

POLYCYCLIC AROMATIC HYDROCARBONS IN BEEF BALANGU SMOKED WITH DIFFERENT FUEL WOOD SPECIES

^{1*}Ribah Mohammed Ibrahim, ²Abubakar Mujahid Tambuwal and ³Goparaju Anumolu

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Abstract

Smoking enhances the microbiological safety and eating quality of meat; however, such treatments contribute to the formation of harmful substances in meat products. Polycyclic aromatic hydrocarbons are the main contaminants formed during the smoking and grilling of meat products. They are created due to the incomplete burning of fuel and leakage of cellular juice from the heat source. These compounds have been implicated in various human cancer cases. This study investigated the presence of PAHs in beef balanga smoked with different fuel wood species. The research consisted of five (5) treatments involving one kilogram (1kg) each of beef smoked with geza (*Combretum micratum*), kalgo (*Piliostigma thonningii*), malga (*Cassia sieberiana*), and sabara (*Guiera senegalensis*) in a laboratory oven. Three samples from each treatment group were used for the determination of PAHs using GC-MS. The result for PAHs indicated that 40 PAH compounds were detected in all 15 samples. Six compounds each were detected in meat smoked with geza and malga, nine PAHs were detected in meat smoked with kalgo, 12 compounds were detected in meat smoked in an electric oven, and seven compounds were detected in meat smoked with Sabara. The PAHs 2-methyl-Naphthalene (10.00%) and 2-(1-dimethyl)-Naphthalene (10.00%) were found to be more prevalent across the treatment groups, followed by Naphthalene (7.50%) and 1,6-dimethyl-Naphthane (5.00%). The less prevalent PAHs were 1-naphthalanol (2.5%) and pylene (2.5%), followed by 5-azulene (2.50%) and 9, 10-anthracenedol (2.50%). 2-methyl-naphthalene was more prevalent across the treatments. It was concluded that from all the treatment

¹Department of Animal Science, School of Agricultural Sciences, Kampala International University, Kampala, Uganda

²Department of Animal Science, Faculty of Agriculture, Kebbi State University of Science and Technology, Aliero, PMB 1144, Birnin Kebbi 1144, Nigeria

³School of Mathematics and Computing, Kampala International University, Kampala, Uganda

Email: ibrahim.ribah73@kiu.ac.ug

Phone Number: +256706708248; +2347036333707

assessed, PAHs 2-methyl-Naphthalene were found to be more prevalent across the treatment, and meat samples smoked with sabara and roasted in oven have higher protein content (26.397 and 26.583, respectively). It is recommended that, Geza and Malga fuel wood species should be utilized by the butchers and households especially during festivities, this is due the fact that they have less number of PAHs detected.

INTRODUCTION

Smoking food, especially meat and fish, has been a traditional way of preparing food because it improves the taste and smell of food and helps it last longer (Mejborn *et al.*, 2019). Mark (2011) explained that smoking meat involves placing the food near wood heat and smoke for a certain time during the cooking process. In hot smoking, hardwood is burned completely with enough air to create light brown smoke with low moisture. The smoke helps keep food safe by removing moisture, and the particles in the smoke stop bacteria from growing, prevents fats from breaking down, and improve the taste of the food, which in turn makes the food last longer (Huang *et al.*, 2016). Using firewood to make heat and smoke is a type of cooking method that changes the food in both physical and chemical ways, depending on how hot the fire is and the conditions used (Wang *et al.*, 2021). Similarly, smoking and grilling are known to help make meat and fish safer and of better quality (Cordeiro *et al.*, 2020). However, there are serious concerns about using these methods because they can create harmful chemical compounds that can make food unsafe. The main harmful substances formed when smoking or grilling meat are polycyclic aromatic hydrocarbons (PAHs). PAHs are a group of environmental pollutants that are produced when fuel such as wood or charcoal is not completely burned or when fats and oils are broken down at high temperatures (WHO, 2006). These compounds are produced when the fuel is not completely burned or when juices from meat touch the heat source, creating a mixture that is absorbed into the food. PAHs form during the smoking and curing of meat, and they build up in meat when smoked at very high temperatures, usually more than 400°C, over open flames. These compounds can cause cancer and other health problems in humans and animals (Anyakora *et al.*, 2008).

According to Nisha *et al.* (2015), the types and amounts of PAHs in smoked food are to a large extent dependent on the smoking process, with factors such as the type of smoke generator, type of wood, moisture content, combustion temperature, and degree of smoking playing a vital role in influencing PAH formation. In Northern Nigeria, meat is commonly processed using different types of firewood because the resulting meat is widely accepted and enjoyed for its unique taste. However, there are growing concerns that meat processed with firewood may contain harmful PAHs such as benzo[a]-pyrene, benzo[a] anthracene, benzo[b] fluoranthene, chrysene, and cyclopenta (c,d) pyrene. These are known to be cancer-causing and can lead to human health issues. Although meat made with firewood that produces a lot of smoke may look and smell appealing, it could still contain PAHs (Maga, 1987). Therefore, there is a need to find better types of firewood that will produce less smoke and reduce the formation of harmful PAHs. This research aims to determine the amount of PAHs present in beef balangu smoked with different types of firewood. The results of this study could help promote the consumption of balanga in the region and help identify the firewood types that produce less smoke and fewer harmful compounds.

MATERIALS AND METHODS

Study area

The experiment was conducted at the Animal Science Laboratory, Abdullahi Fodiyo University of Science and Technology Aliero, Kebbi State, Nigeria.

Sources of the experimental materials

Fresh semi-membranous muscle from an apparently healthy bull was purchased from the central abattoir of Birnin Kebbi in the morning immediately after slaughter. Fuelwood species were purchased from nearby villages where they were available.

Treatments and Experimental Design

The experiments were laid in a completely randomized design consisting of five (5) treatments represented by four fuelwood species Kalgo (*Piliostigma reticulatum*), Sabara (*Guiera senegalensis*), Geza (*Combretum micranthum*), Malga (*Cassia arereh*), and electric oven, to represent T1, T2, T3, T4, and T5, respectively. All treatments were replicated 3 times to give 15 observations.

Sample preparation

Five kilograms (5kg) of meat was purchased and all visible fat and connective tissues were trimmed. The meat was divided into five (5) groups containing 1kg each. The meat from each group was sliced to a thickness of 2 cm according to the balangu method. After slicing, each group of meat was smoked with a corresponding fuelwood species. Treatment one (T1) was smoked with Kalgo (*Piliostigma reticulatum*), treatment two (T2) was smoked with Sabara (*Guierasenegalensis*), treatment three (T3) was smoked with Geza (*Combretum micranthum*), treatment four (T4) was smoked with Malga (*Cassia arereh*), and treatment five (T5) was roasted in the oven. After smoking the meat, each group was collected and carefully cutout three (3) pieces of about 50 ± 5 g. The 3 pieces from each treatment served as the samples used for the determination and quantification of PAHs.

Determination of PAHs

The extraction and cleaning of the samples for analysis were performed using the procedure described by Iwu et al. (2024). We used the European Standard EN 15662:2008, known as the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. The procedure involved a citrate-buffered method. Approximately 5 g of the sample and 10 mL of distilled water were added to a QuEChERS Teflon extraction/centrifuge tube and mixed for 1 min using a vortex mixer. Then, 10 mL of HPLC-grade acetonitrile containing 100 L (0.1 mL) of 0.5 g/mL of surrogate standard polychlorinated biphenyl (PCB) 153 was added to the sample tube and mixed vigorously. The sample was left for 15 min. A pre-mixed extraction salt solution, which included 4 g of coarse anhydrous magnesium sulfate, 1 g of sodium chloride, 0.5 g of disodium hydrogen citrate sesquihydrate, and 1 g of trisodium citrate dehydrate, was added to the sample tube. The tube was then sealed and shaken vigorously for 1 min to distribute the salt into the sample. The mixture was vortexed for 3 min and then centrifuged at 3000 rpm for 5 min. An aliquot of 6 mL of the upper organic phase was transferred into a 15 mL dispersive solid phase (DSP) clean-up Teflon centrifuge tube containing 1.2 g of clean-up salts comprising 900 mg of fine magnesium sulfate, 150 mg of primary secondary amine (PSA), and 150 mg of C18 sorbent. The mixture was vortexed for 5 min and then centrifuged at 3000 rpm for 5 min. The upper layer was again transferred into a 10-mL graduated glass test tube with a cap. To stabilize the extracted upper layer, 5% formic acid was added, and the contents were shaken for 1 min using a vortex shaker. Then, 1 mL of the extract was transferred into a round-bottom flask and evaporated to near dryness at 40°C using a rotary evaporator. The mixture was then reconstituted in 1 mL of a mixture of hexane and acetone in a 4:1 ratio. The extract was then transferred to an auto-sampler amber vial for

analysis using gas chromatography (GC-2010 Plus) coupled with mass spectrometry (MS) QP2010 Ultra (Shimadzu, USA), fitted with a VF5 MS cross-linked capillary column of 30 meters $\times$ 0.25 micrometers $\times$ 0.25 millimeters internal diameter. Helium gas was used as the carrier at a constant rate of 1.2 mL/min. The purge low was 3 mL/min with a linear velocity of 40 cm/s. The GC oven temperature was programed to increase from 60°C at a rate of 1 min, then to 200°C at a rate of 10°C per min (held for 2 min), and finally to 300°C at a rate of 10°C per min (held for 3 min). The splitless injection mode was used with an injection temperature of 250°C and an injection volume of 1 L. The ion source temperature for the MS was set to 200°C, and the interface temperature was set to 250°C. The solvent cut-off time was set at 3 min (Iwu et al., 2024).

Data Analysis

PAH analysis was performed in triplicate ($n = 3$). The chromatograms and data obtained from GC–MS analysis were processed using the computer-based GC software GC–MS Solutions provided by Shimadzu Corporation. Subsequently, the results were transferred to Microsoft Excel version 15.0 and presented in tables of results.

RESULTS AND DISCUSSION

Results

GC-MS Total Ion Chromatogram for Polycyclic Aromatic Hydrocarbons Detected in *Balangu* Fuelwood Smoked with Geza (*Combretum micratum*)

Table 1 shows the GC-MS TIC for PAHs detected in *Balangu* smoked with geza (*Combretum micratum*) fuelwood. Results indicated that a total of six (6) PAH compounds were identified, including 5-azulenemethanol, 2-naphthalenemethanol, 1-naphthalenol, 2-naphthalenemethanol, triphenylene, and perylene. Triphenylene occupied the largest (25.34%) part of the PAH content with a peak height of 975089. Figure 1 shows the PAH peaks in the GC-MS total ion chromatogram of balanga smoked with geza.

Table 1: Peak report of GC-MS Total Ion Chromatogram (TIC) for polycyclic aromatic hydrocarbons detected in *Balangu* smoked with geza (*Combretum micratum*) fuelwood.

Peak	Full Time	Area	Area (%)	Height	Height (%)	Name of the aromatic hydrocarbon
1	12.76	1240146	10.05	404573	8.68	5-Azulenemethanol
2	12.87	1079250	8.74	429062	9.21	2-Naphthalenemethanol
3	13.16	2951569	23.91	1490213	31.97	1-Naphthalenol
4	13.22	1187665	9.62	445515	9.56	2-Naphthalenemethanol
5	25.03	3128557	25.34	975089	20.92	Triphenylene
6	25.91	2757029	22.33	916238	19.66	Perylene
Total		12344216	100.0	4660690	100.0	

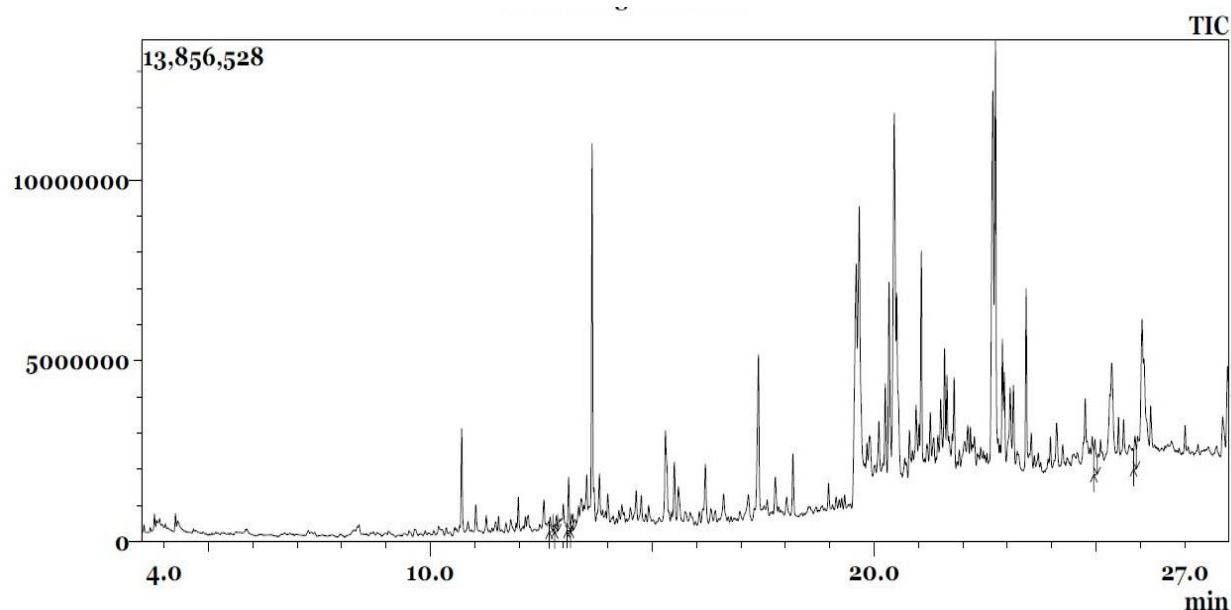


Figure.1: GC-MS total ion chromatogram of balance smoked with geza

GC-MS Total Ion Chromatogram (TIC) for polycyclic aromatic hydrocarbons detected in *balangu* smoked with *kalgo* (*Piliostigma thonningii*) fuelwood.

Table 2 shows the GC-MS TIC for PAHs detected in *Balangu* smoked with alko fuelwood. The results indicated that a total nine (9) PAH compounds were identified, including azulene, 1-methyl-naphthalene, 2-methyl-naphthalene, 2,6-dimethyl-naphthalene, 1,3-dimethyl-naphthalene, 2-(1-dimethylethyl)naphthalene, 4,6,8-trimethylethylazulene, 9,10-anthracenedione, and 10, anthracenediol. 1-methyl-naphthalene occupied the largest 919.75%) part of the PAH content with a peak height of 73972. Figure 2 shows the total ion chromatogram of polycyclic aromatic hydrocarbons detected in *Balangu* smoked with alko fuel wood.

Table 2: Peak report of GC-MS Total Ion Chromatogram (TIC) for polycyclic aromatic hydrocarbons detected in *kalgo* (*Piliostigma thonningii*) fuel wood smoked in *Balangu*.

Peak	Full Time	Area	Area (%)	Height	Height (%)	Name of the aromatic hydrocarbon
1	7.115	194707	16.73	82816	21.55	Azulene
2	8.840	229787	19.75	73972	19.25	1-methyl- Naphthalene
3	9.015	146517	12.59	45621	11.87	2-methyl- Naphthalene
4	10.29	124254	10.68	29510	7.68	2,6-dimethyl- Naphthalene
5	10.440	154465	13.28	55029	14.32	1,3-dimethyl- Naphthalene
6	11.530	52477	4.51	15041	3.91	2-(1-dimethylethyl) Naphthalene
7	12.120	78258	6.73	22303	5.80	4,6,8-Trimethylazulene
8	17.760	141841	12.19	41021	10.67	9,10-Anthracenedione
9	22.395	41166	3.54	18997	4.94	9,10-Anthracenediol
Total		1163472	100.00	384310	100.00	

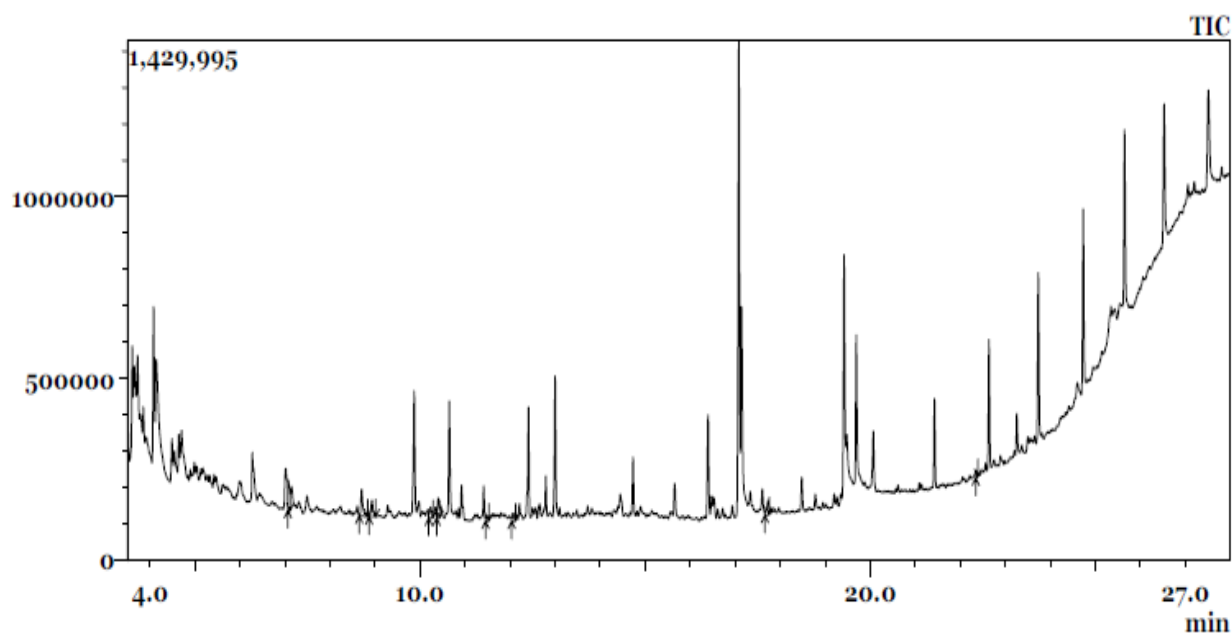


Figure 4.2: GC-MS total ion chromatogram of polycyclic aromatic hydrocarbons detected in *Balangu* smoked with *kalgo* fuel wood.

GC-MS Total Ion Chromatogram (TIC) for Detected Polycyclic Aromatic Hydrocarbons in Balangu Smoked with *Malga Cassia sieberiana* Fuelwood.

Table 3 shows the GC-MS TIC for PAHs detected in *Balangu* smoked with malga fuelwood. The results indicated that a total six (6) PAH compounds were identified including Naphthalene, 1-methylNaphthalene, 1, 6-dimethyl-Naphthalene, 1, 5-dimethyl-Naphthalene, 2-(1-methyl) Naphthalene and 4, 6, 8-Trimethylazulene. 1-methyl-naphthalene occupied the largest (27.55%) part of the PAH content with a peak height of 73972. Figure 3 shows the GC-MS total ion chromatogram (TIC) peak for polycyclic aromatic hydrocarbons detected in *balangu* smoked with malga fuel wood.

Table 3 GC-MS total ion chromatogram (TIC) peak report for polycyclic aromatic hydrocarbons detected in *balangu* smoked with malga fuel wood.

Peak	Full time	Area	Area (%)	Height	Height (%)	Aromatic hydrocarbon names
1	7.115	194707	23.35	82816	29.72	Naphthalene
2	8.840	229787	27.55	73972	26.54	1-methylNaphthalene
3	10.290	124254	14.90	29510	10.59	1,6-dimethyl- Naphthalene
4	10.44	154465	18.52	55029	19.75	1,5-dimethyl- Naphthalene
5	11.530	52477	6.29	15041	5.40	2-(1-methylethyl) Naphthalene
6	12.120	78258	9.38	22303	8.00	4,6,8-Trimethylazulene
Total		833948	100.00	278671	100.00	

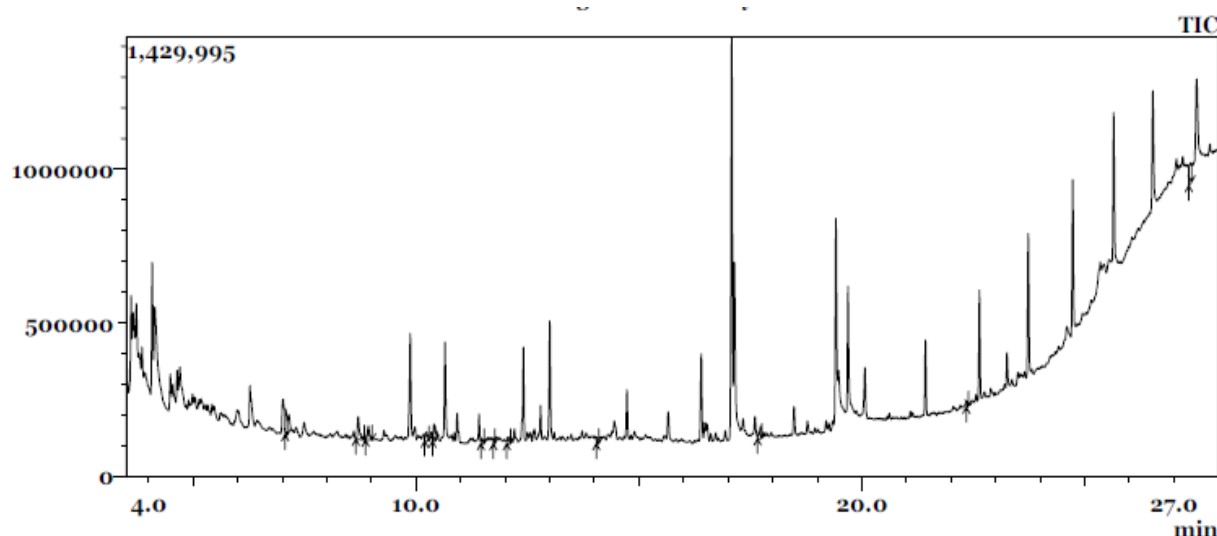


Figure 4: GC-MS total chromatogram of Balangu smoked with *Cassia sieberiana* malga

GC-MS Total Ion Chromatogram (TIC) for polycyclic aromatic hydrocarbons (PAH) detected in *Balangu* roasted in an oven

Table 4 shows the GC-MS TIC for PAHs detected in *Balangu* roasted with Oven. Result indicated that a total twelve (12) PAH compounds were identified, including naphthalene, 2-methyl-naphthalene, 2-methyl-naphthalene, 1,6-dimethyl-naphthalene, 1,3-dimethyl-naphthalene, 2-(1-methylethyl)naphthalene, 1,6,7-trimethyl-naphthalene, 2,3,6-trimethyl-naphthalene, 2,6-diisopropyl-naphthalene, 9,10-anthracenedione, 2-methy, 9,10-anthracenediol, 9,10-dihyd, and 1-naphthalenepropanol. 2-methylnaphthalene occupied the largest (15.55%) part of the PAH content with a peak height of 73972. Figure 4 shows the GC-MS total ion chromatogram (TIC) peak for polycyclic aromatic hydrocarbons detected in *Balangu* roasted with oven.

Table 4 Peak report of GC-MS Total Ion Chromatogram (TIC) for polycyclic aromatic hydrocarbons detected in *Balangu* roasted with oven.

Peak	Full time	Area	Area (%)	Height	Height (%)	Aromatic hydrocarbon names
1	7.115	194707	13.18	82816	16.98	Naphthalene
2	8.840	229787	15.55	73972	15.17	2-methyl- Naphthalene
3	9.015	146517	9.92	45621	9.35	2-methyl- Naphthalene
4	10.290	124254	8.41	29510	6.05	1,6-dimethyl- Naphthalene
5	10.440	154465	10.45	55029	11.28	1,3-dimethyl- Naphthalene
6	10.440	52477	3.55	15041	3.08	2-(1-methylethyl) Naphthalene

7	11.530	29000	1.96	17134	3.51	1,6,7-trimethyl- Naphthalene
8	12.120	78258	5.30	22303	4.57	2,3,6-trimethyl- Naphthalene
9	14.100	58211	3.94	20861	4.28	2,6-Diisopropylnaphthalene
10	17.760	141841	9.60	41021	8.41	9,10-Anthracenedione, 2-methy
11	22.395	41166	2.79	18997	3.89	9,10-Anthracenediol, 9,10-dihyd
12	27.395	226994	15.36	65460	13.42	1-Naphthalenepropanol, .alpha
Total		1477677	100.00	487765	100.00	

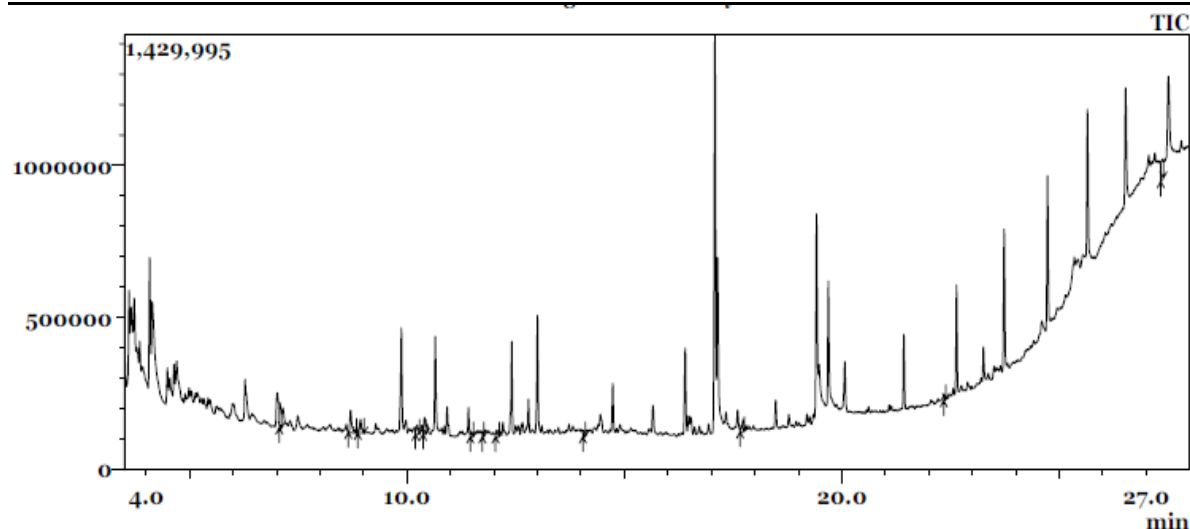


Figure 4: GC-MS Total Ion Chromatogram of Balangu Roasted in an Oven.

GC-MS Total Ion Chromatogram (TIC) for polycyclic aromatic hydrocarbons detected in *Balangu* smoked with *sabara* (*Geira senegelensis*) fuel wood.

Table 5 shows the GC-MS TIC for PAHs detected in *Balangu* smoked with sabara fuelwood. The results indicated that a total seven (7) PAH compounds were identified, including naphthalene, 2-methyl-naphthalene, 2,6-dimethyl-naphthalene, 1,5-dimethyl-naphthalene, 2-(1-methylethyl)-naphthalene, 1,6,7-trimethyl-naphthalene, 9,10-anthracenedione, and 2-ethyl-naphthalene. 1-methyl-naphthalene occupied the largest (23.55%) part of the PAH content with a peak height of 29510. Figure 4.5 shows the GC-MS TIC peak report for polycyclic aromatic hydrocarbons detected in *Balangu* smoked with sahara fuel wood.

Table 5: GC-MS total ion chromatogram (TIC) peak report for polycyclic aromatic hydrocarbons detected in *Balangu* smoked with sahara fuel wood.

Peak	Full Time	Area	Area%	Height	Height%	Name of the aromatic hydrocarbon
1	7.115	194707	19.95	73972	25.90	Naphthalene
2	8.840	229787	23.55	29510	23.14	2-methyl- Naphthalene
3	10.290	124254	12.73	55029	9.23	2,6-dimethyl- Naphthalene
4	10.440	154465	15.83	15041	17.21	1,5-dimethyl- Naphthalene
5	11.530	52477	5.38	22303	4.70	2-(1-methylethyl) Naphthalene
6	12.120	78258	8.02	22303	6.98	1,6,7-trimethyl- Naphthalene
7	17.760	141841	14.54	41021	12.83	9,10-Anthracenedione, 2-ethyl-
Total		975789	100.00	319692	100.00	

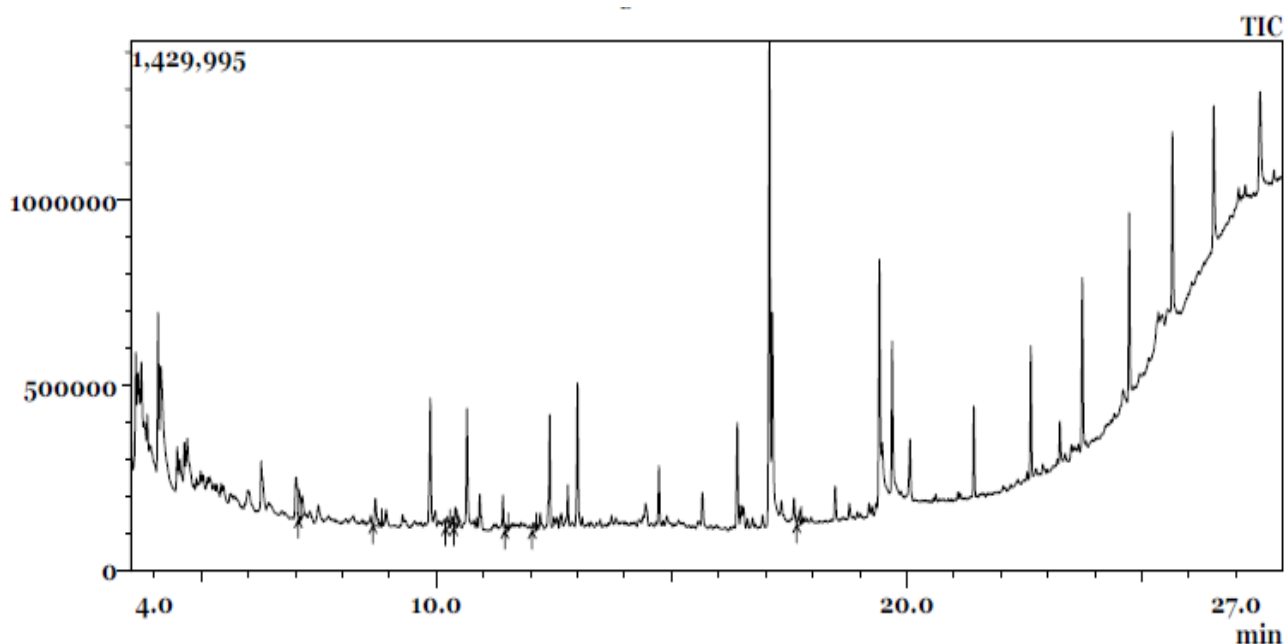


Figure 4.39: GC-MS total chromatogram of beef balanga smoked with *sahara* fuel wood.

Discussion

The most commonly detected PAHs in the processed balangu samples using different smoke sources include various groups of naphthalenes, azulene, methanol, naphthalene, triphenylene, perylene, trimethylazulene, anthracenedione, and anthracenediol. Similar studies have detected similar compounds from related works elsewhere. Andrés *et al.* (2019) reported that the highest concentrations of naphthalene, acenaphthene, fluoranthene, and pyrene were found in meat and fish samples from European markets. Samane *et al.* (2020) reported that anthracene and acenaphthylene were higher in the processed meat samples collected from Iran's market. Tracie *et al.* (2021) phenanthrene, anthracene, and pyrene, and that triple-smoked ham samples showed significantly higher levels of these PAHs than single-smoked ham. Iyekhoetin *et al.* (2020) detected pyrene, fluoranthene, and anthracene when investigating the mutagenic potential of 20 different commercially processed meat and fish products in Finland. Samuel *et al.* (2020) detected pyrene, anthracene, and perylene in processed meat and meat products.

Most of the PAHs identified from the research, especially the naphthalene groups, are of economic benefit and have not shown carcinogenic effects from various studies. However, 5-azulenemethanol has been listed as a non-hazardous substance or mixture according to the EPA. According to EU Directives 67/548/EEC or 1999/45/EC, azulene is toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Although naphthalene was reported as a non-hazardous substance or mixture according to Regulation (EC) No. 1272/2008, its potential health effects may cause eye and skin irritation. Ingestion of the compound may cause digestive tract irritation. It may cause respiratory tract irritation. Triphenylene has shown limited evidence of carcinogenic effect; however, it is a cancer suspect agent. Its potential health effects include eye and skin irritation and may cause digestive tract irritation and cardiac disturbances. May cause central nervous system depression.

Rema *et al.* (2024) reported pyrene in beef meat with an area of 20.84% and peak height of 19.56%. The present results may be similar to other findings because both samples undergo the same method of smoking and generate the same types of PAHs.

Yahaya *et al.* (2017) reported 1-methyl-naphthalene in beef with an area of 28.80% and peak height of 23.90%. Chronic exposure to naphthalene by inhalation or skin contact caused cataracts and retinal hemorrhage U S E PA (2014). The rate and extent of naphthalene absorption in humans exposed by inhalation have not been determined, but there is evidence for uptake via inhalation from case reports and occupational health studies. Prolonged exposure to naphthalene vapors can cause adverse health effects in humans, according to clinical reports Harden *et al.*, (2018). Ajiboye *et al.* (2011) reported 1,5-dimethylnaphthalene in beef with an area of 0,99% and peak height of 1,92%. Naphthalene enters the atmosphere from both emissions from industrial facilities and other localized sources and from mobile sources when wood is burned (IARC, 2002) Naphthalene enters the atmosphere from both emissions from industrial facilities and other localized sources and from mobile sources when wood is burned (IARC, 2002)

CONCLUSION

It was found out that from all the treatment assessed, the PAHs 2-methyl-Naphthalene and 2-(1-dimethyl)-Naphthalene were found to be more prevalent across the treatment, with less occurrences of 9, 10-Anthracenediol, Pylene, 1-Naphthalanol and 5-Azulenemethanol. The compound Naphthalene, effect human Health through inhalation, ingestion and dermal contract leads to a headache, nausea, vomiting, jaundice, irritation and inflammation. The presence of these organic compound in food is concerning and requires continuous monitoring. It is recommended that, Geza and Malga fuel wood species should be utilized by the butchers and households especially during festivities, this is due the fact that they have less number of PAHs detected.

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