Polycyclic Aromatic Hydrocarbon Levels in selected Soup Thickeners Marketed in Yenagoa, Bayelsa State, Nigeria

ABSTRACT

This study investigated the polycyclic aromatic hydrocarbons (PAHs) concentration in selected soup thickeners marketed in Yenagoa, Bayelsa State. Three commonly consumed soup thickeners (*ukpo, ofor* and *achi*) were purchased from Swali market in Yenagoa Bayelsa State and taken to the laboratory for analysis. Sample extraction was done using dichloromethane and PAHs concentrations were determined by Gas Chromatography-Flame Ionization Detection (GC-FID). Data was subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS)and significant differences were determined ($p<0.05$). PAHs detected in the samples in varying concentrations include naphthalene, acenapthylene, fluorene, phenanthrene, pyrene, chrysene, anthracene and benzopyrene. The results were as follows: *ukpo*- (naphthalene-12.69±0.24 mg/kg, acenapthylene 8.17±0.01 mg/kg, fluorene-3.55±0.18 mg/kg, phenanthrene-6.10±0.08 mg/kg, pyrene-2.45±0.11 mg/kg, chrysene-4.27±0.13 mg/kg), *ofor*-(acenapthylene-22.21±0.29 mg/kg, fluorene-18.60±0.32 mg/kg, phenanthrene-14.11±0.03 mg/kg, pyrene-17.26±0.16 mg/kg, chrysene-13.85±0.17 mg/kg) and *achi*-(naphthalene-15.47±0.00 mg/kg, acenapthylene-28.07±0.00 mg/kg, pyrene-19.79±0.00 mg/kg, chrysene-22.06±0.00 mg/kg, anthracene-23.48±0.00 mg/kg, benzopyrene-11.86mg/kg). The findings of this study indicates that soup thickeners marketed in Yenagoa Bayelsa State contained varying concentrations of PAHs. Consumers are therefore advised to consume these soup thickeners with caution due to the toxic effects of PAHs to the body.

Keywords: thickeners, *ukpo, achi, ofor,* toxic, PAHs

INTRODUCTION

Food plays a very vital role in the maintenance of the health of individuals in a population. Every tribe or region in Nigeria has different condiments used in preparation of soups and other dishes. These condiments are used to enhance flavour, provides proteins and vitamins and other organoleptic properties which makes the food appealing and delicious [1]. Soup condiments are used for garnishing soups however they may be rich in protein, can replace fish or meat and aid in dietary strategies to combat obesity due to their high fiber, low carbohydrate, and fat content and may additionally offer health benefits such as boosting brain power, protecting the heart, and preventing cancer [2]. Thus, soup condiments play a significant role in nutrition in Nigeria. *Brachystegia eurycoma,* is a tree species native to Southern Nigeria and Western Cameroon. It typically grows up to 35 meters in height with a diameter of 2.5 meters [3]. The flour prepared from the dry seeds of *B. eurycoma* is called *Achi* in Igbo, *Ekalado* or *Eku* in Yoruba, *Okweri* in Edo, *Akpakpa* or *Taura* in Hausa, *Apaupan* in Ijaw, and *Odukpa* in Ibibio [4]. *Achi* is a good source of carbohydrate and fiber, is used as flavoring and thickening agents for soups in Eastern Nigeria [5]. The seeds are used in folkloric medicine to maintain body temperature, soften stool, and protect against colon and rectal cancer [5]. *Achi* also contains a wide range of bioactive compounds including flavonoids, phenolic compounds, alkaloids, saponins, and tannins [6] as well as nutritive components such as carbohydrate, proteins, lipids, and minerals [7]. Other than its nutritional value, different parts of the plant have been demonstrated to possess biologic/pharmacologic activities, namely, analgesic, anti-inflammatory, anti-microbial, wound healing, anti-oxidant, anti-cancer, and blood glucose lowering activities as well as lipid profile and liver enzyme modulation activities [8]. *Detarium microcarpum* is tree belonging to the Fabaceae family and commonly found in Africa [9,10]. *Detarium microcarpum* grows in dry regions of central and western Africa, where it can reach heights of up to 15-25 m [11]. *Detarium microcarpum* root, stems, leaves and seeds have been extensively used in treatment of diseases/illness such as itches, diarrhoea, meningitis, tuberculosis, diabetes mellitus and haemorrhoids [12] while the fruit and leaves are widely consumed as a vegetable [10]. In some African countries the flour made from the dry seeds of *D. microcarpum* (called *ofor* in Southern Nigeria) is traditionally used as an emulsifying and thickening agent in soups due to its high carbohydrate content [13]. The seed is also rich in nutrients such as carbohydrate, crude protein, crude fibre, moisture and ash as well as essential amino acids (e. g. lysine, cystine, valine, methionine, isoleucine, leucine, phenylalanine and threonine), non-essential amino acids, beta-carotene, phytosterols, phospholipid and glycolipids, linoleic acid and several phytochemicals [14]. *Mucuna sloanei* is a climbing shrub with twining stem that can grow up to a height of 8 m [15]. A special type of soup thickener is prepared by grinding the seed of *M. sloanei* and is called *ukpo* or *okobo* in Igbo, *karasuu*" in Hausa, "*yerepe"* in Yoruba and "*ibabat"* in Efik [16]. In South Eastern Nigeria, *Ukpo* is used in making of soups, [17]. It is also used to improve the texture of various foods thereby increasing their quality [18]. *Ukpo* is rich in protein, crude fibre, carbohydrate, crude fat [16] as well as high concentrations of lysine, methionine, phosphorus and serves as a good source of dopamine, an important neurotransmitter [15]. The rich nutritional composition of *Ukpo* (especially protein)makes it very good in improving the nutritional status of the population especially in combating protein-energy malnutrition (PEM); it is often used to supplement soups prepared from cocoyam and melon [19]. *Ukpo* also has functional properties and pharmaceutical properties; excessive consumption is also known to cause toxicity [20].

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds composed of two or more fused aromatic rings and often referred to as either light (2–3 rings) or heavy (4–6 rings) based on the number of aromatic rings they contain [21]. PAHs can originate from natural sources like forest fires and volcanic emissions, as well as from human activities such as coal burning, vehicle exhaust, engine oils, and cigarette smoke [22]. PAHs are known to have toxic effects on humans when ingested especially cancer [23]. Exposure of humans to PAHs occur through various sources, including diet, inhalation, and skin contact however, diet remains the primary route of exposure accounting for over 70% of PAHs exposure [23]. PAHs can adversely affect human health, primarily through carcinogenic and mutagenic effects, as well as immunosuppressive effects [24]. Due to the significant health impacts of PAHs, it is important to monitor PAHs in local food condiments so as to mitigate likely risks that they may pose.

Due to the important nature of sou thickeners in the diet of Nigerians and the poisonous nature of PAHs which may enter these condiments during preparation, transport or adulteration, this study was conducted to determine the PAHs in *Achi*, *Ofor* and *Ukpo.*

MATERIALS AND METHODS

Collection and preparation of samples

Samples of *ukpo, ofor* and *achi* were purchased from different stalls at Swali market in Yenagoa Bayelsa State and mixed properly to produce a composite. The samples were then taken to the laboratory for analysis.

Determination of polycyclic aromatic hydrocarbons (PAHs)

Sample Extraction

Ten (10) g of sample was added into an amber glass bottle. 10 g Anhydrous sodium sulphate (Na_2SO_4) was also added into the glass bottle containing the sample. The sample was stirred. The addition of Na_2SO_4 was to remove moisture from the sample. 300 µg/ml of surrogate (1-chlorooctadecane) standard was added to the sample. 30 mL of dichloromethane (DCM) was added to the sample as extracting solvent and the bottle containing the sample was corked very tight and transferred to a mechanical shaker. The sample was agitated between 5 to 6 h at room temperature using a mechanical shaker. After agitation, the sample was allowed to settle for 1 h and then filtered through 110 mm filter paper into a clean beaker. The filtrate was allowed to concentrate to 1 mL by evaporation overnight in a fume cupboard.

Sample clean-up

Sample clean-up was performed using glass column. Column preparation was carried out by inserting glass cotton into the column. 10 g Silica gel was dissolved with 50 mL DCM to form slurry, and the slurry was added into the column. 10 g Anhydrous Na_2SO_4 was added into the column followed by addition of pentane. After preparation of the column, the concentrated sample extract was mixed with 20 mL cyclohexane in a beaker and transferred into prepared column. The sample extract was eluted using 30 mL pentane as solvent and eluted sample collected in a beaker below the column. The sample was eluted further by adding more 20 mL pentane into the column. After elution the column was rinsed with 20 mL DCM. The eluted sample was allowed to stand overnight at room temperature in a fume cupboard for evaporation to take place.

Sample Separation and Detection using Gas Chromatograph - Flame Ionization Detector (GC-FID)

The separation and detection of compounds samples were carried out using Agilent 6890N Gas Chromatograph - Flame Ionization Detector (GC-FID) instrument. 3 µl of concentrated sample eluted from column was injected into GC vial. The blank DCM was injected into micro-syringe of GC to clean the syringe (3 times) before taking the sample for analysis. The micro-syringe was further rinsed with the sample. Then the sample was injected into the column for separation of compounds in the sample. After separation the compounds were passed through a flame ionization detector. FID detects the compounds in the sample. The amount of PAH was resolved at a particular chromatogram in mg/kg for each sample.

RESULTS AND DISCUSSION

The results of the study shows that PAHs detected in *Achi* (*Brachystegia eurycoma*) were as follows: Naphthalene, acenapthylene, anthracene and benzo(a)pyrene, pyrene, chrysene (Table 1 and Fig. 1) while fluorene and phenanthrene were not detected. In *Ofor* (*Detarium microcarpum*) PAHs detected were acenapthylene, fluorene, phenanthrene, pyrene and chrysene (Table 2 and fig. 2) while naphthalene, anthracene and benzo(a)pyrene were not detected. In *ukpo* (*Mucuna sloanei*) PAHs detected were naphthalene, acenapthylene, pyrene, chrysene, anthracene and benzo(a)pyrene (Table 3 and Fig. 3) while fluorene and phenanthrene were not detected. PAHs are known toxicants which may contaminate food and cause harm to consumers. Acute effects of PAHs in humans causes symptoms such as inflammation, eye irritation, nausea, skin reactions and vomiting while chronic effects include cancers (especially relating to alimentary canal, lung, skin and bladder), kidney damage, cataracts, liver damage, mutations, cellular damage and heart-related issues [24]. Amongst the PAHs detected in the samples studied, benzo(a)pyrene is highly lipid soluble and easily bioaccumulates in tissues and organs especially small intestine and liver [25,26]. In a study by Pandey *et al.*, [27], PAHs were detected in significant concentration in vegetable oils marketed in India especially benzo(a)pyrene and chrysene. Phenanthrene and benzopyrene have also been reported to be present in linseed and safflower oils with concentrations up to 234mg/kg [28]. Processing methods have also been reported to lead to significant reduction in PAHs concentration in olive oil [29]. In a similar study by Martorell *et al.* [30], average PAHs concentration of up to 38.99mg/kg were detected in meat and meat products in Spain while beef, pork, poultry and lamb samples from China had PAHs concentrations of up to 34.4 mg/kg [31]. Other studies have shown that several condiments (such as beef, fish, bacon ham) used in food preparation contain varying concentrations of PAHs (especially BaA and chrysene) in levels which exceed limits set by regulatory bodies [32,33,34]. Also, Tongo *et al.* [35] evaluated PAHs concentration in different smoked fish in the southern part of Nigeria and reported that *Clarias gariepinus*, *Ethmalosa fimbriata*, *Tilapia zilli*, and *Scomber scombrus* contained significant levels of PAHs. PAHs have also been reported in leafy vegetables. Different researchers have reported PAHs in leafy vegetables from different countries such as China, Ghana, South Korea [36.37]. Commonly detected PAHs include acenaphthylene, acenaphthene, BaA, BaF, and BaP, Chr and pyrene, naphthalene. These findings are consistent with the findings of this current study. PAHs have been detected in herbs and spices such as thyme, basil, black pepper and nutmeg [38].

Although there’s no literature on the occurrence and concentration of PAHs in soup thickeners in Nigeria, this study has shown that PAHs are found in these important traditional soup condiments.

Table 1: Polycyclic Aromatic Hydrocarbons concentration (mg/kg) in *Achi (Brachystegia eurycoma)*

PAHs	Concentration (mg/kg)

Naphthalene	15.47±0.00
Acenapthylene	28.07±0.00
Fluorene	ND
Phenanthrene	ND
Pyrene	19.79±0.00
Chrysene	22.06±0.00
Anthracene	23.48±0.00
Benzopyrene	11.86

Values are presented as mean ± standard deviation (n=2); ND- Not Detected

Fig 1: GC-FID chromatogram of *Achi* (*Brachystegia eurycoma*).

Table 2: Polycyclic Aromatic Hydrocarbons concentration (mg/kg) in *Ofor (Detarium microcarpum)*

PAHs	Concentration (mg/kg)
Naphthalene	ND
Acenapthylene	22.21±0.29
Fluorene	18.60±0.32

Phenanthrene	14.11±0.03
Pyrene	17.26±0.16
Chrysene	13.85±0.17
Anthracene	ND
Benzopyrene	ND

Values are presented as mean ± standard deviation (n=2); ND- Not Detected

Fig 2: GC-FID chromatogram of *Ofor* (*Detarium microcarpum*).

Table 3: Polycyclic Aromatic Hydrocarbons concentration (mg/kg) in *Ukpo (Mucuna sloanei)*

PAHs	Concentration(mg/kg)
Naphthalene	12.69±0.24
Acenapthylene	8.17±0.01
Fluorene	3.55±0.18

Phenanthrene	6.10±0.08
Pyrene	2.45±0.11
Chrysene	4.27±0.13
Anthracene	ND
Benzopyrene	ND

Values are presented as mean ± standard deviation (n=2); ND- Not Detected

Fig 3: GC-FID Chromatogram of *Ukpo* (*Mucuna sloanei*)

CONCLUSION

Soups form an integral component of the food of most Nigerians. These soups are prepared using a wide range of ingredients including soup thickeners such as *achi, ofor and ukpo.* Findings of this study on *achi, ofor* and *ukpo* marketed within Yenagoa, Bayelsa State indicate that they contain various PAHs. PAHs are known to be harmful to humans some of which are carcinogenic. The general public are therefore advised to consume these soup thickeners in moderation as their PAHs could predispose them to long-term harmful effects.

References

1. Zubairu, L. H., Chima, M. P., Daniel, O. O., & Yahaya, M. (2021). Amino Acid Profile and Vitamin C Content of Selected Condiments Used as Thickeners in Soup Preparation. *Asian Food Science Journal*, *20*(9), 21-28.
2. Chukwu, M. N., Nwakodo, C. S., Alozie, Q., & Ndulaka, J. C. (2018). Comparative studies in organoleptic properties of Ogiri-Ahukere and Ogiri- Egusi condiments. *Research Journal of Food Science Quality Control*, 4(1), 11-19
3. Sainge, M. N., Nchu, F., & Peterson, A. T. (2020). Diversity, above-ground biomass, and vegetation patterns in a tropical dry forest in Kimbi-Fungom National Park, Cameroon. *Heliyon*, *6*(1).
4. Bafor, E. E., Chiekwe, O., Ofeimun, J., Amaechina, F., & Ayinde, B. (2017). *Brachystegia eurycoma* harms (Fabaceae) stem bark extract modulates gastrointestinal motility in animal models. *African Journal of Biomedical Research*, *20*(3), 309-316.
5. Okoli, R. I., Turay, A. A., & Mensah, J. K. (2015). The phytochemical analysis and antibacterial effects of stem bark extracts of *Brachystegia eurycoma* harms. *International Journal of Herbs and Pharmacological Research*, *4*(2), 10-16.
6. Sunday, E. A., Ansari, R. A., & Ogbonnaya, E. (2021). Phytochemical screening and GC-MS studies of some soup thickeners in southern Nigeria. *Journal of Pharmacognosy and Phytochemistry*, *10*(1), 1786-1790.
7. Ududua, U., Monanu, M. O., & Chuku, L. C. (2019). Proximate analysis and phytochemical profile of *Brachystegia eurycoma* leaves. *Asian Journal of Research in Biochemistry*, *4*(2), 1-11.
8. Igbe, I., & Okhuarobo, A. (2018). Expanding the insights into the usefulness of Brachystegia eurycoma Harms: A review of its nutritional and medicinal values. *Journal of Intercultural Ethnopharmacology*, *7*(1), 82-94.
9. Gaisberger, H., Kindt, R., Loo, J., Schmidt, M., Bognounou, F., Da, S. S., et al. (2017). Spatially explicit multi-threat assessment of food tree species in Burkina Faso: A fine-scale approach. *PLoS One*, *12*(9), e0184457
10. Aviara, N. A., Onaji, M. E., & Lawal, A. A. (2015). Moisture-dependent physical properties of *Detarium microcarpum* seeds. *Journal of Biosystems Engineering*, *40*(3), 212-223.

11. Burlando, B., Palmero, S., & Cornara, L. (2019). Nutritional and medicinal properties of underexploited legume trees from West Africa. *Critical reviews in food science and nutrition*, *59*(sup1), S178-S188
12. Kagambega, W., Meda, R. N. T., Koama, B. K., Drabo, A. F., Belem, H., Dabire, D., et al., (2021). Polyphenols quantification and antioxidant activity of methanolic and aqueous extracts from eight medicinal plants used to manage avian diseases in Burkina Faso. *Journal of Medicinal Plants Research, 15*(5), 226-231.
13. Adedeji, A. A., Alakali, J., Adewale, P. O., & Ngadi, M. O. (2012). Thermophysical properties of *Detarium microcarpum* seed flour. *LWT*, *47*(2), 233-237.
14. Mariod, A. A., Tahir, H. E., & Komla, M. G. (2019). *Detarium microcarpum*: chemical composition, bioactivities and uses. *Wild Fruits: Composition, Nutritional Value and Products*, 207-217.
15. Nwosu, J. (2011). The effect of storage condition on the rheological/functional properties of Soup thickener *Mucuna sloanei* (Ukpo). *Researcher*, 3, 6-10.
16. Ide, P. E., Eze, P. C., & Eze, C. N. (2019). Comparative studies on the proximate composition and functional properties of *Mucuna sloanei* bean flour varieties. *Journal of Asian Scientific Research, 9*(11), 185-192.
17. Eze, P. C. & and Eze, C. N. (2017). Determination of some physical and mechanical properties of horse eye-bean (*Mucuna sloanei*) from South East Nigeria. *Journal of Experimental Research*, 5, 33-41.
18. Bello, F., & Udo, V. (2017). Effect of Fermentation on the Nutritional, Anti-Nutritional and Functional Properties of Horse Eye Beans (*Mucuna urens*) Flour. *Current Journal of Applied Science and Technology*, *24*(3), 1-7.
19. Owuamanam, C., Obeleagu, S., Ogueke, C., Iwouno, J., Nwakaudu, A. & Nwachukwu, I. (2016). Functional properties of seed flours of *Detarium microcarpum* and *Mucuna sloanei* as affected by sodium chloride and palm oil: A response surface methodology approach," *FUTO Journal of Serology*, 2, 361-378.
20. Okaka, J. C., Enoch, N. T. & Okaka, A. N. (2006). Food and human nutrition. OCJ Academic Public. Enugu, Nigeria.

21. Domingo, J. L., & Nadal, M. (2015). Human dietary exposure to polycyclic aromatic hydrocarbons: A review of the scientific literature. *Food Chemistry & Toxicology, 86*, 144–153.
22. Amirdivani, S., Khorshidian, N., Ghobadi-Dana, M., Mohammadi, R., Mortazavian, A. M., Quiterio, S. L., Barbosa, R. H., & Raices, R. (2019). Polycyclic aromatic hydrocarbons in milk and dairy products. *International Journal of Dairy Technology, 72*
23. Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine, 5*, S1691–S2221.
24. Sampaio, G. R., Guizellini, G. M., da Silva, S. A., de Almeida, A. P., Pinaffi-Langley, A. C. C., Rogero, M. M., de Camargo, A. C., & Torres, E. A. F. S. (2021). Polycyclic Aromatic Hydrocarbons in Foods: Biological Effects, Legislation, Occurrence, Analytical Methods, and Strategies to Reduce Their Formation. *International Journal of Molecular Science, 22*, 6010.
25. Ifegwu, O. C., & Anyakora, C. (2015). Polycyclic aromatic hydrocarbons: Part I. Exposure. In Advances in Clinical Chemistry, 1st ed.; Elsevier: Amsterdam, The Netherlands, 277–304.
26. Katona, B.W., Lynch, J. P. (2018). Mechanisms of gastrointestinal malignancies. In Physiology of the Gastrointestinal Tract; Elsevier: Amsterdam, The Netherlands, 1615–1642.
27. Pandey, M. K., Mishra, K. K., Khanna, S. K., & Das, M. (2004). Detection of polycyclic aromatic hydrocarbons in commonly consumed edible oils and their likely intake in the Indian population. *Journal of American Oil & Chemicals Society,* 81, 1131–1136.
28. Ciecierska, M., & Obiedzinski, M. W. (2013). Polycyclic aromatic hydrocarbons in vegetable oils from unconventional sources. *Food Control,* 30, 556–562
29. Kiralan, S. S., Toptanc, I. & Tekin, A. (2019). Further evidence on the removal of polycyclic aromatic hydrocarbons (PAHs) during refining of olive pomace oil. *European Journal of Lipid Science & Technology*, 121, 1800381
30. Martorell, I., Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M., & Domingo, J. L. (2010). Polycyclic aromatic hydrocarbons (PAH) in foods and estimated PAH intake by the population of Catalonia, Spain: temporal trend. *Environment International*, *36*(5), 424-432.

31. Yu, Y., Wang, X., Wang, B., Tao, S., Liu, W., Wang, X., Cao, J., Li, B., Lu, X., Wong, M. H. (2011). Polycyclic aromatic hydrocarbon residues in human milk, placenta, and umbilical cord blood in Beijing, China. *Environmental Science & Technology*, 45, 10235–10242.
32. Roseiro, L. C., Gomes, A., & Santos, C. (2011). Influence of processing in the prevalence of polycyclic aromatic hydrocarbons in a Portuguese traditional meat product. *Food Chemistry & Toxicology,* 49, 1340–1345.
33. Wretling, S., Eriksson, A., Eskhult, G. A., & Larsson, B. (2010). Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. *Journal of Food Composition Analysis*, 23, 264–272.
34. Ledesma, E., Rendueles, M., & Díaz, M. (2015). Spanish smoked meat products: Benzo(a)pyrene (BaP) contamination and moisture. *Journal of Food Composition Analysis*, 37, 87–94.
35. Tongo, I., Ogbeide, O., & Ezemonye, L. (2017). Human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) in smoked fish species from markets in Southern Nigeria. Toxicology Reports, 4, 55–61.
36. Jia, J., Bi, C., Zhang, J., Jin, X., & Chen, Z. (2018). Characterization of polycyclic aromatic hydrocarbons (PAHs) in vegetables near industrial areas of Shanghai, China: Sources, exposure, and cancer risk. *Environmental Pollution*, 241, 750–758.
37. Mohammed, S., Obiri, S., Ansa-Asare, O. D., Dartey, G., Kuddy, R. & Appiah, S. (2019). Assessment of concentration of polycyclic aromatic hydrocarbons (PAHs) in vegetables from farms in Accra, Ghana. Environmental Monitoring & Assessment, 191, 417.
38. Rozentale, I., Yan Lun, A., Zacs, D., & Bartkevics, V. (2018). The occurrence of polycyclic aromatic hydrocarbons in dried herbs and spices. *Food Control,* 83, 45–53.