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Determination of PAHs in Fresh and Smoke-dried Alligator Pepper

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ABSTRACT

Smoking as a way of preservation could be a source of PAHs in food. This study was aimed at determining poly-aromatic hydrocarbons (PAHs) in fresh and smoke-dried samples of alligator pepper (*Aframoum melegueta*). Fresh and smoke-dried samples of the pepper were bought from Swali Market, Bayelsa State, Nigeria. Seeds were mechanically homogenized in a stainless steel blender. 1 g of the sample was vortexed-mixed for about 5 minutes with a mixture of water, methanol, and chloroform and the mixture finally centrifuged and the chloroform layer collected for analysis with HPLC-FLD. The results showed the absence of any PAH in the fresh alligator pepper while six PAHs (benzo [a] anthracene, chrysene, benzo [b] fluorene, benzo [k] fluorene, dibenzo [a,h] anthracene, and benzo [g,h,i] perylene) were detected in the smoked dried alligator pepper but they were all found to be present at concentrations below quantification levels, suggesting that smoke-dried alligator pepper used for various purposes is free from PAHs and therefore do not pose any health risk in respect of PAHs.

Keywords : Alligator, pepper, PAHs, HPLC, FLD

I. INTRODUCTION

Alligator pepper (*Aframomum melegueta)* is a West African spice which corresponds to the seeds and seed pods of *Aframomumdanielli*, *A. citratum* or *A. exscapum* [1]. The plants which produce alligator pepper are herbaceous perennials of the ginger (*Zingiberaceae*) family of flowering plants, native to swampy habitats along the West African coast.

Alligator pepper is widely used by many people of different cultures in Nigeria for various purposes. It is served along with Kola nuts to guests for entertainment, used for religious rites by diviners for invoking spirits.

It is a common ingredient in pepper soup, a spicy delicacy in most parts of West Africa. Concoctions made of alligator pepper are often used by traditional doctors as medications for various ailments. The seeds of alligator pepper are used by some people belonging to the Yoruba tribe (South-West, Nigeria) to treat wounds, prevent infections, improve the state of drunkenness, and improve the state of indigestion.

However, this seed has been evaluated for its medical values. Sonibare [2] evaluated the antimicrobial, phytochemical properties of *Aframomum melegueta* and reported positive results for these medical conditions. Lawal [3] reported the potent effect on the blood pressure of normotensive and hypertensive patients.

Polycyclic aromatic hydrocarbons (PAH) constitute a class of diverse organic compounds, each of them containing two or more aromatic rings. Some of these compounds are known or suspected to be mutagenic and/or carcinogenic in mammals [4]. PAH containing up to four fused benzene rings are known as light PAH and those containing more than four benzene rings are called heavy PAH. Heavy PAH are more stable and considered to be more toxic than the light ones [4]. Polycyclic aromatic hydrocarbons emanate mainly from incomplete combustion and pyrolysis processes of organic compounds at high temperatures. However, aromatization can occur at lower temperatures ranging from 100 to 150 °C [5]. PAH compounds are emitted from processing of coal, crude oil, petroleum, and natural gas, from production of aluminum, iron and steel, from heating in power plants and homes, burning of refuse, wood fires, and from motor vehicle exhausts [6].

Exposure to PAHs occurs mainly by inhalation of air and by ingestion of food and drinking water ([7], [8]). Although food can be contaminated by environmental factors (air, dust and soil), PAHs in food are mainly formed during industrial processing and food preparation, for example smoking, roasting, baking, drying, frying, or grilling [9].

The importance of analyzing PAHs in various foods using different methods following different extraction methods cannot be over-emphasized ([10], [11], [12], [13], [14], [15]).

The alligator pepper are smoke-dried in order to improve its shelf life before they are taking to the market; smoking could be a source of PAHs in the alligator pepper and PAHs can cause cancer. This study was aimed at determining polyaromatic hydrocarbons (PAHs) in the fresh and smoke-dried samples of alligator pepper (*Aframomum melegueta*) bought from Swali Market, Yenagoa, Bayelsa State of Nigeria.

II. METHODS AND MATERIAL

The chromatographic system comprises an Agilent 1100 series G-1322A, a scanning fluorescence detector (all Agilent Technologies, Palo Alto, USA). An Agilent Technology Chem. Hypersil Green PAH column with guard column, 5 µm, 250 mm × 3 mm i.d. (Thermo Electron Corporation, Runcorn, UK).

Acetonitrile, chloroform, and methanol, all of HPLC grade, were obtained from Labscan (Dublin, Eire). PAHs (Benzo [a] anthracene, chrysene, Benzo [b] fluorine, Benzo [k] fluorene, Benzo [a] pyrene, indeno [1,2,3-cd] pyrene, dibenzo [a,h] anthracene, benzopyrene, and benzo [g,h,i] perylene) were from Ehrenstorfer (Augsburg, Germany).

a. Standards

Stock standard solutions of PAHs were prepared in methanol at concentrations of 50 µg L^{-1}. Solutions were stored at 4ºC in the dark and were stable for approximately one month. Calibration standard solutions of PAHs were prepared for chromatographic analysis.

b. Sample collection/sample preparation

Fresh and smoke-dried samples of alligator pepper (*Afromomum melegueta*) were bought from Swali Market, Bayelsa State. Fresh and smoke-dried samples of alligator pepper (*Afromomum melegueta*) were bought from Swali Market, Bayelsa State. Seeds were mechanically homogenized in a stainless steel blender. 1 g of the sample was vortex mixed in a solvent system of methanol, chloroform, water in the ratio of 3 mL: 6 mL:3 mL. The mixture obtained was centrifuged for 3 min at 10000 rpm. Three phases were formed; liquid phase (water-methanol solution), solid phase (scum containing proteins and other large-molecule compounds), and the second liquid phase (chloroform solution containing PAH). The chloroform phase was filtered through filter paper. The filtered extract was evaporated on a water bath (40ºC) under a stream of nitrogen to almost dryness and diluted with 2 mL methanol and this final sample was introduced into the chromatographic system

c. HPLC Analysis

The fresh and the smoke-dried samples were injected into the HPLC system.. The mobile phase consisted of acetonitrile and water at a flow rate of 1 mL/ min and run with a program outline in Table 1

The identification of PAHs was performed by comparison of their retention times with those obtained with true standards at the same conditions. The detector was set to excitation and emission wavelength as in Table 2

Table 1 : Mobile phase program

The mobile phase gradient Time (min)	Water (%)	Acetonitrile (%)
0	50	50
20	0	100
35	0	100
40	50	50
45	50	50

Table 2: Programmed excitation and emission wavelength with times

Time (min)	**$\lambda_{excitation}$ (nm)**	**$\lambda_{emission}$ (nm)**
Benzo[a]anthracene	248	375
Chrysene	270	385
Benzo[b]fluorene	256	446
Benzo[k]fluorene	295	410
dibenzo[a,h]anthracene	274	507
Benzo[g,h,i]perylene	295	410

III. RESULT AND DISCUSSION

a. Qualitative Analysis

The HPLC-FLD analyses of the fresh, smoke-dried alligator pepper samples, and the mixture of standard PAHs were carried out and the results are shown in Figures 1 and 2. In both figures, chromatogram A is the result of PAH mixtures while chromatogram B in Figure 1 represent the analysis of fresh alligator pepper. Chromatogram B in Figure 2 shows the result of the analysis of the smoke-dried pepper samples. When the retention times were matched, it shows that no PAHs were detected in the fresh alligator pepper while six PAHs (benzo [a] anthracene, chrysene, benzo [b] fluorene, benzo [k] fluorene, dibenzo [a,h] anthracene, and benzo[g,h,i] perylene) were detected in the dried sample.

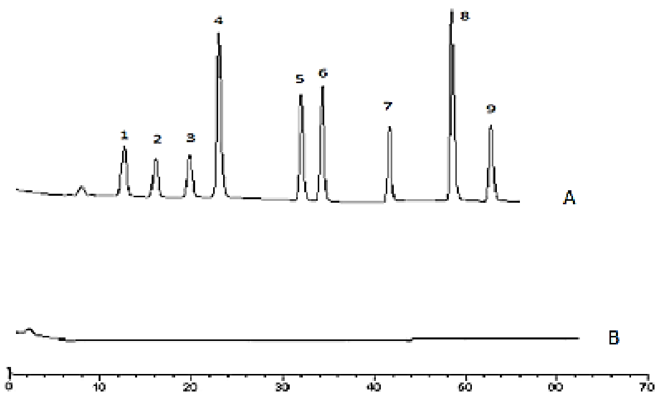

Figure 1. HPLC analysis. Trace A, PAH standards, 1 = benzo [a] anthracene, 2 = chrysene, 3 = benzo [b] fluorene, 4 = Benzo [k] fluorene, 5 = Benzo [a] pyrene, 6 = indeno [1,2,3-cd] pyrene, 7 = dibenzo [a,h] anthracene, 8 = benzopyrene, and 9 = benzo [g,h,i] perylene, trace B, fresh alligator pepper.

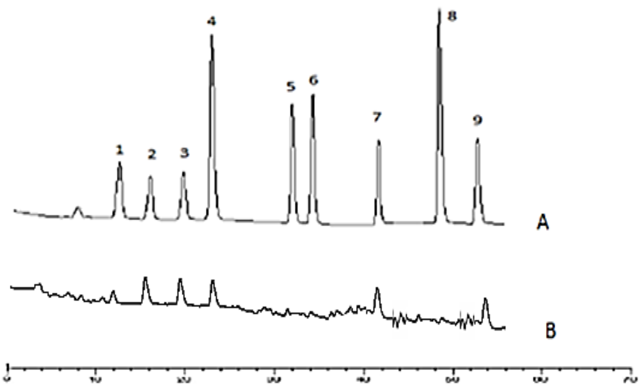

Figure 2. HPLC analysis. Trace A, PAH standard, trace B, smoke-dried alligator pepper. 1= benzo [a] anthracene, 2 = chrysene, 3 = benzo [b] fluorene, 4 = Benzo [k] fluorene, 7 = dibenzo [a,h] anthracene, and 9 = benzo [g,h,i] perylene

b. Quantitative Analysis

The quantitative analysis of the smoke-dried sample of alligator pepper shows concentrations of the six PAHs below quantitation limits as indicated in Table 3.

Table 3. The result of the quantitative analysis

S/N	**PAH**	**mg/kg**
1	Benzo [a] anthracene	BQL
2	Chrysene	BQL
3	Benzo [b] fluorene	BQL
4	Benzo [k] fluorene	BQL
5	Dibenzo [a,h] anthracene	BQL
6	Benzo [g,h,i] perylene	BQL

BQL = below quantitation limit; Limit of quantification of PAHs, 0.02 - 1.6 ng/g

IV. CONCLUSION

The results show that PAHs were present in the smoke-dried alligator pepper seeds only at concentration below quantitation limits. PAHs were not detected in the fresh samples of alligator pepper. Hence, even the smoke-dried alligator pepper is free from PAH-contamination and therefore can be used for their medicinal purposes without any threat of health hazards.

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