International Journal of Academic and Applied Research (IJAAR)
ISSN: 2643-9603
Vol. 4, Issue 4, April – 2020, Pages: 35-39

Microwave-Assisted Extraction and Identification of Polycyclic Aromatic Hydrocarbons (PAHs) in Grilled Meat (Suya) products in basawa Zaria, Northern Nigeria.

Olabimtan Olabode.H[*1], Okibe Ora. P[2], Suleman Stephen.M[3], Benedict Odjobo[4] & Agboni Mercy.O[5]

[1,2]Department of Industrial and Environmental Pollution, National Research Institute for Chemical Technology, Zaria Kaduna State, Nigeria.
[3]Chemistry Department, Nigeria Army University, Biu Borno State, Nigeria.
[4]Bioresources Development Center, National Biotechnology Development Abuja, Nigeria.
[5]Ogel Chemicals and Services Zaria Kaduna State, Nigeria.

Corresponding author email: Olabode4angel@gmail.com

Abstract *Grilled meat or suya in local parlance in Nigeria is the traditional and commonly practice manner of cooking meat which involves direct contact with wood combustion fumes, which was the agent of PAHs contamination was analyzed (identification) with a rapid method involving microwave-assisted saponification and simultaneous extraction, separation, and identifications (GCMS). Sixteen (16) Environmental Protection Agency (EPA) US relevant PAHs were isolated as low molecular weight [flourene, phenanthrene, anthracene, fluoranthene, pyrene, naphthalene, acenaphthylene, and acenaphthene] and high molecular weight [Benz (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, dibenzo(a, h) anthracene, benzo(g, h, i)perylene, indenol(1,2,3,-c,d) pyrene] PAHs. GCMS peak number 1(phenanthrene), 2(acenaphthene) 3(fluorene), 4(acenaphthylene), 5(anthracene), 6(fluoranthene), 7(naphthalene), 9(pyrene) and 16(benzo (g, h, i) perylene) respectively and as non-carcinogenic PAHs, while 8(Chrysene), 10(Benzo (a) anthracene), 12(Benzo (b) fluoranthene), 13(Benzo (k) fluoranthene), 14[Indenol(1,2,3-c,d)pyrene] respectively and as potential carcinogens & 11(Dibenzo (a,h) anthracene) as the only direct carcinogenic PAH in grilled meat product (Suya) obtained in basawa Zaria Kaduna State, Nigeria.*

Keywords: Grilled meat, polycyclic aromatic hydrocarbons, microwave extraction, low and high molecular PAHs and carcinogenicity.

1.0 INTRODUCTION

PAHs as the conventional abbreviation of polycyclic aromatic hydrocarbons are a series of environmental contaminants emanating from partial combustion of natural organic materials and have obtained a good deal of interest over the years because of their carcinogenicities [1]. It is properly recognized that uncooked meat does not comprise appreciable degrees of carcinogenic PAHs and no accumulation along the food chain has been observed for these contaminants in animal fat tissue [2]. All food processing involving thermal method with high temperature and/or direct contact with combustion gases, such as smoking, toasting, roasting or grilling may additionally be responsible for excessive PAH ranges in processed foodstuffs. The quantity of PAHs generated at some stage in the thermal meal processing relies upon several parameters such as temperature, duration of the treatment, distance from the supply of heating, oxygen accessibility, fat content, and the kind of flammable used [3]. Significant contamination stages are projected when food is smoked over an open flame, whilst charcoal grilling usually yields small quantities of PAHs [3]. Smoking is an age-old approach that brings preferred flavors, improves color and appearance, has a tenderizing action, and preserves ingredients [4]. Traditional direct smoking, in which the smoke is generated in the same chamber the product is processed, exposes it to greater PAH content material than indirect smoking, which makes use of a different compartment for smoke generation. With the recent case, it is likely to reduce the degree of PAHs through channeling the smoke via washers, filters or through cooling it earlier before contacting the food. In developed nations, computerized smoking chambers with exterior smoke generators, and emission and temperature controlled systems, capable to minimize PAH production, have generally replaced direct smoking systems with the usage of traditional kilns [3].In different international locations such as Nigeria, typical direct smoking structures are nearly solely used even today. Different processes have been proposed for hazard characterization of the PAH combination in food, the most famous being the use of benzo[a]pyrene(BaP) and the toxic equivalency factor (TEF) approaches as a marker.

Based on an examination of PAH profiles in meals and on comparison of carcinogenicity research of two coal tar combinations in mice, the scientific committee on food (SCF) and the association WHO/FAOprofessional committee on food additives recommended that BaP must be adopted as an indicator of the prevalence and influence of PAHs in foodstuff [3]. However, recently the TEF strategy used to be reassessed and it used to be deemed as being no longer scientifically valid because of the lack of records from oral carcinogenicity studies on individual PAHs, their different modes of action, and the evidence of terrible predictivity of the carcinogenic potency of PAH combinations based on the presently proposed TEF values [3].In 2005, the European Commission introduced for BaP chosen as a pointer of the prevalence and carcinogenic potency of the whole type of carcinogenic and genotoxic PAHs with the highest level of 5 mg kg^{-1} in smoked fish and meat [5]. Before the introduction of this Regulation, lower permitted limits for BaP existed in a range of European Union Member States such as Belgium (2 mg kg^{-1}), the Slovak Republic, and Germany (1 mg kg^{-1})[5]. Up to now no maximum allowable grades for PAHs and/or BaP have been defined by the government of Nigeria. Based on newly assessable data, in 2008 the EFSA board established that BaP is now not an appropriate indicator for the manifestation of eight excessive molecular weight PAHs in food, and a subgroup of four PAHs, are the most suitable indicators for PAHs and cautioned with the procedure of the limit of exposure method for danger assessment [6]. Generally, smoked foods may additionally contribute significantly to PAH dietary consumption if such ingredients are the phase of the usual food plan [3]. The consumption of historically smoked products could be accountable for the greater incidence of primary liver and abdominal cancer in Nigeria compared with that in Europe and the United States [4]. To our knowledge, there are no latest consumption facts for grilled meat in Nigeria. Alonge (1988) mentioned BaP contents from 10.5 to 66.9 mg kg^{-1} in traditionally smoked meat from Nigeria, while Bababunmi et al. said 8.5 mg $kg^{-1}$of BaP in suya meat (a spiced skewered meat very famous in West Africa, commonly grilled over an open fire)[7]. More recently, Duke and Albert (2007) determined BaP contents ranging from 6.5 to 21.5 mg kg^{-1} in suya meat from 4 exceptional selling points [7]. PAHs resolution in meat and fish samples, when carried out by capacity of a standard sampling technique, with the application of a solvent and is, therefore, time-consuming and can induce analyte losses and a stream of contamination[8].

To overcome these disadvantages, a new extraction process with microwave radiation was used to operate simultaneous rapid extraction and saponification of the samples matrices of a variety of PAHs fraction in some grilled meat products normally consumed in Zaria Kaduna State in northern Nigeria. Also involves GCMS qualitative determination of Environmental Protection Agency (EPA)-priority PAHs [9]

2.0 MATERIALS AND METHODS

2.1 Chemical reagents

Standard PAH mixture, acetone, distilled water, acetonitrile, methylene chloride, n-hexane, potassium hydroxide (KOH), ethanol and silica.

2.2 Apparatus and instruments

Beakers, separating funnel, retort stand, rotary evaporator, vial bottle, dropping pipette, measuring cylinder, spatula, hand gloves, round bottom flask, masking tape, refrigerator, GCMS Hp5972 Series with F.I.D detector, weighting alance and sonicator.

2.3 Sample collection

Some grams of suya meat was purchased from suya spot Basawa in Zaria, Kaduna State of Nigeria.100 g of each of the samples were lyophilized, pulverized and packed in aluminum foil wraps and stored in the freezer at –20^{o}C before analysis.

2.4 PAHs extraction using microwave radiation

This was according to the method described by Albero et al, with few modifications. 200 mg of the lyophilized sample was taken into a vessel and added to 0.8 ml of distilled water, 4 ml saturated methanolic KOH and 10ml of n-hexane. A microwave-assisted saponification/extraction was carried out at 120^{o}C for 20 min with Kenmore 85962 microwave model. Once cooled, vessels were opened and a measured amount of 7.5 ml of the organic extract, corresponding (with a relative uncertainty of less than 2%) to three-quarters of the added amount of n-hexane, was withdrawn with a volumetric pipette and concentrated to a few microliters using a rotary evaporator and then a nitrogen flow. Objectively, minimizing the losses of the volatile PAHs, the remaining chemical solvent was volatilized to naturally to dryness at room temperature, and the residue was then dissolved with 200 ml of n-hexane [10].

2.5 PAHs extracts purification

A measured part of the sample extract (100 ml) was directly loaded on a 250 mg silica cartridge (Supelco), previously conditioned with 1ml of dichloromethane and 1ml of n-hexane. After sample loading, the PAH fraction was eluted with 2.5ml of n-hexane/dichloromethane (70/30). The fraction so obtained was gently concentrated to a few microliters under a nitrogen flow and then left to evaporate spontaneously to dryness at room temperature [11].

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2.6 GCMS analysis of PAHs

The sample was analyzed for Environmental Protection Agency (EPA)-priority PAHs by high-resolution GCMS Hp5972 Series with F.I.D. 0.5ml aliquot of the extract was manually injected with a syringe. The injection temperature was 250°c. The temperature program was initially set at 60°c 1min followed by a 250°c/min ramping at 300°c and then held there for 10min. Helium was used as a carrier gas of a flow rate of 1.36 ml/min.

3.0 RESULTS AND DISCUSSION

Figure1.Spiced grilled meat (suya) bought from basawa Zaria.

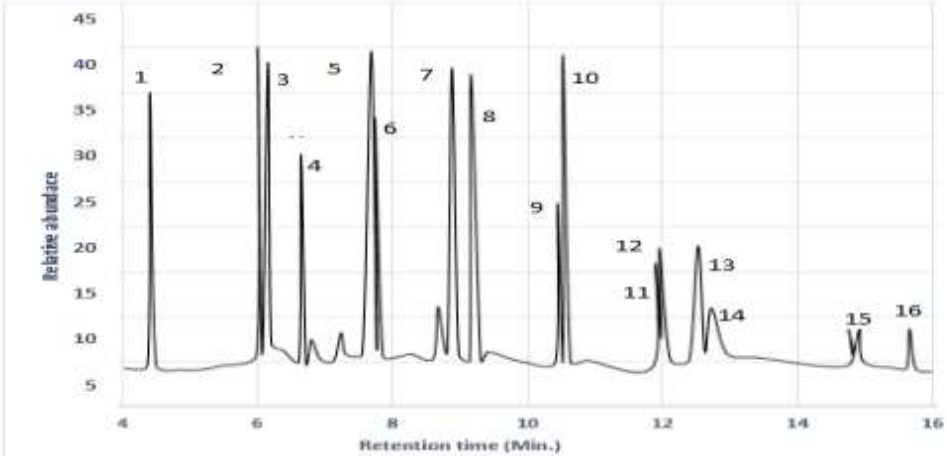

Figure 2. GCMS spectrum of PAHs of grilled meats obtained from Basawa Zaria Kaduna State, Nigeria.

Table 1.Qualities and detected (GCMS) PAHs on grilled meats from Basawa Zaria, Kaduna State, Nigeria.

Peak Number	Compound	Chemical Formula	Mass (m/z)	Retention Time (Min.)	Ring Number	Carcinogenicity
1	Phenanthrene	$C_{14}H_{10}$	178	4.50	3	Z
2	Acenaphthene	$C_{12}H_{10}$	154	5.97	3	Z
3	Flourene	$C_{13}H_{10}$	166	6.37	3	Z
4	Acenaphthylene	$C_{12}H_{8}$	152	6.69	3	Z
5	Anthracene	$C_{14}H_{10}$	178	7.74	3	Z
6	Fluoranthene	$C_{16}H_{10}$	202	7.81	4	Z
7	Naphthalene	$C_{10}H_{8}$	128	9.03	2	Z
8	Chrysene	$C_{18}H_{12}$	228	9.52	4	Y2
9	Pyrene	$C_{16}H_{10}$	202	10.84	4	Z
10	Benzo (a) anthracene	$C_{18}H_{12}$	228	10.87	4	Y2
11	Dibenzo[a,h]anthracene	$C_{22}H_{14}$	228	12.01	5	Y1
12	Benzo (b) flouranthene	$C_{20}H_{12}$	252	12.34	5	Y2
13	Benzo (k) flouranthene	$C_{20}H_{12}$	252	12.52	5	Y2
14	Indenol[1,2,3cd]pyrene	$C_{22}H_{12}$	276	11.03	6	Y2
15	Benzo (e) pyrene	$C_{20}H_{12}$	252	15.05	5	X
16	Benzo (g,,h,i) perylene	$C_{22}H_{12}$	276	15.82	6	Z

A-Carcinogenic to human; Y1-Probably carcinogenic to humans; Y2-Possibly carcinogenic to humans; Z-Not classifiable as carcinogenic to humans [12]

Figure 1 is the spiced grilled meat that is ready to be consumed. The GCMS profile of the PAHs components of the activated (lyophilized) grilled meat as displayed (Figure 2). Sixteen EPA recognized PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, naphthalene, acenaphthylene, acenaphthene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo(K) fluoranthene, benzo(a) pyrene, dibenzo(a,h) anthracene, benzo (g,h, i) perylene and indeno(1,2,3,-c,d)pyrene.) were identified with respect to their chemical formula, mass to charge ratio, retention time in GCMS column, ring number and carcinogenicity (Table 1.)

4.0 CONCLUSION

Grilled meat popularly regarded as 'Suya' in the northern part; basawa Zaria Kaduna state of Nigeria has been affirmed as a medium of PAHs especially with carcinogenic Dibenzo(a,h) anthracene. The partial combustion of flame against the product (meat) precipitates direct carbon as soot and this triggers possibly triggers some reactions against some atmospheric radicals, that resulted in various degrees of PAHs. Critical approach (s) is /is required in controlling these exposures. Moreover, other PAHs as well could accumulate and induce cancer over time as margin of exposure and lowering of their concentration cannot be managed by the consumers.

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