas chromatography.

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1.0 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds comprising of two or more fused aromatic rings which are formed by incomplete pyrolysis of organic matter and during certain industrial processes.¹ In the past decade PAHs were evaluated by the International Programme on Chemical Safety (IPCS), the Scientific Committee on Food (SCF) and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). SCF and JECFA concluded that 15 PAHs 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.² They suggested the use benzo[*a*]pyrene as a marker of occurrence and effect of the carcinogenic PAHs. For different food categories and subcategories, the data on PAH8, PAH4 and PAH2 were then used for the exposure calculation as well as the estimation of margins of exposure (MOEs) based on the bench mark dose lower confidence limit for a 10% increase in the number of tumour bearing animals compared to control animals (BMDL₁₀).²

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The two highest contributors to the dietary exposure were cereals and cereal products, and sea food and sea food products. Food can be contaminated by environmental PAHs that are present in air, soil or water, by industrial food processing methods (e.g. heating, drying and smoking processes) and by home food preparation (e.g. grilling and roasting processes).

Studies have reported the contaminations of PAHs in both edible and non- edible substances. Edibles substances such as rice,^{3,4,5,6} wheat, beans, soya bean, bambara nut, pigeon peas, guinea corn,^{7,8} maize and snacks,^{9,10,11,12} toasted bread,^{13,14} pastas,¹⁵ meat, cow hides,^{16,17,18,19} beverages,²⁰ infant formulae,²¹ fish,^{22,23,24,25,26} roasted food snacks-mackerel, suya beef and plantain,^{27,28} yam, cassava, orange and papaya,²⁹ vegetable oils,^{30,31,32,33,34} fruits, leafy and underground vegetables,^{35,36} milk, water,¹⁷ have been contaminated by PAHs. While non-edible such as sediments,³⁷ soil, air, coal, creosote have also been reported to be contaminated with PAHs.

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Bambara groundnut, *vigna subterranean* is a legume indigenous to Africa and is cultivated across the semi-arid sub-saharan African region.³⁸ It is a sustainable crop and contains carbohydrates, 64.4%, plant-based protein, 23.6%, fat, 6.5%, fiber, 5.5%, unsaturated fatty acids and essential minerals as Magnesium, Iron, Zinc and Potassium.³⁹ Bambara groundnut is a hardy crop and has been recognized as an important nutritious food source when food is scarce.⁴⁰ It is resilient to adverse environmental conditions and can yield on poor soil. It is consumed in Nigeria in different forms.

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Pigeon peas, *Cajanus cajanifolia* are both a food crop (dried peas, flour, or green vegetable peas) and a forage/cover crop. They are in some areas an important crop for green manure, providing up to 90 kg nitrogen per hectare.⁴¹ The woody stems of pigeon peas can also be used as firewood, fencing and thatch. It is an important ingredient of animal feed used in West Africa, especially in Nigeria. Leaves, pods, seeds and the residues of seed processing are used to feed all kinds of livestock.⁴² Pigeon peas contain high levels of protein and the important amino acids methionine, lysine, and tryptophan.

Wheat, *Triticum species* is a cereal grain, originally from the Levant region of the Near East but now cultivated worldwide.⁴³ Globally, wheat is the leading source of vegetable protein in human food, having a higher protein content than other major cereals. Wheat contains water 12%, carbohydrate, 70%, protein, 12%, fat, 2%, minerals 1.8%, crude fiber 2.2%, thiamin, riboflavin, niacin, vitamin A.⁴⁴ It is a major diet component because of the wheat plant's agronomic adaptability. In addition to agronomic adaptability, wheat offers ease of grain storage and ease of converting grain into flour for making edible, palatable, interesting and satisfying foods. It is the most important raw material for making bread, pasta (spaghetti and macaroni), cookies, crackers,

cake.⁴⁴ The popularity of foods made from wheat flour creates a large demand for the grain, even in economies with significant food surpluses.

Sixteen PAHs have been listed by United State Environmental Protection Agency, USEPA as priority pollutants present in the air, water and soil.⁴⁵ They are detrimental to public health due to their toxicity, carcinogenic and bioaccumulation properties and has raised alarm globally. This study therefore proposed to determine the PAHs contamination levels and assess the health risk associated with the consumption of wheat, bambara groundnut and pigeon peas grains from markets in South East Nigeria.

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2.0 Materials and Methods

2.1 Equipment and Reagents

Gas chromatography/flame ionization detector (HP 6890 Powered with HP ChemStation) , rotary evaporator, borosilicate beaker, glass column, sonicator.

All reagents and solvents were of analytical grade and were purchased from Sigma Aldrich US A. These included hexane, dichloromethane, activated alumina as well as four deuterated (surrogate) standard namely acenaphthalene d₁₀, chrysene d₁₂, phenanthrene d₁₀ and perylene d₁₂. The analysis was carried out in Multi Environmental Management Consultants Ltd, Plot 4/5 Laara Sownmade Rd, off Igbe Rd, Ijede Ikorodu, Lagos, Nigeria.

2.2 Sampling

Eighteen (18) samples which included different types of wheat, *Triticum specie* (Hard and Soft) bambara groundnut, *Vigna subterranean* (Pure white and Mixed white): pigeon peas, *Cajanus cajanifolia* (White and Red) were purchased from some major markets in Enugu and Anambra states of Nigeria. The markets included New market, Gariki market and Ogbete main market in Enugu East, Enugu South and Enugu North Local Government Areas of Enugu State respectively, Nsukka main market in Igboetiti Local Government Area of Enugu State. Awka central market in Awka South Local Government Area of Anambra State, Umunze main market in Orumba South L. G. A. of Anambra State. The samples were picked to remove sand and other impurities, ground and put in labeled amber sample bottles ready for extraction.

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2.3 Extraction of Samples:

Recovery experiments to optimize PAH extraction from grain samples were carried out. Three mixed standard solutions of concentrations 100, 500 and 1000 µg/mL were prepared using four deuterated PAHs (d-PAHs). These were used to spike three 5 g portions of ground grain samples which were extracted by sonication using 3:1 dichloromethane-hexane mixture as solvent. The extracts were cleaned-up in an alumina column using the same solvent mixture. ⁴⁶

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2.4 Determination of PAHs

PAHs concentrations were determined with a gas chromatography equipped with flame ionization detector, GC-FID, (HP 6890). The initial oven temperature programme was 60°C for 5 mins, raised to 250 °C, first at a rate of 15°C/min and maintained at this temp for 14 mins and at second rate of 10°C/min for 5 mins. Nitrogen gas was used as the carrier gas at the flow rate of 1 cm³/min at a pressure of 30 psi. Following recoveries of 94.0 to 99.2%, the grain samples were extracted and PAHs determined by the same procedure. ⁴⁶

Comment [u22]: Do you self-contaminate the samples? Ordinarily the natural concentrations of PAHs in the samples should be determined and compared for this research to be original. What is the essence of sample spiking? If it self-contamination, then the research work isn't necessary. Note: explain and indicate in this section of the write up the reason for sample spike with prepared PAHs.

2.5 Statistical Analysis

Analysis of variance and Pearson Correlation Coefficient at 95% confidence level were carried out using SPSS version 16.00 on the data obtained.

2.6 Daily Estimated Intake (EDI)

The dietary intakes of the 16 PAHs were estimated, using a deterministic approach. A fixed value for the consumption of an individual food was multiplied by a fixed value for the contaminant concentration in that food. ⁴⁷ The total exposure was obtained by summing the intakes from all foods, using the following equation:

Estimated daily Intake (EDI) = \sum Consumption rate × Occurrence

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Body Weight

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Body weight for adult female = 60 Kg and body weight for adult male = 70 Kg were used. ⁴⁸

2.7 Risk Assessment

For risk assessment, the margin of exposure approach (MOE) as adopted by the EFSA Scientific Committee in the Opinion related to substances which are both genotoxic and carcinogenic. ² The uncertainty in the assessment objectives is considered to be negligible. Margins of exposures (MOEs) were calculated by dividing the lowest BMDL₁₀ values among the models with acceptable fits by the mean and high level estimates of dietary exposure to benzo[a]pyrene, PAH2, PAH4 and PAH8. However, for high level consumers the MOEs are close to or less than 10,000, which as proposed by the EFSA Scientific Committee ² indicates a potential concern for consumer health and a possible need for risk management action. But for lower level consumers, the MOEs of 10,000 or higher would be of low concern for human health and might be considered low priority for risk management actions. ¹⁹

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The risk was estimated using the Margin of Exposure (MOE) approach according to the following equation: ⁴⁹

$$\text{MOE} = \frac{\text{BMDL}_{10}}{\text{EDI}}$$

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Where BMDL₁₀ is the benchmark dose lower confidence limit at 10% incidence level. Considering a BMDL₁₀ of 0.07, 0.17, 0.34 and 0.49 all in mg/kg bw per day for BaP, PAH2, PAH4 and PAH8, respectively, for adult and children scenario, where:

BaP = Benzo[a] pyrene

PAH2 = Benzo [a]pyrene and chrysene

PAH4 = Benzo [a]anthracene, benzo[a] pyrene, benzo [b] fluoranthene and chrysene

PAH8 = The sum of eight carcinogenic PAHs: benzo [a] anthracene; benzo [b]fluoranthene; benzo [k] fluoranthene; benzo[g,h,i]perylene; benzo [a]pyrene; chrysene; dibenz[a,h]anthracene; and indeno[1,2,3-C,d] pyrene.

3.0 RESULT AND DISCUSSION

3.1 RESULT

Table 1: PAHs Concentration ($\times 10^{-2}\mu\text{g}/\text{kg}$) in Bambara nut

PAHs	Pure White Mean \pm SD	Mixed White Mean \pm SD
Naphthalene	0.024 \pm 0.004	0.025 \pm 0.002
Acenaphthylene	0.037 \pm 0.009	0.053 \pm 0.026
Acenaphthene	1.428 \pm 2.112	0.591 \pm 0.734
Fluorene	0.186 \pm 0.294	1.217 \pm 1.867
Phenanthrene	3.31 \pm 2.902	3.309 \pm 2.914
Anthracene	5.439 \pm 2.335	4.745 \pm 3.695
Fluoranthene	1.372 \pm 1.402	2.132 \pm 2.089
Pyrene	4.69 \pm 2.015	8.315 \pm 3.649
Benzo[a]anthracene	5.213 \pm 1.192	3.65 \pm 3.811
Chrysene	2.586 \pm 4.035	2.592 \pm 4.030
Benzo[b]fluoranthene	0.402 \pm 0.110	0.433 \pm 0.094
Benzo[k]fluoranthene	0.383 \pm 0.075	0.517 \pm 0.319
Benzo[a]pyrene	5.458 \pm 0.433	4.71 \pm 3.743
Indeno[1,2,3-cd]pyrene	0.076 \pm 0.096	0.095 \pm 0.131
Dibenzo[a,h]anthracene	0.143 \pm 0.194	0.177 \pm 0.254
Benzo[g,h,i]perylene	0.356 \pm 0.150	0.332 \pm 0.157
Σ 16 PAHs	31.103 \pm 17.358	32.893 \pm 27.515
Σ LMW PAHs	10.423 \pm 6.661	9.940 \pm 4.742
Σ HMW PAHs	20.679 \pm 3.293	22.953 \pm 14.125
PAH2	8.044 \pm 3.659	7.302 \pm 7.102
PAH4	13.658 \pm 2.509	11.385 \pm 10.249
PAH8	14.617 \pm 2.949	12.506 \pm 11.098

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Table 2: PAHs Concentration ($\times 10^{-2}\mu\text{g}/\text{kg}$) in Wheat

PAHs	Soft Wheat Mean \pm SD	Hard Wheat Mean \pm SD
Naphthalene	0.028 \pm 0.001	0.026 \pm 0.002
Acenaphthylene	0.055 \pm 0.016	0.047 \pm 0.004
Acenaphthene	3.425 \pm 1.228	4.012 \pm 2.059
Fluorene	1.115 \pm 1.093	1.13 \pm 1.072
Phenanthrene	2.255 \pm 0.216	2.421 \pm 0.451
Anthracene	6.472 \pm 1.175	6.317 \pm 0.955
Fluoranthene	2.104 \pm 0.744	2.017 \pm 0.620
Pyrene	2.853 \pm 1.518	2.791 \pm 1.606
Benzo[a]anthracene	2.979 \pm 0.415	2.852 \pm 0.594

Chrysene	5.497±2.658	5.113±2.115
Benzo[b]fluoranthene	0.288±0.068	0.276±0.085
Benzo[k]fluoranthene	0.374±0.021	0.346±0.059
Benzo[a]pyrene	4.72±0.619	4.467±0.978
Indeno[1,2,3-cd]pyrene	0.129±0.055	0.116±0.037
Dibenzo[a,h]anthracene	0.208±0.108	0.174±0.060
Benzo[g,h,i]perylene	0.252±0.004	0.231±0.025
∑16 PAHs	32.75±2.471	32.33±1.880
∑LMW PAHs	13.349±1.542	13.952±2.396
∑HMW PAHs	19.402±0.928	18.381±0.515
PAH2	10.217±2.039	9.579±1.137
PAH4	13.483±1.556	12.707±0.458
PAH8	14.445±1.702	13.574±0.470

Table 3: PAHs Concentrations(× 10⁻² µg/kg) in Pigeon Peas

PAHs	White Mean±SD	Red Mean±SD
Naphthalene	0.037 ± 0.00	0.025 ± 0.011
Acenaphthylene	0.111 ± 0.125	0.051 ± 0.026
Acenaphthene	1.324 ± 1.859	0.546 ± 0.531
Fluorene	0.158 ± 0.242	0.101 ± 0.146
Phenanthrene	4.389 ± 4.668	2.727 ± 2.374
Anthracene	4.151 ± 2.573	5.386 ± 2.872
Fluoranthene	0.679 ± 0.286	0.878 ± 0.600
Pyrene	3.243 ± 4.056	9.335 ± 6.618
Benzo[a]anthracene	3.839 ± 2.811	6.74 ± 2.19
Chrysene	0.876 ± 0.994	2.339 ± 3.650
Benzo[b]fluoranthene	0.404 ± 0.111	0.44 ± 0.035
Benzo[k]fluoranthene	0.312 ± 0.209	0.543 ± 0.306
Benzo[a]pyrene	5.074 ± 2.372	6.737 ± 0.431
Indeno[1,2,3-cd]pyrene	0.042 ± 0.033	0.094 ± 0.129
Dibenzo[a,h]anthracene	0.094 ± 0.118	0.238 ± 0.219
Benzo[g,h,i]perylene	0.271 ± 0.098	0.313 ± 0.160
∑16 PAHs	25.004±20.553	36.493±20.305
∑LMW PAHs	10.169±5.477	8.837±4.766
∑HMW PAHs	14.835±7.178	27.657±14.190
PAH2	5.950±1.381	9.076±4.042
PAH4	10.193±4.191	16.256±6.260
PAH8	10.913±4.295	17.444±7.023

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Table 4: ESTIMATED DAILY INTAKE, EDI ($\times 10^{-2}$ $\mu\text{g}/\text{kg}/\text{bw}/\text{day}$) OF ADULT MALE INDIVIDUALS ON WHEAT, BAMBARA NUT AND PIGEON PEAS

PAHs	Wheat		Bambara nut		Pigeon Peas	
	Soft Wheat	Hard Wheat	Pure white	Mixed white	White	Red
BaP	0.674	0.638	1.403	1.211	0.808	0.77
PAH2	1.46	1.368	2.068	1.878	0.68	1.037
PAH4	1.926	1.815	3.512	2.928	1.165	1.858
PAH8	2.064	1.939	3.759	3.216	1.247	1.994

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Table 5: ESTIMATED DAILY INTAKE, EDI ($\times 10^{-2}$ $\mu\text{g}/\text{kg}/\text{bw}/\text{day}$) OF ADULT FEMALE INDIVIDUALS ON WHEAT, BAMBARA NUT AND PIGEON PEAS

PAHs	Wheat		Bambara nut		Pigeon Peas	
	Soft Wheat	Hard Wheat	Pure white	Mixed white	White	Red
BaP	0.944	0.894	1.455	1.256	0.677	0.898
PAH2	2.044	1.916	2.145	1.947	0.793	1.21
PAH4	2.696	2.542	3.642	3.037	1.359	2.167
PAH8	2.89	2.714	3.898	3.335	1.455	2.326

Table 6: MARGIN OF EXPOSURE, MOE FOR ADULT MALE EXPOSED INDIVIDUALS ON WHEAT, BAMBARA NUT AND PIGEON PEAS

PAHs	Wheat		Bambara nut		Pigeon Peas	
	Soft Wheat	Hard Wheat	Pure white	Mixed white	White	Red
BaP	103858	109718	49893	57803	86633	90909
PAH2	116438	124269	82205	90522	250000	163934
PAH4	176532	187328	96811	116120	291845	182992
PAH8	237403	252708	130353	152363	392943	245737

Table 7: MARGIN OF EXPOSURE, MOE FOR ADULT FEMALE EXPOSED INDIVIDUALS ON WHEAT, BAMBARA NUT AND PIGEON PEAS

PAHs	Wheat		Bambara nut		Pigeon Peas	
	Soft Wheat	Hard Wheat	Pure white	Mixed white	White	Red
BaP	74152	78300	48110	55732	103397	77951
PAH2	83170	88728	79254	87314	214376	140496
PAH4	126112	133753	93355	111953	250184	156899
PAH8	169550	180545	125705	146927	336770	210662

Table 8: SOURCE DETERMINATION

PAHs RATIO	Pure white Bambara nut	Mixed white Bambara nut	Soft Wheat	Hard Wheat	White Pigeon peas	Red Pigeon peas
ANT/ANT + PHE	0.622	0.589	0.755	0.723	0.486	0.664
FLA/FLA + PYR	0.226	0.204	0.424	0.42	0.173	0.086
I[cd]P/I[cd]P + B[ghi]P	0.176	0.222	0.339	0.334	0.134	0.231
B[a]A/B[a]A + CHR	0.668	0.585	0.351	0.358	0.814	0.742

3.2 DISCUSSION

The sixteen PAHs were detected in the analyzed grain samples. The tables 1, 2 and 3 showed the data obtained from the PAHs analysis of bambara groundnut, wheat and pigeon peas respectively. From table 1, the $\Sigma 16$ PAHs obtained in mixed white bambara groundnut (32.893 ± 27.515) is higher than that of pure white (31.103 ± 17.358). The sum total of LMW PAHs in pure white and mixed white were 10.432 ± 6.661 and 9.940 ± 4.742 respectively while the HMW PAHs were 20.697 ± 3.293 and 22.953 ± 14.125 . The probable carcinogenic PAH8 obtained were respectively 14.617 ± 2.949 and 12.506 ± 11.098 in pure white and mixed white bambara groundnut. Table 2 displayed the data gotten from the analysis of wheat grain, the $\Sigma 16$ PAHs detected in the soft and hard wheat were 32.750 ± 2.471 and 32.33 ± 1.880 respectively. Σ LMW and Σ HMW for soft wheat were respectively 13.349 ± 1.542 and 19.402 ± 0.928 while Σ LMW and Σ HMW for hard wheat were 13.952 ± 2.396 and 18.381 ± 0.515 respectively. PAH8 detected for

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soft and hard wheat respectively recorded 14.445 ± 1.702 and 13.574 ± 0.470 . From the table 3, $\Sigma 16$ PAHs obtained in white and red pigeon peas were respectively 25.004 ± 20.553 and 36.493 ± 20.305 . The sums of LMW-PAHs and HMW-PAHs in both white and red pigeon peas were 10.169 ± 5.477 , 14.835 ± 7.178 and 8.837 ± 4.766 , 27.657 ± 14.190 respectively. The PAH8 determined for white and red pigeon peas were 10.913 ± 4.295 and 17.444 ± 7.023 . Comparing the three analyzed grains, the highest level of 16 PAHs were detected in red type of pigeon peas, followed by mixed-white bambara groundnut and the two types of wheat. The high molecular weight, HMW-PAHs were detected highest in red type of pigeon peas seconded by mixed-white bambara groundnut and followed by pure white. The eight probable carcinogenic PAHs dominated in red type of pigeon peas followed by pure white bambara nut and then soft wheat.

The high concentration of PAHs in red pigeon peas can be attributed to the presence of higher level of gluten protein. The PAH concentrations obtained in this present study was two orders of magnitude lower than that obtained by Muntean *et al.*⁸ in analyzed wheat flour ($0.07 \mu\text{g}/\text{kg}$). Al-Rashdem *et al.*¹³ reported no detectable amount of B[a]P in some of samples of bread baked from white wheat flour supplied by the Kuwait flour mills. In their investigation, the original white wheat flour and brown wheat flour were analyzed in parallel to the toasted white bread and there was detection of B[a]P in bread samples baked from brown wheat flour. The presence of Fla and Pyr was observed in the range of 1.19–2.19 and 0.71–1.66 $\mu\text{g}/\text{kg}$ for brown and white wheat flour respectively. Some LMW-PAH were detected, such as Naph, Fla and Phe in white wheat flour and brown wheat flour. The PAH concentrations obtained were comparably higher than those obtained in this study. Generally all the PAH concentrations obtained in this study were below the legal permissible limit of $1.0 \mu\text{g}/\text{kg}$ established for cereals and cereal based – products by EFSA.¹

Tables 4 and 5 showed the daily estimated intakes of the three analyzed grains-wheat, bambara groundnut and pigeon peas among male and female Nigerians using the BaP, PAH2, PAH4, PAH8 indicators. From Table 4 which comprised of the exposed adult male individuals who consume these grains, the total dietary exposure of adult male ($\times 10^{-2} \mu\text{g}/\text{kg bw}/\text{day}$) for BaP and PAH2 were respectively 1.312 and 2.828; 2.614 and 3.946; 1.578 and 1.717 in wheat, bambara groundnut and pigeon peas. While that of PAH4 and PAH8 were 3.741 and 4.003; 6.44 and 6.975; 3.023 and 3.241 respectively in wheat, bambara groundnut and pigeon peas. From

table 5, the total dietary exposure of adult female ($\times 10^{-2}$ $\mu\text{g}/\text{kg}$ bw/day) for BaP and PAH2 were respectively 1.838 and 3.96; 2.711 and 4.092; 1.575 and 2.003 in wheat, bambara groundnut and pigeon peas. For PAH4 and PAH8, the total dietary exposure were 5.238 and 5.604; 6.671 and 7.233; 3.526 and 3.781 in wheat, bambara groundnut and pigeon peas respectively.

Comparing tables 4 and 5, adult female individuals have higher intakes of the three analyzed grains, in other words they are more exposed to health risk when compared to adult male individuals. The total dietary exposure of male (41.42 $\mu\text{g}/\text{kg}$ bw/day) was less than that of female (48.24 $\mu\text{g}/\text{kg}$ bw/day).

The total values of the indicators detected in this study were lower than the values reported by Udowelle et al.¹⁴ and Iwegbue et al.²¹ Udowelle et al.¹⁴ reported detection of total BaP concentration of 6.7 $\mu\text{g}/\text{kg}/\text{bw}/\text{day}$ and PAH8 of 9.13 $\mu\text{g}/\text{kg}$ in bread consumed in Nigeria while Iwegbue et al.²¹ presented the not detected, (nd – 2.67; nd – 5.29; nd – 11.20; nd – 34.96) $\mu\text{g}/\text{kg}/\text{bw}/\text{day}$ respectively in BaP, PAH2, PAH4 and PAH8.

Tables 6 and 7 presented the margin of exposures, MOE for adult male exposed individuals and adult female exposed individuals on wheat, bambara groundnut and pigeon peas respectively. From table 6, the MOE ranged from 103,858 to 252,708 in wheat, 49,893 to 152,363 in bambara groundnut and 86,633 to 392,943 in pigeon peas. From table 7, the MOE varied from 74,152 to 180,545 in wheat, 48,110 to 146,927 in bambara groundnut and from 77,951 to 336,770 in pigeon peas. The values of margin of exposure, MOE obtained for all the indicators were much higher than 10000 which according to EFSA indicate low concern for human health and considered low priority for risk management actions. The values were in line with the values obtained by Iwegbue et al.¹⁴, Ihedioha et al.¹⁵ and Lee et al.³⁴. The studies of Iwegbue et al.²¹ on Polycyclic aromatic hydrocarbon concentrations on commercially available infant formulae in Nigeria reported that the values of all the indicators-BaP, PAH2, PAH4, PAH8 were greater than 10,000 and that of Ihedioha et al.¹⁵ on Risk assessment of polycyclic aromatic hydrocarbons in pasta products consumed in Nigeria revealed that the MOE values for adult consumers were far higher than 10,000 indicating no health risk from consumption of the products. Also the study by Lee et al.³⁴ on the Occurrence and Risk characterization of PAHs of edible oils reported the values of MOEs (between 66094 and 1729776) were over 1.0×10^4 indicating that the risk of 4 PAHs in edible oils were of low concern from a public health point of view.

From Table 8, for Ant/Ant+Phe all the sample concentrations were > 0.1 ; B[a]A /B[a]A + Chr for all the samples were > 0.35 , for I[c,d]P/I[c,d]P + B[g,h,i]P ratio, the values of most analyzed samples lied between 0.2 – 0.5 indicating fuel combustion emission source. Fla/ Fla +Pyr ratio obtained were < 0.4 in some samples indicating petrogenic emission source.

So the two sources of PAH emission in the analyzed samples were petrogenic and combustion. Combustion (or pyrolytic) being the dominant emission source.⁵⁰

From the analysis of variance of all the analyzed grains, $p>0.05$ indicating that there was no significant difference between the PAH concentrations of the analyzed grains although the concentration levels of PAHs in white pigeon pea was comparably lower than the rest of the analyzed grains. It was also ascertained from the analysis that the PAHs concentrations of grains were homogeneous. The Pearson correlation coefficient analysis indicated strong positive correlation among the PAH concentrations of all the analyzed grains. The values of diagnostic ratios were used extensively to distinguish between petrogenic and pyrogenic source of PAHs.

The three analyzed grains contained the sixteen PAHs but at the level very much lower than 1.0 $\mu\text{g}/\text{kg}$ which is the permissible limit established by EFSA for cereals and cereal based products. Estimation of daily intake revealed that adult female individuals are more exposed when compared to their male counterpart. The values of margin of exposures, MOEs obtained for all the indicators were much higher than 10000 which according to EFSA indicate low concern for human health and considered low priority for risk management actions. This study grants safety of consuming these grains. The data from this study can be used by the regulatory bodies to establish limits for legume grains (bambara groundnut and pigeon peas). The environmental substances like foods, soil, water and air should be on regular chemical analysis to ensure their safety with respect to PAHs and other hazardous compounds.

Reference

1. European Food Safety Authority, EFSA. (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European commission on

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polycyclic aromatic hydrocarbons in food. *Euro Food Saf Auth J.* 2008; 724: 1-114.

2. European Food Safety Authority, EFSA. (2005). Opinion of the scientific committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic. *Euro Food Saf Auth J.* 282:1-31.
3. Odika, I. M. and Okoye, C. O. B. (2018). Polycyclic aromatic hydrocarbons, PAHs contamination levels in Nigeria staple grains. *International Journal of Innovative Science Research Technology*, 3(10): 752-757
4. Escarrone, A. L., Caldas, S. S., Furlong, E. B., Meneghetti, V. L., Fagundes, C. A., Arias, J. L. and Primel, E. G. (2014) Polycyclic Aromatic Hydrocarbons in Rice Grain dried by Different Processes: Evaluation of a Quick, Easy, Cheap, Effective, Rugged and Safe Extraction Method. *Food Chemistry*, 1 (146):597-602.
5. Bertinetti, I. A., Ferreira, C. D., Monks, J. L.F., Sanches Filho, P. J. and Elias, M. C. (2018). Accumulation of Polycyclic Aromatic Hydrocarbons in rice subjected to drying with different fuels plus temperature, industrial processes and cooking. *Journal of Food Composition and Analysis*, 66: 109-115.
6. Akan, J. C., Dawa, J. Y., Bukar, L. I. and Muhammed, Z. (2018). Polycyclic Aromatic Hydrocarbons in different varieties of Rice (*Oryza sativa*) from Yobe State Nigeria. *Environment and Pollution*, 7(2): 21-31.
7. Ifeoma Maryrose Odika, Gloria Chinenye Nwanisobi, Uche Virginia Okpala, Evangelina Chinyere Obi-uchendu and Mediatix Obiageli Odionyenma (2022). Determination of polycyclic aromatic hydrocarbons (PAHs) contamination levels in underutilized grains (guinea corn, pigeon peas and bambara nut) in South East Nigeria. *American Journal of Applied Chemistry*, 10(2): 43-47.
8. Muntean, N., Muntean, E. and Duda, M. (2013) Contamination of some plant origin food products with polycyclic aromatic hydrocarbons. *Bulletin UASMV serie Agriculture*, 70(2): 383-386.
9. Olabemiwo, O. M. (2013). Levels of Polycyclic Aromatic Hydrocarbons in Grilled/Roasted Maize and Plantain Sold in Ogbomoso, Nigeria. *International Journal of Basic & Applied Sciences*, 13(3):87-93.

10. Olabemiwo, O. M., Tella, A. C., Omodara, N. B., Esan A. O. and Alabede Oladapo. (2013). Polycyclic Aromatic Hydrocarbons in Three Local Snacks in Ogbomoso, Nigeria. *American Journal of Food and Nutrition*, 3(2): 90-97.
11. Embbey, K. O., Chukwujindu, M. A., Ajogungbe, E. E. and Godswill O. T. (2015). Polycyclic Aromatic Hydrocarbon and Metal Concentrations in Imported Canned Maize. *Turkish Journal of Agriculture - Food Science and Technology*, 3(1): 53-58.
12. De Lima, R. F. and Reichert Junior, F.W. (2017) Polycyclic aromatic hydrocarbons in corn grains submitted to drying with firewood. *Food Chemistry*, 215: 165-170
13. Al-Rashdan, A., Murad, H., Ahmed, N., Ibtisam, A. and Al-Ballam Z. (2010). Determination of the Levels of Polycyclic Aromatic Hydrocarbons in Toasted Bread using Gas Chromatography Mass Spectrometry. *International Journal of Analytical Chemistry*. 2010 , <http://dx.doi.org/10.1155/2010/821216> Retrieved 2010-6-21
14. Udowelle, N. A., Igweze, Z. N., Asomugha, R. N., Orisakwe, O. E.(2017). Health risk assessment and dietary exposure of polycyclic aromatic hydrocarbons, lead and cadmium from bread consumed in Nigeria. *Roczniki Panstwowego Zakladu Higieny*, 68(3): 269-280. http://wydawnictwa.pzh.gov.pl/roczniki_pzh/
15. Ihedioha, N. J., Okali, E. E., Ekere, N. R. & Ezeofor, C.C. (2019). Risk Assessment of Polycyclic Aromatic Hydrocarbons in Pasta Products Consumed in Nigeria. *Iran J Toxicol*, 13(1):19-26.
16. Ogbonna, I. and K. Nwaocha, (2015). Determination of levels of polycyclic aromatic hydrocarbons on singed cowhide (punmo) and charcoal grilled meat (suya). *Archives of Applied Science Research*, 7(4): 1-6
17. Eze C. Woko, C. O. Ibegbulem, Chinwe S. Alisi (2020). Polycyclic Aromatic Hydrocarbons, Heavy Metals and Derivable Metabolic Water and Energy of Cattle Hides(Kanda) processed by Singeing. Doi: <https://doi.org/10.1101/2020.03.18.994962>
18. Mottier, P., Parisod, V. and Turesky, R. J. (2000). Quantitative Determination of Polycyclic Aromatic Hydrocarbons in Barbecued Meat Sausages by Gas Chromatography Coupled to Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 48(4): 1160–1166.

19. Rozentale, I., Stumpe-Viksna, I., Zac, D., Siksna, S. I., Melngaile, A. & Bartkevics, V. (2015). "Assessment of dietary exposure to polycyclic aromatic hydrocarbons from smoked meat products produced in Latvia" *Food Control*, 54:16-22
20. Pau Lian Peng and Lee Hoon Lim (2022). Polycyclic Aromatic Hydrocarbons Sample Preparation and Analysis: A Review. *Food Analytical Methods*. 15:1042-1061.
21. Iwegbue, C. M., Edeme, J. N., Tesi, G. O., Bassey, F. I., Markincigh, B. S. and Nwajei, G. E. (2014). Polycyclic aromatic hydrocarbon concentrations in commercially available infant formulae in Nigeria: Estimation of dietary intakes and risk assessment. *Food Chem Toxicol*, 72:221-227. doi:10.1016/j.fct.2014.06.026
22. Johnson, Y.S. (2012). Determination of Polycyclic Aromatic Hydrocarbons in Edible Seafood by QuEChERS-Based Extraction and Gas Chromatography-Tandem Mass Spectrometry. *Journal of Food Science*, 77(7): 131–137.
23. Forsberg, N. D., Wilson, G. R. and Anderson, K. A. (2011). Determination of Parent and Substituted Polycyclic Aromatic Hydrocarbons in High-Fat Salmon Using a Modified QuEChERS Extraction, Dispersive SPE and GC–MS. *Journal of Agricultural and Food Chemistry*, 59(15):8108–8116.
24. Ramalhosa, M. J., Paíga, P., Morais, S., Delerue-Matos, C. and Oliveira, M. (2009). Analysis of Polycyclic Aromatic Hydrocarbons in Fish: Evaluation of a Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction Method. *Journal of Separation Science*, 32(20): 3529–3538.
25. Jánská, M., Tomaniová, M., Hajšlová, J. and Kocourek V. (2006). Optimization of the Procedure for the Determination of Polycyclic Aromatic Hydrocarbons and their Derivatives in Fish Tissue: Estimation of Measurements Uncertainty. *Food Additives and Contaminants*, 23(3): 309–325.
26. Hassan, J. and Farahani, A. (2011). GC–MS Determination of PAHs in Fish Samples Following Salting-out-Assisted Solvent Extraction-Gel Permeation Chromatography. *Chromatographia*, 74:477–482.
27. Amos-Tautua, B.M.M., Inengite, A. K., Abasi, C. Y. and Amirize, G. C. (2013). Evaluation of Polycyclic 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.

28. Viegas, O., Novo, P., Pinho, O. and Ferreira, I. M. (2012). A Comparison of the Extraction Procedures and Quantification Methods for the Chromatographic Determination of Polycyclic Aromatic Hydrocarbons in Charcoal Grilled Meat and Fish. *Talanta*, 88: 677–683.
29. Nwaichi Euchari O., Agbam Promise, Iwu, P. I. (2017). Polycyclic Aromatic Hydrocarbons and some Trace Metals in Yam, Cassava, Orange and Papaya from two oil and Gas flaring impacted Communities in Southern Nigeria. *Journal of Applied Sciences and Environmental Management*, 21(16): 1057
30. Pau Lian Peng and Lee Hoon Lim (2022). Polycyclic Aromatic Hydrocarbons Sample Preparation and Analysis: A Review. *Food Analytical Methods*. 15:1042-1061.
31. Camargo, M. C., Antonioli, P. R. and Eduardo, V. (2011). HPLC-FLD Simultaneous Determination of 13 Polycyclic Aromatic Hydrocarbons: Validation of an Analytical Procedure for Soybean Oils *Journal of the Brazilian Chemical Society*. 22(7): 1354-1361.
32. Veyrand, B., Brosseaud, A., Sarcher, L., Varlet, V., Monteau, F., Marchand, P. Andre, F. and Le Bizec, B. (2007). Innovative Method for Determination of 19 Polycyclic Aromatic Hydrocarbons in Food and Oil Samples using Gas Chromatography Coupled to Tandem Mass Spectrometry based on an Isotope Dilution Approach. *Journal of Chromatography*, 1149(2):333–344.
33. Hossain M. A. and Salehuddin, S. M. (2012). Polycyclic aromatic hydrocarbons (PAHs) in edible oils by gas chromatography coupled with mass spectroscopy. *Arabian Journal*, 5(3): 391-396
34. Lee, J.G., Suh, J. H. and Yoon, H. J. (2019). Occurrence and risk characterization of polycyclic aromatic hydrocarbons in edible oils by margin of exposure (MOE) approach. *Appl Biol Chem*, 62: 51(2019). <https://doi.org/10.1186/513765-019-0454-0>
35. Tuteja, G. Rout, C. and Bishnoi, N. (2011). Quantification of polycyclic aromatic hydrocarbons in leafy and underground vegetable. A case study of panipat city, *Haryana Indian J Environ Sci Technol*, 4:611- 620.

36. Wennrich, L., Popp, P & Zeibig, M (2001). Polycyclic aromatic hydrocarbon burden in fruit and vegetables species cultivated in allotments in an industrial area. *Int. J. Environ. Anal. Chem.*, 82: 677-690.
37. Wang, X., Sun, S., Ma, H., and Liu, Y. (2006). Sources and Distribution of Aliphatic and Polyaromatic Hydrocarbons in Sediments of Jiaozhou Bay, Qingdao, China. *Marine Pollutant Bulletin*, 52(2): 129–138.
38. Hillocks, R. J., Bennett, C., Mponda, O. M. (2012) Bambara nut: A Review of utilization, market potential and crop improvement. *African Crop Sci J.* 20: 1-16
39. Aziman Halimi R., Barkla, B. J., Mayes, S. , King, G. J. (2019) The potential of the underutilized pulse bambara groundnut (*Vigna Subterranea*) for nutritional food security. *J Food Compos Anal.* 77: 47-59
40. Mbosso, C. Boulay, B., Padulosi, S., Meldrum, G., Mohamadou, Y., Niang, A. B., et al. (2020) Fonio and Bambara groundnut value chains in Mali: Issues, needs and opportunities for their sustainable promotion. *Sustain.* 12: 4766. 10.3390/su12114766
41. Adu-Gyamfi, Joseph J.; Myaka, Fidelis A.; Sakala, Webster D.; Odgaard, Rie; Vesterager, Jens M.; Jensen, Henning Høgh (2007). "Biological nitrogen fixation and nitrogen and phosphorus budgets in farmer-managed intercrops of maize-pigeon pea in semi-arid southern and eastern Africa". *Plant and Soil.* 295 (1–2): 127–136. doi:10.1007/s11104-007-9270-0.
42. Heuzé V., Thiollot H., Tran G., Delagarde R., Bastianelli D., Lebas F., (2017). Pigeon pea (*Cajanus cajan*) seeds. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. <https://www.feedipedia.org/node/329>
43. Shewny, P. R. (2009). Wheat. *Journal of Experimental Botany*, 60(5): 1537-1553.
44. Wheat Production, Types, Nutrition, Uses and Facts. <https://www.britannica.com>> plant Retrieved 2022-8-17
45. International Agency for Research on cancer, IARC: (2010). Some non-teterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC monograph on Evaluation of carcinogenic risk to human. Vol 92.

46. Hiba-Abdalla, M. (2015). Polycyclic aromatic hydrocarbons in food samples: Methods of extraction- A review. *International Journal of current research*, 7(12): 23603-23606.
47. Kroes, R., Muller, D., Lambe, J., Lowik, M.R.H., Van Klaveren, J., Kleiner, J. et al. (2002). Assessment of intake from the diet. *Food Chem Toxicol.* 40(2-3):327-385.
48. Average body weight of a Nigerian weight of a man and a woman in kg in Nigeria (2020) (<https://nimedhealth.com.ng/average>). Retrieved 2022-5-11
49. Food and Agriculture Organization (FAO), World Health Organization (WHO). Safety Evaluation of certain Food Additive and contaminants. (WHO Food Additive series n. 58).Geneva: FAO/WHO, 2007. P: 209-267.
50. Yunker, M. B., Macdonald, R. W., Vingarzan, R., Mitchell, R. H., Goyette, D., and Sylvestre, S. (2002). PAHs in the Fraser River Basin: A Critical Appraisal of PAH Ratios as Indicators of PAH Source and Composition. *Organic Geochemistry*, 33(4): 489–515.