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COMPARISON OF THE CONCENTRATION OF PAHs IN RAW AND ROASTED BEEF SAMPLES FROM EKPOMA

OBRIFOR, E. B.[1], EGBON, E. E.[1], ISAH, A.[1], ERHABOR, D. O.[3], OGONEGBU, A. C.[2]

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[1]*Ambrose Alli University, Ekpoma, Edo State*
[2]*Dennis Osadebay University, Anwai, Asaba*
[3]*Federal College of Education (Technical), Umunze, Anambra State*

Corresponding author: essayshype@yahoo.com; Tel: +2348067502568

ABSTRACT

The determination of the concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in raw and roasted beef samples from Ekpoma, Edo State was the aim of the study. Samples were obtained from different suya outlets for the roasted samples, while the fresh beef were obtained from different spots in Ekpoma main market. Sixteen (16) polycyclic aromatic hydrocarbons (PAHs) in fresh and roasted beef samples were evaluated in this study. Extraction was done using a mix solvent of cyclohexane and acetone in a ratio of 50:50. Analysis was performed by Gas Chromatography-Mass Spectrometry (G.C-MS). The procedure involved extraction, and adsorption column for clean-up. The cleaned fluid from samples was concentrated using a rotary evaporator and compressed air. The resulting solution was stored in vial bottles ready for PAH determination. The part of beef used for the experiment was the tissue. The various samples were run through the G.C-MS chromatography column. Results of each sample were obtained graphically. Findings revealed that of all sixteen (16) PAHs targeted, only two (2) PAHs were detected: Pyrene and Fluoranthene. Bulk of the PAHs were below detectable limits. The raw beef samples all had no PAHs detected (below detectable limits). The analysis of the raw and roasted beef samples was done in triplicates. Only roasted meat 1 (RTMT1) had the presence of Pyrene, with a concentration of 1.714 mg/kg, while roasted meat 2 (RTMT2) and roasted meat 3 (RTMT3) had the same concentration of 0.009 mg/kg of Fluoranthene. From the findings, it was observed that fresh beef samples normally do not contain PAHs, but during processing by roasting, PAHs can be introduced in some cases. Bulk of the PAHs in the roasted beef samples were below detectable limits. From the findings, the concentration of PAHs was below the recommended level: 5.0 μg/kg Benzo(a)pyrene (BaP) in smoked meat and fishery products.

Keywords: *Polycyclic Aromatic Compound, Fresh and Roasted Beef, Bos taurus*

Introduction

The increase in the case of cancerous growth is quite alarming in contemporary times. Several factors have been attributed to this effect. One of the substances which has been partly identified by scientists among the many factors is the presence of polycyclic aromatic hydrocarbons (PAHs) in food consumed, especially when roasted.

Polycyclic aromatic hydrocarbons are hydrocarbons—organic compounds containing only carbon and hydrogen—that are composed of multiple aromatic rings. PAHs are uncharged, non-polar molecules found in coal and tar deposits. They are also produced by the thermal decomposition of organic matter (Wikipedia, 2018).

PAHs occur in varying amounts in water, soil, air, and even in some food and food products like fish and meat in trace amounts (Erhabor & Edjere, 2018). In Nigeria, meat preservation is either by smoking or drying, and in the process of preservation by drying, the shelf life is prolonged.

When food items are processed by subjecting them to heat treatment such as drying, smoking, roasting, baking, and frying, it has been reported that the amount of PAHs in them increases (Ishizaki et al., 2010). Humans are reported to obtain most of this PAH contamination from their diet (Farhadian et al., 2011). Olabemiwo (2013) reported an increase in the level of PAHs in grilled/roasted maize and plantain. The flames contain PAHs that adhere to the surface of the meat (Cross & Sinha, 2004). In barbecued meat, PAH levels were found to increase from average background values usually in the range of 0.01 to 1

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g/kg in uncooked foods (meats, fish, vegetables) to 130 g/kg (Gómez-Guillén et al., 2009).

The presence of variable quantities of PAHs has been reported in different food categories and beverages including vegetables and fruits, cereals, oils and fats, smoked products (e.g., meat), coffee, and tea (Tuteja et al., 2011).

In a study carried out to investigate the levels of PAHs in singed and unsinged hides and skins of animals slaughtered at three district abattoirs (Obosi, Uga, and Kwata) in Anambra State, Ofomata et al. (2020) found the total PAHs of raw and singed cattle hides to be respectively 0.80 and 12.33 g/kg for Obosi district, 0.56 and 6.96 g/kg for Uga district, and 8.30 and 16.24 g/kg for Kwata district. The total PAHs levels in raw and singed goat skins were respectively 2.75 and 9.00 g/kg for Obosi district, 1.76 and 6.42 g/kg for Uga district, and 1.30 and 5.19 g/kg for Kwata district. The levels of some PAHs in singed hides and skins were significantly (pi0.05) higher than in the unsinged samples.

Also, in research conducted by Akpoghelie (2018) on the assessment of PAHs in smoked fish and suya meat consumed in Warri, Nigeria, the results revealed that the PAH values for grilled meat/smoked fish are far higher than those obtained from boiled water, indicating that roasting introduces and increases PAHs in food (meat).

Analysis of charcoal-roasted common food items has proven the presence of PAHs such as benzo(a)pyrene, anthracene, chrysene, benzo(a)anthracene, and indeno(1,2,3-c,d)pyrene (Linda et al., 2011). Most of these PAHs have been found to be carcinogenic, while some are not (Lijinsky, 1999). Research carried out by Akpoghelie (2018) also supports this finding.

Polycyclic aromatic compounds, as chemical compounds, are known potent carcinogenic compounds. A major source of exposure is through food contaminated with PAHs (Ishizaki et al., 2010). These food substances can be contaminated either through processing (drying methods) or from the environment from which the meat is bred.

At ambient temperatures, PAHs are solids with low vapor pressure and very low water solubility, which tends to decrease with increasing molecular weights. They attach strongly to soil and other particles, break down slowly, undergo photodecomposition when exposed to ultraviolet light from solar radiation, react readily with pollutants such as ozone, nitrogen oxides, and sulfur dioxide, and can be degraded by some microorganisms in the soil. They are soluble in many organic solvents and are highly lipophilic, meaning they mix more easily with oil than water. The larger compounds are less water-soluble and less volatile (i.e., less prone to evaporation). Because of these properties, PAHs in the environment are found primarily in soil, sediments, and oily substances. However, they are also a component of concern in particulate matter suspended in the air (Majid Kermani et al., 2023). When PAHs attach to dust or ash, they could cause lung irritation. Skin contact with PAHs may cause redness, blistering, and peeling (Hui Jin et al., 2024). The following health effects can occur after several years of exposure to PAHs:

- **Cancer:** Many PAHs are carcinogenic, producing tumors (cancer) such as benzo[a]pyrene, methyl cholanthrene, and dibenz[a,f]anthracene. Although studies in experimental animals on individual PAHs, notably benzo[a]pyrene (B[a]P), have shown various toxicological effects, including hematological effects, reproductive and developmental toxicity, and immunotoxicity, the critical effects are genotoxicity and cancer (Luch, 2005). Benzo(a)pyrene has been shown to cause lung and skin cancer in laboratory animals. Other PAHs are not known to have this effect. Extracts of various types of smoke containing PAHs have caused lung tumors in laboratory animals (Luch, 2005).

- **Toxicity:** PAH toxicity is very structurally dependent, with isomers (PAHs with the same formula and number of rings) varying from being nontoxic to extremely toxic. Highly carcinogenic PAHs may be small or large. One notable PAH compound, benzo(a)pyrene, is known as the first chemical carcinogen discovered (Luch, 2005).

Materials and Methods

Location

Ekpoma is a town in Edo State, Nigeria. It is the administrative headquarters of the Esan West Local Government Area. Ekpoma lies on the geographical coordinates of latitude 6°45N and longitude 6°08E. The town has an official Post Office and is home to the Ambrose Alli University. The roasted beef (suya)

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samples were collected from various suya outlets in Ekpoma, Esan West L.G.A. of Edo State, Nigeria. The raw beef samples were collected from different spots in the Ekpoma main market.

Materials

Meat Samples

1. Bos taurus commonly called beef meat (RAW)
2. Bos taurus commonly called beef meat (ROASTED)

Chemicals and Reagents

All reagents and chemicals used were of analytical grade and included the following:

- Acetone (Sigma-Aldrich Corporation, United States)
- Dichloromethane (Sigma-Aldrich Corporation, United States)
- Cyclohexane (Sigma-Aldrich Corporation, United States)

The three solvents were redistilled before use to keep them free from impurities.

Sodium Sulphate: Granular and anhydrous. The sodium sulphate granules were purified by heating at 400°C for 4 hours in a shallow tray and cooled in a desiccator.

Mix 26: The internal standard or surrogate, Mix 26, comes in 1 ml vials. It was prepared by diluting a 1 ml solution containing 4000 ng/μl in 100 ml of dichloromethane. The solution, which contains 40 ng/100 ml, has a fluorescent green color.

Equipment/Apparatus

The equipment/apparatus used include:

- Water bath
- Vials and corks
- Test tubes
- Round bottom flasks
- Rotary evaporator
- Thermometer
- Silica gel packed cartridges
- Conical flasks
- Retort stands
- Weighing balance
- Spatula
- Beakers
- Volumetric pipettes: 1 ml, 5 ml, and 10 ml
- Micro syringes: 10 μL, 100 μL, 250 μL, 500 μL, 1000 μL
- Drying oven
- Desiccator
- Blender
- Ultrasonic bath
- SPE cartridges with stand
- Gas chromatography instrument
- Oven and drier

Method

Sampling Area

Ekpoma, Esan West L.G.A. of Edo State, Nigeria, was the sampling location.

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Sample Collection

The roasted meat samples, Bos taurus commonly called suya, were collected from three different locations in Ekpoma:

- Ujuolen junction axis (RTMT 1), a very busy junction with high traffic and vehicular activity
- Ekpoma market square axis (RTMT 2)
- New market axis (RTMT 3)

Composite sampling was applied at these locations to obtain representative samples.

The fresh cow meat samples, Bos taurus commonly called beef meat, were collected from three different locations in Ekpoma:

- Ekpoma market square axis (FRMT 1)
- New market axis (FRMT 2)
- Hausa market axis (FRMT 3)

Composite sampling was also used at these locations.

The samples were kept in polythene bags, properly labeled, placed in coolers with ice, and transported to Earth Quest Laboratory, Otokutu, Warri, Delta State, where they were stored in a refrigerator at 4°C prior to further processing.

Sample Preparation

The fresh meat samples from the sampling sites were washed and re-rinsed with distilled water. The fresh and roasted samples were separately ground using a Mikachi meat grinder, homogenized using a laboratory pestle and mortar, and then stored in a refrigerator at 4°C prior to extraction and analysis.

Extraction and Clean-Up of Samples

The fresh and roasted meat samples were separately ground using a blender (Mikachi meat grinder). The blender was washed, rinsed, and re-rinsed with distilled water after each use. Fifty grams of each meat sample was mixed with 25 g of sodium sulphate and 200 ml of a 50/50 cyclohexane/acetone mixture in a tightly covered bottle, with 10 ml of Mix 26 internal standard added to each bottle. The bottles containing the sample, solvents mixture, sodium sulphate, and internal standard were placed inside an ultrasonic bath (Astrabro ultrasonic cleaner, model 7E) for 2 hours, with shaking every 10 minutes.

Twenty-five milliliters of the extract was collected using a pipette and filler, concentrated using a rotary evaporator to 5 ml, and then further concentrated to 1 ml using compressed air. The clean-up was done using a solid phase extractor, and cyclohexane was employed as the eluting solvent. The cleaned-up sample was concentrated to 1 ml using compressed air, stored in 1 ml vials, and subjected to GC analysis using an FID detector.

Gas Chromatography Operating Procedure

Instrument type: Gas chromatography system 6890 series
Product: HP
Detector type: FID

The basic chromatography parameters for the analysis of polycyclic aromatic hydrocarbons are as follows:

- Initial Temperature: 100°C
- Rate 1: 4°C/min
- Final Temperature: 330°C
- Detector Temperature: 300°C

One milliliter extracts of both the Clarias gariepinus (raw and roasted) and Bos taurus (raw and roasted) kept in vials were analyzed using GC-MS model QP2010SE, Shimadzu, Japan.

Results and Discussion

Table 1. Polynuclear Hydrocarbon Content (mg/kg)

The summary of the concentration of various Polynuclear Aromatic Compounds (PAHs) present in raw and roasted meat samples is shown in Table 1. Table 2 shows the comparison of results between raw meat and roasted meat samples. This research was conducted to determine the presence and concentration of 16 PAHs in raw and roasted meat samples from Ekpoma, Edo State. The results showed that not all 16 targeted PAHs were present in the raw and roasted meat samples. A total of only two PAHs were observed in the samples: Pyrene and Fluoranthene. The concentration of PAHs in the samples is shown in Table 2.

No PAHs were detected in any of the raw meat samples labeled FRMT 1, FRMT 2, and FRMT 3. This result is consistent with other researchers' findings

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Table 1: Polynuclear Hydrocarbon Content (mg/kg)

COMPONENT	FRMT1	FRMT2	FRMT3	RTMT1	RTMT2	RTMT3
Naphthalene	0.000	0.000	0.000	0.000	0.000	0.000
Acenaphthalene	0.000	0.000	0.000	0.000	0.000	0.000
Acenaphthene	0.000	0.000	0.000	0.000	0.000	0.000
Florene	0.000	0.000	0.000	0.000	0.000	0.000
Phenanthrene	0.000	0.000	0.000	0.000	0.000	0.000
Anthracene	0.000	0.000	0.000	0.000	0.000	0.000
Fluoranthene	0.000	0.000	0.000	0.000	0.009	0.009
Pyrene	0.000	0.000	0.000	1.714	0.000	0.000
Benzo(a)anthracene	0.000	0.000	0.000	0.000	0.000	0.000
Chrysene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(b)fluoranthrene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(a)pyrene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(k)fluoranthrene	0.000	0.000	0.000	0.000	0.000	0.000
Indeno(1,2,3-cd)pyrene	0.000	0.000	0.000	0.000	0.000	0.000
Dibenzo(a,h)anthracene	0.000	0.000	0.000	0.000	0.000	0.000
Benzo(g,h,i)perylene	0.000	0.000	0.000	0.000	0.000	0.000
Total PAH (mg/kg)	BDL	BDL	BDL	1.714	0.009	0.009

Note: Each of the coded samples are averages of triplicate measurements of representative samples from each sampling spot.

KEY: RTMT – ROASTED MEAT; FRMT – RAW MEAT; BDL – BELOW DETECTABLE LIMIT

that raw foods typically do not contain high levels of PAHs, which are generally formed during processing such as roasting, baking, smoking, or frying (Amos-Tauta, Inengite, Abasi, & Amirize, 2013). For example, a study by Ofomata, I. B. et al. (2020) investigating PAH levels in singed and unsinged hides and skins of animals slaughtered at three district abattoirs (Obosi, Uga, and Kwata) in Anambra State found that total PAHs in raw and singed cattle hides were 0.80 and 12.33 g/kg for Obosi district, 0.56 and 6.96 g/kg for Uga district, and 8.30 and 16.24 g/kg for Kwata district. Similarly, total PAHs in raw and singed goat skins were 2.75 and 9.00 g/kg for Obosi district, 1.76 and 6.42 g/kg for Uga district, and 1.30 and 5.19 g/kg for Kwata district. The levels of some PAHs in singed hides and skins were significantly (pi0.05) higher than in unsinged samples.

The concentration of PAHs in roasted meat (suya) samples in our study, RTMT 1, RTMT 2, and RTMT 3, ranged between 0.009 to 1.714 mg/kg. The total average PAHs level in suya was 0.576 mg/kg.

Table 2. Concentration (mg/kg) levels of PAHs in raw and roasted meat

[h!]

The results of the raw beef samples were comparable with the report of Amos-Tauta et al. (2013), where the PAH concentrations were below detectable limits. However, the roasted meat (suya) samples showed the presence of Pyrene and Fluoranthene. This could be due to the processing location of the roasted meat. RTMT 1 was sourced from Ujuoelen junction axis, a very busy spot with heavy vehicle activity. Akpoghelie (2018) noted that poor road networks and potholes can increase traffic congestion, leading to higher PAH emissions. To determine the source of PAHs in samples, whether from pyrolytic, combustive, petrogenic, or petroleum hydrocarbon origins, the ratios of Fluoranthene (Fla) to Pyrene (Pyr) and Phenanthrene (Ph) to Anthracene (An) are often used. A ratio of Fluoranthene to Pyrene greater than one (Fla/Pyr ¿ 1) indicates a pyrolytic source, while Fla/Pyr ¡ 1 suggests a petroleum hydrocarbon source. Similarly, a Phenanthrene to

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Table 2: Concentration (mg/kg) levels of PAHs in raw and roasted meat

PAHs AVERAGE	FRMT1	FRMT2	FRMT3	AVERAGE	RTMT1	RTMT2	RTMT3
Pyrene 0.570	0.000	0.000	0.000	0.000	1.714	0.000	0.000
Fluoranthene 0.006	0.000	0.000	0.000	0.000	0.000	0.009	0.009
TOTAL 0.576	0.000	0.000	0.000	0.000	1.714	0.009	0.009

Anthracene ratio less than ten (Ph/An ¡ 10) indicates a combustion source, while Ph/An ¿ 10 suggests a petrogenic source. In this study, the PAHs in the roasted meat can be attributed to a pyrolytic source, as the ratio is ¿1.

The PAHs concentration of RTMT 1 was higher than that of RTMT 2 and RTMT 3. Apart from the busy location where the sample (RTMT 1) was sourced, other factors that could affect the results include the part of the beef (muscle) used, as some parts may have higher fat content; the method of preparation; the length of cooking; and the amount of heat used (Akpoghelie, 2018).

Figure 1: Polycyclic Aromatic Hydrocarbon Content (mg/kg)

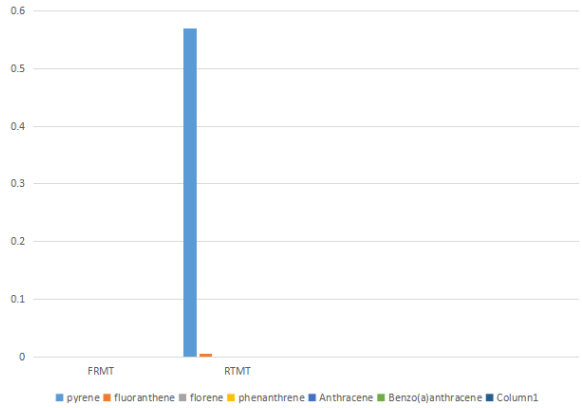

Figure 1: Polycyclic Aromatic Hydrocarbon Content (mg/kg)

KEY: ROASTED MEAT: RTMT; RAW MEAT: FRMT

The highest concentration of total PAHs was detected in RTMT 1, with a concentration of 1.71 mg/kg, and the lowest in FRMT 1, FRMT 2, and RTMT 3, all having concentrations below detectable limits. The total PAHs concentration can be attributed to the temperature of heating and the particle size of PAHs sorbed into organic matter. High-temperature heating tends to produce high molecular weight PAHs (Choi et al., 2010). High molecular weight PAHs, which have more than four rings, are more likely to be formed. Pyrene was detected in RTMT 1. Additionally, two- to four-ringed PAHs volatilize sufficiently to appear in the atmosphere in gaseous form, with their physical state depending on temperature (Walker et al., 2013). High temperatures generate high molecular weight PAHs, while low temperatures generate low molecular weight PAHs (Wikipedia, 2018). This could explain the high concentration of Pyrene.

Conclusion

The results of the six (6) samples in this study showed a total of two (2) PAH compounds present. Total PAH concentrations for roasted meat samples were higher than those of raw meat. This is because, when food, particularly meat and meat products, are smoked, roasted, barbecued, or grilled, PAHs are formed as a result of incomplete combustion or thermal decomposition of the organic material (WHO, 2006). According to the results of this research, the concentration of PAHs is above the recommended level of 5.0 g/kg or 0.005 g/g BaP in smoked meat and the 2 g/kg fixed by European standards (Akpoghelie, 2018). No PAHs were detected in the fresh beef samples. It was also observed that when raw meat is roasted, the amount of PAHs increases, and new PAHs can be formed in the process.

From the foregoing analysis, caution must therefore be taken because of the adverse effects posed by exposure to PAHs through food, such as genotoxicity, mutagenicity, and carcinogenicity. This research sought to compare the concentration of samples based on location, as this is a factor in the presence and

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concentration of PAHs in food samples.

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