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Quantification of Polycyclic Aromatic Hydrocarbons, Pahs in Grain Legumes from Markets in Anambra and Enugu States of Nigeria

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ABSTRACT: Polycyclic aromatic hydrocarbons are harmful persistent organic pollutants generated by the incomplete combustion of organic matter. They have a high carcinogenic and mutagenic potential since they can interfere with the normal function of DNA. Legumes also known as pulses are important staple foods not only for human food and animal feed but also an important sources of proteins, minerals, lipids, vitamins, starch, sugars and other non-starchpolysaccharides. This study therefore determined the contamination levels of these PAHs in the staple grain legume samples from major markets eastern Nigeria. The samples which included different varieties of beans, soya beans, pigeon peas and bambara nut were purchased, picked and ground into powdered form. The extraction was by sonication and determination of sixteen priority PAHs was carried out using gas chromatography coupled with flame ionization detector, GC-FID. The PAH2 concentration levels in the analyzed samples varied from (5.98 to 8.59) × 10^{-2}µg/kg in soya beans and bambara nut respectively with the percentages of 29.5 and 26.1 of the total PAHs. The PAH4 concentration levels ranged from (9.20 to 14.33) × 10^{-2}µg/kg in soya beans and bambara nut respectively with 45.75% and 43.60% of the total PAHs. While the PAH8 concentrations in the samples ranged from (10.72 to 15.39) × 10^{-2}µg/kg in beans and bambara nut respectively with 41.6% and 46.8% of the total PAHs. So bambara nut has the highest occurrence of B[a]P, PAH2, PAH4 and PAH8. The 16 PAHs were detected in all the samples, diagnostic ratio calculated showed that fuel combustion is the major source of emission. The result of TTEC for cPAHs of the analyzed legumes indicated non-toxicity of the samples. PAHs though detected at a very low concentrations in the samples can at certain significant concentration level be very dangerous to human health. There is great need for setting permissible limits for PAHs in grain legumes as in the case of cereals and cereal products.

KEYWORDS; Polycyclic aromatic hydrocarbons, legumes, concentrations, food.

I INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are aromatic hydrocarbons with two or more fused benzene rings in various structural configurations (Jiang et al.,2014) and do not contain hetero atom or carry substituents. PAHs are harmful persistent organic pollutants generated by the incomplete combustion of organic matter. They have a high carcinogenic and mutagenic potential since they can interfere with the normal function of DNA (Kao et al., 2012).PAHs containing up to four rings are refer to as light PAHs and are called low-molecular weight (LMW) polycyclic aromatic hydrocarbons. They include naphthalene, acenaphthylene, acenaphthene, fluorine, phenathrene and anthracene. While those that contain more than four rings are heavy PAHs and are referred to as high-molecular weight (HMW) PAHs. They include fluoranthene, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene. Heavy PAHs are more stable and more toxic than the light PAHs (Kuppusamy et al.,2016). The HMW PAHs are even more detrimental to the environment and human health. The two primary factors which contribute to the persistence of HMW PAHs in the environment are PAHs molecule stability and hydrophobicity.

PAHs are an environmental concern because they are toxic to aquatic life and some are suspected human carcinogens (Van Metre, 2006). The United States Environmental Protection Agency, US EPA (USEPA,1990) has established 16 PAHs on its list of priority pollutants to be monitored in water and wastes. The most important property driving PAH remediation is toxicity (or carcinogenicity). PAHs ranked 9th on the 2015 Agency of Toxic Substances and Disease Registry. (ATSDR, 2015). Priority list of Hazardous Substances(PLHS) based on their toxicity, frequency of occurrence at USEPA National Priorities list (ie superfund) sites and potential for human exposure. From a review of data collected from the member States, EFSA's CONTAM Panel, in 2008, concluded that benzo[a]pyrene was not a suitable indicator for the occurrence of PAHs in food. The Panel found that PAH4 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) and PAH8 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi] perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) were the most suitable indicators for PAHs in food with PAH8 not providing much added value compared to PAH4.In accordance with these findings, the legislation was updated and separate maximum limits are now set for benzo[a]pyrene and PAH4 (Commission Regulation (EU) No 835/2011).

The known routes of exposure to PAHs in the general population is from breathing ambient indoor air, eating food containing PAHs, smoking cigarettes, or breathing smoke from open fireplaces, from the fossil fuels that we use to drive our cars, cook our food (Zhang et al.,2015), warm our home. PAHs in agricultural crop leaves, contributes to the exposure of organisms to these chemicals through the dietary pathway(Sun et al.,2016). The major route of exposure is consumption of food contaminated by PAHs. Their presence in the environment is reflected in their presence at detectable levels in many types of uncooked food. Studies have revealed detection of PAHs in cassava tuber, oil bean (Nwaichi, et al., 2016), corn\maize, (Odika and Okoye, 2018); rice,(Akan et al.,2018), meat(Ogbonna and Nwaocha2015); (fish, Iwegbue et al. 2015);fruits and vegetables, (Abou-Arab et al., 2014); cereal products (noodles, spaghetti and macaroni) (Ikedioha et al., 2018).

Legumes are grown agriculturally, primarily for human consumption, for livestockforage and silage, and as soil-enhancing green manure. Well-known legumes include alfalfa, clover, beans, peas, chickpeas, lentils, lupins, soybeans, peanuts, and tamarind. They are cultivated for their seeds, which are used for human and animal consumption or for the production of oils for industrial uses. Grain legumes include beans,soyabeans, pigeon peas, bambranut,lentils, lupins, peas,peanuts and clover. Legumes also known as pulses, are important staple foods for human food and animal feed. They have long been a part of the Mediterranean diet, and their health benefits stem from the fact that they are important sources of adequate proportions of different nutrients, such as proteins, minerals, lipids, vitamins, starch, sugars and other non-starch polysaccharides. Legume grains have a multitude of uses, from direct consumption to production of flours that can be used in breads and cakes, substantially increasing their protein content. (Vasconcelos and Gomes, 2016). Some of the most commonly consumed legumes in the eastern part of Nigeria include beans, soya beans, pigeon peas and bambara nut. However a review of studies of PAHs in foods showed limited information on grain legumes. Therefore this study was carried out in order to provide a baseline information on PAHs quantification and to ascertain their contamination levels in these grain legumes commonly consumed in theEastern part of Nigeria.

II MATERIALS AND METHODS

Sample collection and preparation: The grain legume samples were purchased from five major markets in the eastern region of Nigeria. Varieties of each grain were pooled into composite samples as follows: beans (14), soya beans (4), pigeon peas (6) and Bambara nut (6). The total of 30 composite samples were used. The samples were picked to remove sand and other impurities, ground into powder, sieved and put in labeled amber sample bottles ready for extraction.

Equipment and Reagents: All reagents and reagents were of analytical grade and included; hexane, dichloromethane, activated alumina. Four PAHs surrogate standard mixtures-acenaphthalene $d_{10,}$ chrysene $d_{12,}$ phenathrene d_{10} and perylened$_{12}$ .were purchased from Sigma Aldrich U S A.Gas chromatography/flame ionization detector (HP 6890 Powered with HP ChemStation) , rotary evaporator, borosilicate beaker, glass column, sonicator.

Extraction: Recovery experiments to ensure the effectiveness of PAHs 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) which included, acenaphthalene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. Each of the three concentrations (100, 500, 1000) µg/mL was used to spike three 5 gram portion of ground samples before the extraction by sonication using 3:1 dichloromethane – hexane mixture as solvent. The extracts were cleaned-up in alumina column using the same solvent mixture and were concentrated afterwards.

Determination of PAHs: Following recoveries of 94.0 to 99.2%, the grain samples were extracted by the same procedure. PAHs concentrations were determined in each sample using a gas chromatography equipped with flame ionization detector, GC-FID (HP 6890).

Statistical Analysis: The statistical analysis of the data obtained in different samples was carried out using SPSS version 16.00 to calculate analysis of variance and Pearson correlation coefficient at 95% confidence level

Toxic Equivalency Factor (TEF): The Total Toxic Equivalence Factor (TTEF) for each analyzed sample was calculated from the concentrations of cPAHs obtained using the expression:

$(TTEC) = \sum C_n \times TEF_n$

Calculation of PAH Diagnostic Ratios: The sources of the PAHs detected in this study will be calculated using PAH diagnostic ratios of Ant/(Phe+Ant), Fla/(Pyr+Fla), I[cd]P/(I[cd]P+B[ghi]P) and B[a]A/B[a]A +Chr.(Tobiszewski and Namie'snik, 2012). The PAHs involved in each ratio have close molar mass, so it is assumed that they have similar physicochemical properties based on the PAH isomer ratios in the source identification complied by Yunker et al.,(2002)

III. RESULT AND DISCUSSION

Result

Concentrations of PAHs in the Analyzed Legume Grains

Table 1: The Average PAH Concentrations ($\times 10^{-2}$ µg/kg) in the Analyzed Legume Grains

PAH	Beans	Soya beans	Pigeon peas	Bambara nut
Naphthalene	0.027	0.070	0.032	0.025
Acenaphthylene	0.098	0.161	0.081	0.045
Acenaphthene	1.296	0.639	0.935	1.030
Fluorene	0.138	0.137	0.129	0.142
Phenanthrene	4.749	1.970	3.558	3.339
Anthracene	4.349	2.699	4.768	5.523
Fluoranthene	1.082	1.089	0.779	1.084
Pyrene	3.335	2.557	6.289	6.314
Benzo[a]anthracene	2.939	2.642	5.290	5.322
Chrysene	1.750	1.974	1.607	2.584
Benzo[b]fluoranthene	0.522	0.626	0.422	0.419
Benzo[k]fluoranthene	0.422	0.304	0.427	0.471
Benzo[a]pyrene	4.691	3.956	5.906	6.013
Indeno[1,2,3-cd]pyrene	0.068	0.256	0.068	0.086
Dibenzo[a,h]anthracene	0.049	0.099	0.172	0.160
Benzo[g,h,i]perylene	0.260	0.919	0.292	0.344

	Total	25.773	20.101	30.755	32.901
	Average	1.611	1.345	1.922	2.056
Stdev1.792	1.477	2.356	2.420		
∑PAH2		6.441	5.9307.513	8.597	
∑PAH4		9.902	9.198	13.225	14.338
∑PAH8		11.142	10.776	14.184	15.339

TABLE 2: PAH Diagnostic Ratios Analysis

SAMPLE	Ant/Ant+Phe	Fla/Fla+Pyr	I[cd]P/I[cd]P+ B[ghi]P	B[a]A/B[a]A+ Chr
Beans	0.536	0.245	0.207	0.627
Soya beans	0.628	0.21	0.201	0.684
Pigeon peas	0.573	0.11	0.189	0.767
Bambara nut	0.623	0.172	0.2	0.673
Total	2.360	0.737	0.797	2.751
Average	0.590	0.184	0.199	0.688

The result of recovery experiment ranging from 94.0 to 99.2 % showed high efficiency of the extraction method. The detection and quantification of limit (LOD and LOQ) were (0.03 to 0.09) $\times 10^{-3}$ µg/L and (0.09 to 0.25) $\times 10^{-3}$ µg/L respectively also showed high efficiency of the determination procedure.

The 16 PAHs were detected in all the samples. The total LMW- PAHs concentration ($\times 10^{-2}$µg/kg) levels in the analyzed grain legume samples ranges from 5.68 to 10.66 respectively in soya beans and beans with 28.24 to 41.35% of the total PAHs. The total HMW-PAHs concentration ($\times 10^{-2}$µg/kg) levels in the analyzed samples ranged from 14.35 to 22.80 in soya beans and bambara nut respectively with 71.75% and 69.29%. The EFSA established that the better indicators for ifying the effects of PAHs in food rather than B[a]P are PAH2, PAH4 and PAH8. The B[a]Pconcentration ($\times 10^{-2}$µg/kg) detected in analyzed samples varied from 3.956 to 6.013 in soya beans and bambara nut respectively. The PAH2 concentration levels in the analyzed samples varied from (5.98 to 8.59) $\times 10^{-2}$µg/kg in soya beans and bambara nut respectively with the percentages of 29.5 and 26.1 of the total PAHs. The PAH4 concentration levels ranged from (9.20 to 14.33) $\times 10^{-2}$µg/kg in soya beans and bambara nut respectively with 45.75% and 43.60% of the total PAHs. While the PAH8 concentrations in the samples ranged from (10.72 to 15.39) $\times 10^{-2}$µg/kg in beans and bambara nut respectively with 41.6% and 46.8% of the total PAHs. So bambara nut has the highest occurrence of B[a]P, PAH2, PAH4 and PAH8. The PAH2, PAH4 and PAH8 respectively constituted 24.4% to 29.5%, 38.5% to 45.8% and 41.6% to 53.6% of the sixteen PAHs

Table 2 showed diagnostic ratios for eight PAHs. Ant/Ant+Phe ratio ranged from 0.538 to 0.628 being > 0.1 indicates combustion emission source. Fla/ Fla +Pyr ratio showed the range of 0.110 to 0.245 being < 0.4 indicating petrogenic emission source. While B[a]A /B[a]A + Chr varied from 0.627 to 0.765 being > 0.35 showed fuel combustion source.

Then for I[c,d]P/I[c,d]P + B[g,h,i]P ratio varied from 0.189 to 0.207, being <0.2 and lying between 0.2 – 0.5 showed both petrogenic and fuel combustion sources.The total equivalence concentrations for cPAH of the analyzed grain legume ranged from (4.9-6.69) × 10-2 mg/kg which are five orders of magnitude lower in comparison to method B clean-up level of the chemical B[a]P given as 0.137 mg/kg.

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Discussion

Literature has not reported permissible limit set for PAHs in grain legumes, also much work have not been reported on grain legumes. However the average concentrations of the PAHs determined these analyzed grain legumes were below the permissible limit (1.0 µg/kg) established by EFSA for cereals and cereal products. The PAHs concentrations in bambara nut was highest among the analyzed samplesshowing high content of gluten protein. (Mohammed el al., 2012). Thus the PAHs concentration increases with gluten content.The average total PAHs concentrations (in 10^{-2} µg/kg) detected the studied samples were respectively 25.773, 20.101, 30.755 and 32.901.

The mean total concentrations of 16 PAHs detected in beans (0.026 µg/kg) in this study was 0.6 µg/kg higher than that obtained by Baldyga et al. (2005) in beans (0.02 µg/kg). The average total concentrations of PAHs obtained in soyabeans(0.02 µg/kg) was much lower than that detectedby Garcia et al. (2012) in whole beans which ranged 0.8 to 38.7 µg/kg. A study by Rojo Camargo et al. (2012) revealed high concentrations of 16 PAHs in soybean oil, the authors reported that the total PAHs in crude soybean oil varied from 10 to 316 µg/kg and that of deodorized soybeans oilranged from 3 to 69 µg/kg. This is comparable very high to the PAHs concentrations obtained in this study. Another study by Hossain et al. (2012) on soybeans oil, analyzing four PAHs (naphthalene, anthracene, benzo[a]pyrene and benzo[a]anthracene) detected total 4-PAH concentrations of 0.297 µg/kgwhich was a bit higher compared to the total concentration of the same four PAHs(0.094 µg/kg) obtained in this study.

Analysis of variance showed that $p>0.05$ indicating no significant difference between the PAHs concentration of the analyzed grain legumes. The Pearson correlation coefficient studied indicated strong positive correlation of all the analyzed legumes. From the diagnostic ratio in source identification, Ant/Ant + Phe ratio being >0.1 indicated fuel combustion source.Fla/ Fla +Pyr ratio obtained was < 0.4 showing petrogenic emission source. B[a]A/B[a]A + Chr for all the samples were > 0.35 also indicating fuel combustion source. I[c,d]P/I[c,d]P + B[g,h,i]P ratio lies between 0.2 and 0.5 indicating fuel combustion source. So the primary emission source of PAHs in the analyzed grain legumes was fuel combustion. The result from the TTEC for cPAHs compared very low with that of reference chemical, B[a]P showing non- toxicity of the analyzed grain legumes with respect to PAHs.

IV.CONCLUSION

The sixteen priority PAHs were detected in the analyzed grain legume samples at a very low concentrations. Dignostic ratio showed that most PAHs get into food items mainly through fuel combustion during transportation. Human beings can become exposed to these PAHs by ingesting contaminated foods, inhaling contaminated air or dermal contact with contaminated soil. Although very low concentrations of PAHs was detected in the samples, these PAHs at certain significant concentration level can be very dangerous to human health. There is great need for setting permissible limits for PAHs in grain legumes as in the case of cereals and cereal products. This study has provided base values for future monitoring of contamination values of the grain legumes in Nigeria. There should be continuous demand for analytical monitoring and increased research efforts concerning food contaminants to ensure adequate protection of human health.

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