American Journal of Applied Chemistry
2022; 10(2): 43-47
http://www.sciencepublishinggroup.com/j/ajac
doi: 10.11648/j.ajac.20221002.12
ISSN: 2330-8753 (Print); ISSN: 2330-8745 (Online)

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) Contamination Levels in Underutilized Grains (Guinea Corn, Pigeon Peas and Bambara Nut) in South East Nigeria

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To cite this article:

Ifeoma Maryrose Odika, Gloria Chinenye Nwansiobi, Uche Virginia Okpala, Evangeline Chinyere Obi-Uchendu, Mediatrix Obiageli Odionyenma. 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*. Vol. 10, No. 2, 2022, pp. 43-47.
doi: 10.11648/j.ajac.20221002.12

Received: February 18, 2022; **Accepted**: March 14, 2022; **Published**: April 9, 2022

Abstract: Polycyclic aromatic Hydrocarbons, PAHs are harmful persistent organic pollutants formed by incomplete combustion of organic matter. They are carcinogenic and mutagenic and can occur in foods. These underutilized grains- guinea corn, pigeon peas and bambra nut are commonly consumed in South East Nigeria. This study aimed to quantitatively determine the polycyclic aromatic hydrocarbons contamination levels in these underutilized grains (guinea corn, pigeon peas and bambara nut) commonly consumed in Eastern part of Nigeria. The sixteen PAHs were determined in the grain samples using gas chromatography coupled with flame ionization detector, GC- FID after extraction by sonication. The concentrations ($\times 10^{-2}$ µg/kg) of ∑16 PAHs in the analyzed samples were in the range of 24.07 to 36.49 in red guinea corn and red pigeon peas respectively. The lower molecular weight polycyclic aromatic hydrocarbons, LMW-PAHs total mean concentrations ($\times 10^{-2}$ µg/kg) varied from 6.217±0.442 in red guinea corn to 10.423±6.661 in pure white bambara nut at the percentages of 25.83% and 33.51% respectively. While the high molecular weight polycyclic aromatic hydrocarbon, HMW-PAHs varied from 14.835±7.178 to 27.657±14.190 respectively in white pigeon peas and red pigeon peas at the percentages of 59.34% and 75.79%. The total mean concentration levels ($\times 10^{-2}$ µg/kg) of ∑8 carcinogenic PAHs in samples ranged from 10.913±4.215 in white pigeon peas to 17.444±7.023 in red pigeon peas. The sixteen priority PAHs were detected in all the samples at a very low level, below the maximum allowable limit- 1.0µg/kg established by European Food Safety Authority, EFSA for cereals and cereal-based products. But this can be harmful to human being at long time accumulation in the body. Permissible limit of PAHs in legume grains should also be established.

Keywords: Polycyclic Aromatic Hydrocarbons, Bambara Nut, Underutilized, Food

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are comprised of two or more fused aromatic rings. It has been shown that PAHs are produced by pyrolysis of organic substances and incomplete combustion [1, 2]. Except for smokers, injection of food is the predominant route of exposure of humans to these carcinogens [3, 4]. PAHs occur in foods, not only due to environmental conditions, but can be created by heat treatment such as grilling, smoking, and smoke-drying [4, 5]. Based on toxic potency, benzo(a)anthracene (BaA), chrysene(Chr), benzo(b)fluoranthene(BbF) and benzo(a)pyrene (BaP) are the most important PAHs, and their sum has been defined as PAH4 [4]. Chronic exposure to PAHs can result in skin inflammation, cataracts, asthma-like symptoms and kidney damage. Also, teratogenic, https://cvr.inecnigeria.org/, iand immunotoxic effects of these compounds have been documented [6]. The International Agency for Research on Cancer (IARC) has categorized BaP as a known carcinogen to humans (group 1A). In addition, BaA, Chr, and BbF are listed as possibly carcinogenic to humans (group 2B) [3].

Underutilized crops are those marginalized by farmers and consumers due to agronomic, genetic, economic, environmental and cultural reasons, which were once important and major crop in the community [7]. Underutilized or underused crops are domesticated plant species that have been used for centuries or more for their food, fibre, fodder, oil or medicinal properties, but have been reduced in importance over time. Reductions in use may pertain to, among other things: supply or consumption constraints, poor shelf life, unrecognized nutritional value, poor consumer awareness, and reputational problems (famine food or "poor people's food", sometimes due to the modernization of agricultural practices). Some crops have been so neglected that genetic erosion of their genepools has become so severe that they are often regarded as lost crops [8]. Neglected crops are primarily grown by traditional farmers. These species may be widely distributed beyond their centres of origin but tend to occupy special niches in the local production and consumption systems. They are important for the subsistence of local communities, yet remain poorly documented and neglected by the mainstream research and development activities [7]. Many staple crops, especially in the developing world, are poorly studied by researchers. Some of the underutilized grains in Nigeria are pigeon peas, guinea corn, bambara nut.

Pigeon peas are both a food crop (dried peas, flour, or green vegetable peas) and a forage/cover crop. In combination with cereals, pigeon peas make a well-balanced meal and hence are favoured by nutritionists as an essential ingredient for balanced diets. The dried peas may be sprouted briefly, then cooked, for a flavor different from the green or dried peas. Sprouting also enhances the digestibility of dried pigeon peas via the reduction of indigestible sugars that would otherwise remain in the cooked dried peas [9]. Pigeon peas are in some areas an important crop for green manure, providing up to 90 kg nitrogen per hectare [10]. 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 [11]. Pigeon peas contain high levels of protein and the important amino acids methionine, lysine, and tryptophan [12].

Although it represents a common food staple in semi-arid area of Africa, the Bambara nut remains one of the crops less investigated [13] but one with a great nutritional potential. Actually, the Bambara nut is known for its resistance to drought and the reasonable yield when grown on poor soils. The Bambara nut is the second most important food legume and the third food crop, after maize and groundnut, grown by the small-scale farmers in many African countries. It is also cultivated both as an intercrop with maize, cowpeas and melon and as a sole crop [14]. In accordance with literature data on African samples [15], glutamic (209.5 mg/g crude protein) and aspartic acids (146.1 mg/g crude protein) are the major non-essential amino acids, while leucine (102.1 mg/g crude protein) and lysine (80.2 mg/g crude protein) are the principal essential amino acids, thus indicating a protein quality very similar to that assessed for different legumes [16]. It serves as an important source of protein in the diets of a large percentage of the population in Africa, particularly to poorer people who cannot afford expensive animal protein [13]. Though, grown extensively in Nigeria, it is still one of the lesser utilized and unexploited legume [17].

Guinea corn has been for Centuries, one of the most important staple foods for millions of poor rural people in the semi-arid tropics of Asia and Africa. For some impoverished regions of the world, guinea corn remains a principal source of energy, vitamins and minerals. It grows in harsh environments where other crops do not grow well, just like other staple foods, such as cassava, that are common in impoverished regions of the world. It is usually grown without application of any fertilizers or other inputs by a multitude of small-holder farmers in many countries [11]. Most cultivated varieties of guinea corn can be traced back to Africa where they grow on savanna lands. The seeds and stalks are fed to cattle and poultry. Some varieties have been used for thatch, fencing, baskets, brushes and brooms while stalks have been used as fuel. Sorghum seeds can be popped in the same manner as popcorn (i.e. with oil or hot air), although the popped kernels are smaller than popcorn. Since 2000, sorghum has come into increasing use in homemade and commercial breads and cereals made specifically for the gluten-free diet. In southern Africa, sorghum is used to produce beer, including the local version of Guiness. In recent years, sorghum has been used as a substitute for other grain in gluten-free beer. Since these underutilized grains are consumed by people to some extent there is great need for them to be analyzed to ensure their safety for consumption.

2. Materials and Methods

2.1. Reagents

All reagents and solvents were of analytical grade and were purchased from Sigma Aldrich U S A. These included hexane, dichloromethane, activated alumina as well as four deuterated (surrogate) standard namely acenaphthalene d_{10}, chrysene d_{12}, phenathrene d_{10} and perylene d_{12} 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. Equipment and Instruments

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

2.3. Sampling

Eighteen (18) samples which included different types of bambara nut (pure white and mixed white), pigeon peas (white and red) and guinea corn (red and white) 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, 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.

2.4. 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 [18].

2.5. Determination of PAHs

PAHs concentrations were determined with a gas chromatography equipped with flame ionization detector, GC-FID, (HP 6890). Following recoveries of 94.0 to 99.2%, the grain samples were extracted and PAHs determined by the same procedure [18].

2.6. 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.

3. Results

The two main known types of bambara nut analyzed were pure white and mixed white type. The average total PAH concentrations in pure white bambara nut was 31.103 and that of mixed white was 32.839 with mean concentrations of 1.944±2.162 and 2.052±2.371. The PAH concentrations in mixed bambara nut was a bit higher than that of white.

The total mean concentrations (in $\times 10^{-2}$) of all the probable carcinogenic PAHs- PAH2, PAH4, PAH8 in pure white bambara nut are respectively 8.044±3.659, 13. 659±2.509 and 14.617±2.949 and are higher than their concentrations in mixed white bambara nut which are 7.302±7.102, 11.385±10.249 and 12.506±11.018 respectively. The total mean concentrations (in $\times 10^{-2}$) of the lower molecular weight PAHs (LMW) in pure white and mixed white bambara nut are 10.423±6.661 and 9.940±4.742 respectively with 33.51% and 30.17%. While that of higher molecular weight PAHs, HMW in pure white and mixed white type are respectively 20.679±3.293 and 22.953±14.125 with 66.49% and 69.83%

Table 1. *PAHs Concentration levels ($\times 10^{-2}$µg/kg) in Bambara nut.*

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

The two known types of pigeon peas analyzed were white and red. The average total PAH concentrations in white and red pigeon peas were respectively 24.937 and 36.493 with mean concentrations of 1.559±1.869 and 2.281±3.037. So the PAH concentrations in red pigeon pea analyzed was much higher than white type.

Table 2. *PAHs Concentration levels ($\times 10^{-2}$ µg/kg) in Pigeon Peas.*

	PAHs	White	Red
1	Naphthalene	0.037 ± 0.00	0.025 ± 0.011
2	Acenaphthylene	0.111 ± 0.125	0.051 ± 0.026
3	Acenaphthene	1.324 ± 1.859	0.546 ± 0.531
4	Fluorene	0.158 ± 0.242	0.101 ± 0.146
5	Phenanthrene	4.389 ± 4.668	2.727 ± 2.374
6	Anthracene	4.151 ± 2.573	5.386 ± 2.872
7	Fluoranthene	0.679 ± 0.286	0.878 ± 0.600
8	Pyrene	3.243 ± 4.056	9.335 ± 6.618
9	Benzo[a]anthracene	3.839 ± 2.811	6.74 ± 2.19
10	Chrysene	0.876 ± 0.994	2.339 ± 3.650
11	Benzo[b]fluoranthene	0.404 ± 0.111	0.44 ± 0.035
12	Benzo[k]fluoranthene	0.312 ± 0.209	0.543 ± 0.306
13	Benzo[a]pyrene	5.074 ± 2.372	6.737 ± 0.431
14	Indeno[1,2,3-cd]pyrene	0.042 ± 0.033	0.094 ± 0.129
15	Dibenzo[a,h]anthracene	0.094 ± 0.118	0.238 ± 0.219
16	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

In the analyzed types of pigeon peas, the total mean concentrations ($\times10^{-2}$ µg/kg) of the probable carcinogenic PAHs – PAH2, PAH4 and PAH8 obtained in red type are respectively 9.076±4.042, 16.256±6.260 and 17.444±7.023 which are much higher than their concentrations in white pigeon peas recorded respectively as 5.95±1.381, 10.193±4.191 and 10.913±4.215. The total mean concentration ($\times10^{-2}$ µg/kg) of the lower molecular weight PAHs, LMW and high molecular weight, HMW in red type of pigeon peas are respectively 8.837±4.766 with 24.21% and 27.657±14.190 with 75.79%. While the total mean concentrations of LMW and HMW PAHs in white pigeon peas are 10.169±5.477 with 40.79% and 14.835±7.178 with 59.21% respectively.

Also the two types of guinea corn analyzed were red and white. The average total PAH concentrations in white guinea corn was 28.581 with the mean concentration of 1.786±2.579 and 24.07 in red guinea corn with mean concentration of 2.832±2.205. So the PAH concentrations in white guinea corn were higher than in red type.

The total mean concentrations ($\times10^{-2}$ µg/kg) of the probable carcinogenic PAHs – PAH2, PAH4 and PAH8 obtained in white type of guinea corn were respectively 7.034±0.221, 12.459±1.072 and 13.391±0.657 and are a bit higher than their concentrations in red type which were 6.589±0.442, 11.019±2.374 and 11.685±2.252 respectively. The total concentrations ($\times10^{-2}$ µg/kg) of LMW and HMW PAHs in red type were respectively 6.217±0.442 with 25.89% and 17.837±1.44 with 61.07%. While in white type, the total concentrations of LMW and HMW obtained were 7.786±2.309 with 32.35% and 20.792±2.437 with 72.76% respectively.

Table 3 showed the PAH concentrations in the analyzed guinea corn.

Table 3. *PAHs Concentration levels ($\times10^{-2}$ µg/kg) in Guinea Corn.*

PAHs	Red	White
Naphthalene	0.026 ± 0.001	0.03 ± 0.008
Acenaphthylene	0.036 ± 0.008	0.036 ± 0.008
Acenaphthene	0.193 ± 0.069	0.166 ± 0.066
Fluorene	0.018 ± 0.01	0.027 ± 0.022
Phenanthrene	1.838 ± 1.768	1.891 ± 1.68
Anthracene	4.121 ± 2.142	5.636 ± 3.884
Fluoranthene	0.56 ± 0.159	0.724 ± 0.220
Pyrene	5.592 ± 0.652	6.679 ± 2.874
Benzo[a]anthracene	4.046 ± 2.397	4.915 ± 1.510
Chrysene	0.233 ± 0.051	0.294 ± 0.043
Benzo[b]fluoranthene	0.384 ± 0.120	0.51 ± 0.218
Benzo[k]fluoranthene	0.399 ± 0.127	0.515 ± 0.227
Benzo[a]pyrene	6.356 ± 0.045	6.741 ± 0.264
Indeno[1,2,3-cd]pyrene	0.02 ± 0.005	0.026 ± 0.001
Dibenzo[a,h]anthracene	0.026 ±0.006	0.04 ± 0.023
Benzo[g,h,i]perylene	0.222 ± 0.006	0.351 ± 0.164
∑16 PAHs	24.07±7.56	28.581±11.213
∑LMW PAHs	6.217±0.442	7.78±2.309
∑HMW PAHs	17.837±1.44	20.792±2.437
PAH2	6.59±0.0962	7.034±0.221
PAH4	11.019±2.374	12.459±1.072
PAH8	11.685±2.252	13.391±0.657

4. Discussion

There were limited literature on the studied grains, much work has not been reported on them. The 16 priority PAHs were detected in all the studied grain samples. In Table 1, the ∑LMW-PAHs concentration in pure white bambara nut (10.423×10^{-2} µg/kg) was a bit higher than that of mixed white bambara nut (9.940×10^{-2} µg/kg). While ∑HMW-PAHs concentration levels in mixed white bambara nut (22.953×10^{-2} µg/kg) was higher than that of pure white bambara nut (20.679×10^{-2} µg/kg). The high concentration level of PAHs in bambara may be attributed to the presence of gluten protein. From Table 1, the total concentration level of PAHs in mixed white type of bambara nut (32.893×10^{-2} µg/kg) was a bit higher than the pure white type (31.103×10^{-2} µg/kg) showing the higher content of gluten protein in mixed white bambara nut. In Table 2, The red type of pigeon pea had comparatively high PAHs concentration (36.493×10^{-2} µg/kg) pyrene being the most abundant (9.335×10^{-2} µg/kg). This may also be attributed to high content of gluten protein. The ∑PAH8 in red type (17.444×10^{-2} µg/kg) also recorded highest among all the analyzed grain samples. This implied red pigeon peas being more toxic than others. In Table 3, the total concentration of the 16 PAHs in white type of guinea corn (28.581×10^{-2} µg/kg) was higher than the red type (24.07×10^{-2} µg/kg). Guinea corn had the lowest PAHs concentration among the analyzed grains, this may be attributed to its gluten-free content and small size. High concentration levels of probable carcinogenic (∑PAH8) were detected in red pigeon peas, pure white bambra nut and white guinea corn. Generally all the PAHs concentration levels obtained in this study were in two orders magnitude below the legal permissible limit of 1.0 µg/kg established for cereals and cereal based- product by European Food Safety Authority, EFSA. [19]

5. Conclusion

All the sixteen priority PAHs were detected in the analyzed grain samples and were all below the permissible limit. Although the PAHs were below the safety limit recommended by EFSA for cereal and cereal-based products, these PAHs at certain significant concentration levels can be very dangerous to human health. There is assurance of safety of grains sold in Nigerian markets with respect to PAHs contamination levels. All the environmental substances such as foods, soil, water and air should always be on regular analysis to ensure that they are not contaminated by PAHs beyond permissible limit. The regulatory bodies should establish permissible limits for legume grains.

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