

Research paper

Carcinogens: Polycyclic Aromatic Hydrocarbons and Heavy Metal Content in Some Locally Processed Foods in South Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors COU and FNU, designed the study. Authors COU, CUI, FNU and CSA, performed the statistical analysis, authors KLI and COU wrote the protocol, and the first draft of the manuscript. Authors COU and KLI, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript

ABSTRACT

This study investigated the presence of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in commonly consumed roasted meats and smoked fish in Owerri, Imo State, Nigeria. The processed foods were group as; freshly processed- 6 to 12 hours heat/flame/smoke exposure and long processed- 7 days heat/flame/smoke exposure. Freshly processed (FP) and long processed (LP) beef, fatty beef, chicken, fish and pork samples were purchased from road-side food vendors, wrapped in clean labelled paper foil and taken to the laboratory for analysis. Gas chromatography with flame ionization detector (GC-FID) was used for the evaluation of PAH and atomic absorption spectrophotometer (AAS) was used to determine heavy metals. The total concentration of PAHs in LP food samples was higher when compared to FP samples, whereas variations in concentration of individual PAHs in both samples were observed. Benzo(a)pyrene and other PAHs which are probable human carcinogen, were present in varying concentrations in the samples. Toxic heavy metals such as lead and mercury were present at varying concentrations in the samples, while mercury was not detected in any of the samples. This study have added to the numerous evidences that processing of food by exposure to heat, flame and smoke encourages the generation of toxic compounds and the nature of the food chain and certain environmental activities can impact negatively on their nutritional content. This therefore places consumers at potential health risk.

Keywords: PAHs, Benzo(a)pyrene, Mutagens, Cancer, Toxicants, smoke, roasting

1. INTRODUCTION

Studies have shown cancer to be a major cause of human mortality among the numerous diseases afflicting mankind. Human diet and dietary habits are factored in the aetiology of cancer, making nutrition to account for one third of the total cause of cancer (1). Cancers most commonly associated with diet include esophageal, stomach, colon, liver and the prostate. Several industrial and daily human activities release toxic and harmful compounds into the environment such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals. PAHs are fused aromatic rings (2), formed from incomplete combustion of organic materials such as; coal, oil, gas, wood, fossil fuels and biomass.

Some PAHs are identified as mutagenic and carcinogenic. In mammalian cells PAHs undergo metabolic activation to diol epoxides that bind covalently to cellular macromolecules, such as DNA, leading to errors in DNA replication and mutations that can initiate carcinogenesis (3). Studies have shown that most food intake of PAHs comes from vegetables and cooked meats (4). Exposure includes breathing in PAHs in tobacco smoke, auto emissions or industrial exhausts, consumption of charcoal broiled food and contaminated water. They can also be absorbed through the skin when in contact. The impact of PAHs exposure are observed largely at the lungs, breast, oropharynx, genitourinary and gastrointestinal tracts (5). Several animal and human studies have implicated colon cancer and other forms of cancer to dietary exposure to PAH (6).

Heavy metals when released in the environment are toxic to humans and animals (7). Some heavy metals are beneficial to the body while others are toxic to the system. The largest man-made sources include combustion of fossil fuels and industrial processes. Exposure to metals may occur through the diet, medications, the environment, or in the course of work or play. Chronic illness may be caused by heavy metal toxicity from the environment and food supply. Toxic heavy metals target sites which includes; membrane structural proteins, enzymes, or DNA molecules. Once at the target site, they can displace (mimic) an important mineral from its binding site (8).

In South Eastern Nigeria, some foods are prepared by roasting and smoking. These foods are often consumed by several people including the upper, middle and lower classes, for their nutritional value and easy access. They can be purchased from food centres often sited at strategic locations, especially at road sides and street junctions. This research was carried out to determine the presence and concentration of polycyclic aromatic hydrocarbons and heavy metals in high protein containing food such as lean beef, fatty beef, fish, chicken and pork. The result may serve as pointers to potential health hazards to consumers.

2. MATERIALS AND METHOD

2.1 Preparation of Food Samples

The samples used were non fatty beef (this will be referred here as beef) and fatty beef (known as "Suya"), chicken, pork and mackerel fish. The locally processed food samples were grouped into fresh processed (FP) and long processed (LP). The fresh processed samples were exposed to 6 - 12 hours heat/flame/smoke generated by firewood and charcoal. The long processed samples were exposed initially (6-12 hours) to heat/flame/smoke from firewood and mild/consistent heating for 7 days. The fish sample was processed in similar way but were exposed to smoke generated by firewood. Samples were wrapped in clean labelled paper foil and taken to the laboratory for analysis. The foods were processed by food vendors under the supervision of the researchers. The food vendors were located along streets of Ihiagwa market and mammy market Obinze in Owerri West Local Government, Imo State. These areas witnesses heavy vehicle movement and road construction activities at the time of study.

2.2 Determination of PAHs by Gas Chromatography (GC)

To start the cold extraction 2 g of each sample was weighed into a clean extraction container and 10 ml of dichloromethane was added, thoroughly mixed and allowed to settle. The mixture was carefully filtered into clean solvent and rinsed into extraction bottle, using filter paper fitted into Buchner funnel. The Extract was concentrated to 2 ml and then transferred for cleanup and separation (This involved further purification of the extract prior to gas chromatographic analysis). To achieve this, 1 cm of moderately packed glass wool was placed at the bottom of 10 mm ID × 250 mm long chromatographic column. Slurry of 2 g activated silica in 10 ml dichloromethane was prepared and placed into the chromatographic column. To the top of the column 0.5 cm of sodium sulfite was added.

The column was rinsed with additional 10 ml of dichloromethane. The column was pre-eluted with 20 ml of dichloromethane and this was allowed to flow through the column at a rate of about 2 minutes until the liquid in the column was just above the sulfite layer. Immediately, 1 ml of the extracted sample was transferred into the column. The extraction bottle was rinsed with 1 ml of dichloromethane and added to the column as well. The stop-cork of the column was open and the eluant was collected with a 10 ml graduated cylinder.

Prior to exposure of the sodium sulfite layer to air, dichloromethane was added to the column in 1-2 ml increments. Accurately measured 10 ml of the eluant was collected and labeled aliphatic. The concentrated aliphatic fractions were transported into labeled glass vials with teflon or rubber crimps caps for GC analysis. 1 µL of the concentrated sample was injected by means of syringe through a rubber septum into the column. Separation occurs as the vapour constituents partition between the gas and liquid phases. The sample was automatically detected as it emerges from the column (at a constant flow rate) by the Flame Ionization Detection (FID) detector whose response was dependent upon the composition of the vapour.

2.3 Determination of Heavy Metal Content

A sample weight of 5 g was burnt to ash and ash obtained was dissolved in 5 ml of 10 % HCl. The concentration of lead (Pb), cadmium (Cd), mercury (Hg), zinc (Zn), and iron (Fe) was determined by atomic absorption spectrophotometer as described by James (9).

2.4 Statistical Analysis

The data were expressed as mean ± standard deviation and the significant difference among the group was assessed using one way analysis of variance (ANOVA).

3. RESULTS

3.1 Concentration of Polycyclic Aromatic Hydrocarbons in Freshly Processed Samples

The content and concentration of different PAHs in fresh processed samples are shown in Table 1. The total PAH varied from 0.021 mg/kg in FP pork to 0.213 mg/kg in FP fish. The concentrations of 16 PAHs compounds were analysed in the freshly processed food samples. Acenaphthylene and acenaphthene were not detected in chicken. Also acenaphthene, fluorene and phenanthrene were not detected in fatty beef. Fluorene was not detected in beef while Naphthalene was not detected in all the samples. The presence of benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene classified as probably carcinogenic to humans (Group 2A) is very important. The concentrations of benzo[a]pyrene were 7.88×10^{-4} , 1.25×10^{-4} , 6.48×10^{-5} , 5.20×10^{-6} and 1.76×10^{-3} in beef, fatty beef, fish, chicken and pork respectively. The concentrations of benz[a]anthracene were 4.59×10^{-5} , 2.26×10^{-7} , 1.73×10^{-4} , 3.44×10^{-6} and 6.10×10^{-6} in beef, fatty beef, fish, chicken and pork respectively. Also the concentrations of dibenz[a,h]anthracene were 8.0×10^{-8} , 3.24×10^{-5} , 9.10×10^{-5} , 3.46×10^{-5} and 8.05×10^{-6} in beef, fatty beef, fish, chicken and pork respectively. Some of the detected PAHs are possibly carcinogenic to humans (Group 2B) or not classifiable as to its carcinogenicity to humans (Group 3).

3.2 Concentration of Polycyclic Aromatic Hydrocarbons in Long Processed Samples

The concentration of various PAH compounds of long processed samples are presented in Table 2. The results showed that total PAHs ranged from 0.015 mg/kg in LP pork to 0.934 mg/kg in LP fish. The concentrations of benzo[a]pyrene were 3.12×10^{-5} , 5.01×10^{-5} , 5.205×10^{-4} , 1.258×10^{-5} and 3.204×10^{-4} in beef, fatty beef, fish, chicken and pork respectively. The concentrations of benz[a]anthracene were 3.2×10^{-5} , 1.19×10^{-4} , 7.5×10^{-4} , 3.1105×10^{-5} , and 7.076×10^{-6} in beef, fatty beef, fish, chicken and pork respectively. Similarly, the concentrations of dibenz[a,h]anthracene were 8.66×10^{-5} , 2.874×10^{-4} and 1.59×10^{-5} in beef, fish and chicken respectively. Dibenz[a,h]anthracene were not detected in fatty beef and pork.

3.3 Heavy Metal Content of Fresh and Old Processed Food Samples

Table 3 presented the concentration of heavy metals in fresh and long processed samples. The concentration of iron ranged from 14.92 ± 0.63 mg/kg in LP beef to 43.52 ± 0.41 mg/kg in LP chicken. Mercury was less than 0.001 mg/kg in all the samples and was termed not detected. Zinc content of the various food samples were observed to be higher in concentration than other elements determined. The concentration of zinc varied from 20.80 ± 0.80 mg/kg in FP chicken to 109.40 ± 0.70 mg/kg in FP beef. Appreciable amount of zinc was observed in LP beef (76.25 ± 0.35 mg/kg) and pork (85.00 ± 0.25 mg/kg) and in FP fatty beef (83.45 ± 0.70 mg/kg). Cadmium concentration ranged from 0.180 ± 0.011 mg/kg in FP fatty beef to 2.345 ± 0.35 mg/kg in LP beef. The concentration of lead varied from 0.885 ± 0.08 mg/kg in FP pork to 9.910 ± 0.39 mg/kg in LP beef.

In the beef sample cadmium and Pb concentrations were higher ($P < 0.05$) in LP than in FP samples. Significantly higher concentration of Fe was observed in FP beef than in LP beef. Also concentrations of Zn in FP beef were in beef higher ($P < 0.05$) than in LP beef samples. Iron concentration in fatty beef was significantly higher ($P < 0.05$) in LP than in FP. Zn and Pb concentrations in fatty beef were higher ($P < 0.05$) in FP than in LP fatty beef. Cd concentration in the LP fatty beef was significantly higher than in FP fatty beef.

Concentration of iron and Zinc in LP fish was higher ($P < 0.05$) than the concentrations of iron and zinc in the FP fish. Cd concentration in the FP fish was significantly higher than in LP fish. The concentration of Pb in LP fish was higher ($P < 0.05$) than the concentration in FP roasted fish. Zn and Fe concentrations in LP chicken were significantly higher when compared to FP chicken. Concentration of cadmium in LP chicken varied non-significantly with FP chicken. Pb concentration in FP chicken was higher ($P < 0.05$) than the concentration in LP chicken. The concentrations of Zn and Pb in LP pork were significantly higher. The higher Cd concentration in FP pork varied non-significantly when compared to the LP.

4. DISCUSSION

Polycyclic aromatic hydrocarbons are a group of chemicals that result from incomplete burning of coal, oil, gas, wood, fossil fuels in general and biomass (5). Studies have shown that most food intake of polycyclic aromatic hydrocarbons come from vegetable and cooked meats (4). PAHs have been identified as mutagenic and carcinogenic compounds and benzo(a)pyrene is among the most potent and best documented carcinogen (10,11). Studies in experimental animals have shown various toxicological effects, such as haematological effects, reproductive and developmental toxicity and immunotoxicity. The most important is the carcinogenic and genotoxic (DNA-damaging) potential of these compounds. A number of PAHs have shown carcinogenicity in experimental animals and genotoxicity and mutagenicity in vitro and in vivo.

The results obtained in this study showed that the total concentration of PAHs in long processed food were higher than the concentration in the freshly processed. This could be attributed to longer duration

processing (12). Also roasting very close to the heat source at very high temperature is an important factor. This ensures properly drying for long time storage and spoilage prevention (13,14). The highest amount of PAHs observed in fresh processed fish compared to other fresh processed samples could be due to the type of fuel used (15) and the exposure of the sample to smoke generated by the firewood. The local processing of fish allowed smoke from incomplete wood burning to come into direct contact with the fish. This can lead to its considerable contamination with various PAHs because the process is poorly controlled. The least concentration of total PAHs obtained in fresh processed pork compared to other fresh processed samples indicates the effect of processing. Food vendors pre-cook the pork sample before roasting. This procedure may reduce both the fat content and time of cooking on open flame. The fresh roasting of the pork may be to impart flavours and colours (16). Higher concentration of the PAHs in old roasted fatty beef compared to the freshly roasted fatty beef may be as a result of higher fat content of meat (17), and pyrolysis resulting from increased amount of melted fat dropped on heat source (15). Also the lower amount of total PAHs in beef compared to fatty beef can attributed to fat content as reported by Mottier *et al.* (18) who found significantly higher levels of carcinogenic PAHs in high fat containing lamb sausages.

A cursory look at the individual PAHs showed that naphthalene was not detected in any of the samples. This may be due to its volatile nature. Naphthalene is the lightest amongst the PAHs with 2-fused benzene rings and easily sublimates at room temperature (19). However, study of Houessou *et al.*, (20) on the formation of PAHs during the roasting process indicated a possible transformation of low molecular PAHs to high molecular PAHs as roasting temperature increased. This transformation may be implicated in the increased concentration of high molecular weight PAHs which are largely carcinogenic (i.e benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene) in the long processed samples

The concentration benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene which are classified as probable carcinogens to humans (Group 2A) are of great health concern to consumers. Benzo[a]pyrene is regarded as the most potent PAHs. Its metabolism in living system increases its toxicity (21). As the living system attempts to degrade Benzo[a]pyrene, it forms reactive metabolite which binds to DNA causing disruption to the genetic functioning of the affected cells inducing a genotoxic effect. (21, 22, 23). The highest concentrations of benzo[a]pyrene were obtained in freshly processed pork (1.757×10^{-3} mg/kg) and long processed fish (5.01×10^{-4} mg/kg). It was a surprise to find highest amount of benzo[a]pyrene in FP pork which total PAHs ranked among the least. Indeno (1,2,3,-cd)pyrene and Benzo(k)fuoranthene at concentrations of 6.6×10^{-2} mg/kg and 3.64×10^{-3} mg/kg respectively were among the highest detected carcinogenic PAHs in long processed beef sample. Similar pattern was observed in all LP samples. These observations indicate the assertion that roasting imparts carcinogenic compounds in food (24,25).

Studies have shown some heavy metals to be toxic while some referred to as trace elements are beneficial to life in certain quantities. The results (Table 3) of this study showed the presence of lead, cadmium, zinc, and iron. Mercury was not detected. The concentration of lead varied from 0.71 ± 0.01 mg/kg in FP chicken to 9.91 ± 0.39 mg/kg in LP beef. Lead value of 5.13 ± 0.23 mg/kg in FP beef and 3.70 ± 0.4 mg/kg in LP fish were also observed. Lead is number 2 on the Agency for Toxic Substances and Disease Registry's (ATSDR) top 20 hazardous substances. Lead is an important paediatric heavy metal poisoning (26). It affects bones, brain, blood, kidneys, and thyroid gland on exposure (27,28).

Acute lead exposure can affect cardiac function, and chronic exposure may affect the electrical and mechanical activity of the heart via the alteration of vascular smooth muscle function as observed in experimental animals. Intracellular lead substitutes calcium in calcium-dependent signaling pathways by interacting with calmodulin and calcium-dependent potassium channels. Lead can affect the intercellular messenger protein kinase C and its role in smooth muscle contraction (29). Lead absorption is enhanced by deficiencies of iron, calcium, and zinc (30).

The concentration of cadmium ranged from 0.18 ± 0.01 mg/kg in FP beef to 0.895 ± 0.01 mg/kg in FP fish. Cadmium is present in insecticides, fungicides, sludge, and fertilizers used in agriculture. About 50% of absorbed cadmium is through respiration and about 7% absorbed in the gastrointestinal system is taken

up with food. Organs such as the liver, placenta, kidneys, lungs, brain, and bones can be affected by cadmium exposure (26,28). Cadmium and its compounds accumulation have a possible risk of irreversible effects on human health. A primary mechanism for cadmium toxicity is its effect on cells which has been ascribed to the oxidative stress promoting cadmium action, as observed in vivo, and most importantly, the depletion of glutathione and alteration of sulfhydryl homeostasis, thus indirectly increasing oxidative stress and lipid peroxidation (29). Cadmium and Lead concentration content in the samples may be due to metal bioaccumulation in food chain and the period of exposure and closeness of samples to site of industrial activities such as road construction, exposure to dust and emissions from disposal and burning of hazardous wastes such as electronics and batteries (31).

Iron and Zinc are nutritionally essential for a healthy life. The presence of iron and zinc may be as a result of its natural occurrence in food stuff. For example studies have shown low fat roasted beef as one of the top five foods rich in zinc (32). Iron and zinc are important for proper immune system function. Iron is a constituent of red blood cells and functions as primary carrier of oxygen throughout the body. Zinc supports normal growth and development, and is important for protein and DNA synthesis (32). Metals such as zinc, iron, copper and cobalt serve as catalyst of enzymatic reactions. Most enzymes require ions to either activate or stabilize them. For example, iron (a stabilizing ion) is a structural and functional component of the cytochrome P-450 enzyme system and zinc (an activating ion) forms a temporary bond with alkaline phosphatase (an enzyme needed for bone formation) (33). Generally, the presence of these heavy metals in elevated levels could be as a result of bioaccumulation over time. They could be picked up through diet and transferred through the food chain even before they are processed (34).

4.1 Conclusion

This study has shown that polycyclic aromatic hydrocarbons and heavy metals are present in fresh and prolonged processed foods. Since the metabolites of PAHs targets cellular macromolecules such as DNA and this may lead to mutations and carcinogenesis while toxic heavy metals targets membrane structures, enzymes or DNA. Exposure to these compounds can impact negatively on living system causing serious adverse health problems to living organisms

Considering the findings and observations of this study, we recommend the following measures: Increased awareness on the toxicity and risk of PAHs and heavy metals. This will encourage better processing methods of foods by individuals in order to reduce the health risk. If roasting must take place, it should be done in places free from heavy metal emissions, and food samples should not be placed too close to the heat source. PAHs and heavy metal tolerable limits and standard in food stuff should be developed by regulatory bodies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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386 **Table 1: Concentration (mg/kg) of Polycyclic Aromatic Hydrocarbons in Freshly Processed**
 387 **Samples**

Type of PAH	Beef	Fatty beef	Fish	Chicken	Pork
Naphthalene	ND	ND	ND	ND	ND
Acenaphthylene	2.45×10^{-5}	7.27×10^{-6}	5.47×10^{-4}	ND	1.12×10^{-4}
Acenaphthene	9.94×10^{-6}	ND	1.14×10^{-4}	ND	3.56×10^{-5}
Fluorene	ND	ND	1.42×10^{-4}	9.34×10^{-6}	5.32×10^{-6}
Anthracene	2.41×10^{-5}	2.6×10^{-8}	5.19×10^{-4}	1.83×10^{-5}	2.12×10^{-4}
Phenanthrene	8.49×10^{-6}	ND	8.21×10^{-4}	2.22×10^{-5}	1.553×10^{-5}
Fluoranthene	2.04×10^{-5}	4.64×10^{-6}	1.02×10^{-3}	2.86×10^{-5}	4.573×10^{-5}
Pyrene	82.0×10^{-5}	2.08×10^{-6}	3.75×10^{-4}	3.11×10^{-6}	4.323×10^{-5}
Benz(a)anthracene	4.59×10^{-5}	2.26×10^{-7}	1.73×10^{-4}	3.44×10^{-6}	6.10×10^{-6}
Chrysene	5.72×10^{-5}	3.61×10^{-6}	8.01×10^{-4}	2.60×10^{-6}	3.049×10^{-5}
Benzo(b)fluoranthene	3.83×10^{-2}	7.81×10^{-3}	1.84×10^{-2}	2.22×10^{-3}	3.14×10^{-3}
Benzo(k)fluoranthene	4.95×10^{-6}	2.67×10^{-6}	1.88×10^{-5}	9.0×10^{-8}	7.1×10^{-7}
Benzo(a) pyrene	7.88×10^{-4}	1.25×10^{-4}	6.48×10^{-5}	5.20×10^{-6}	1.76×10^{-3}
Indeno (1,2,3-ed)pyrene	1.34×10^{-3}	3.50×10^{-2}	1.93×10^{-1}	2.94×10^{-2}	1.53×10^{-2}
Dibenz(a,h)anthracene	8.0×10^{-8}	3.24×10^{-5}	9.10×10^{-5}	3.46×10^{-5}	8.05×10^{-6}
Benzo(g,h,i) perylene	9.32×10^{-4}	1.22×10^{-4}	2.37×10^{-4}	3.0323×10^{-4}	6.293×10^{-5}

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 389 **ND → Not Detected**
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403 **TABLES 2: Concentration (mg/kg) of Polycyclic Aromatic Hydrocarbons in the in long processed**
 404 **Samples**

Type of PAH	Beef	Fatty beef	Fish	Chicken	Pork
Naphthalene	ND	ND	ND	ND	ND
Acenaphthylene	1.6×10^{-6}	1.247×10^{-4}	4.25×10^{-5}	4.79×10^{-5}	5.882×10^{-4}
Acenaphthene	1.04×10^{-5}	ND	1.843×10^{-4}	0.869×10^{-5}	2.48×10^{-5}
Fluorene	1.44×10^{-5}	ND	2.5×10^{-5}	1.198×10^{-5}	ND
Anthracene	0.162×10^{-5}	6.008×10^{-4}	1.85×10^{-4}	1.264×10^{-4}	ND
Phenanthracene	3.7×10^{-5}	ND	7.63×10^{-4}	0.527×10^{-5}	ND
Fluoranthene	1.08×10^{-4}	4.428×10^{-4}	3.09×10^{-2}	5.385×10^{-5}	3.077×10^{-5}
Pyrene	3.3×10^{-5}	2.619×10^{-5}	1.941×10^{-3}	5.188×10^{-5}	2.007×10^{-5}
Benz(a)anthracene	3.2×10^{-5}	1.19×10^{-4}	7.5×10^{-4}	3.1105×10^{-5}	7.076×10^{-6}
Chrysene	8.47×10^{-5}	1.654×10^{-4}	5.24×10^{-4}	4.7112×10^{-5}	4.87×10^{-6}
Benzo(b)fluoranthene	3.64×10^{-3}	3.02×10^{-2}	8.477×10^{-1}	1.6076×10^{-1}	8.92×10^{-3}
Benzo(k)fluoranthene	2.1×10^{-6}	1.96×10^{-4}	5.37×10^{-5}	6.35×10^{-6}	ND
Benzo(a) pyrene	3.12×10^{-5}	5.01×10^{-5}	5.205×10^{-4}	1.258×10^{-5}	3.204×10^{-4}
Indeno (1,2,3-cd)pyrene	6.6×10^{-2}	6.5×10^{-2}	4.954×10^{-2}	3.254×10^{-2}	ND
Dibenz(a,h)anthracene	8.66×10^{-5}	ND	2.874×10^{-4}	1.59×10^{-5}	ND
Benzo(g,h,i) perylene	1.796×10^{-5}	1.895×10^{-3}	2.654×10^{-5}	3.53×10^{-5}	2.698×10^{-5}

405 **ND → Not Detected**

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Table 3. Heavy metal content (mg/kg) of fresh processed and long processed sample

SAMPLE IDENTITY	IRON	MERCURY	ZINC	CADMIUM	LEAD
Fresh processed beef	41.69±0.84	<0.001	109.4±0.7	0.633±0.04	5.13±0.23
Long processed beef	14.93±0.63	<0.001	76.25±0.35	2.345±0.35	9.91±0.39
Fresh processed fatty beef	20.27±0.35	<0.001	83.45±0.7	0.18±0.01	2.245±0.07
Long processed fatty beef	43.50±0.40	<0.001	25.55±0.4	0.265±0.04	2.045±0.15
Fresh processed fish	26.20±0.40	<0.001	23.70±0.6	0.895±0.01	0.705±0.01
Long processed fish	40.85±0.62	<0.001	51.95±0.49	0.51±0.02	3.70±0.40
Fresh processed chicken	25.14±0.24	<0.001	20.80±0.8	0.415±0.3	0.705±0.01
Long processed chicken	43.52±0.41	<0.001	44.00±0.30	0.443±0.04	3.70±0.40
Fresh processed pork	23.85±0.39	<0.001	62.20±0.20	0.355±0.06	0.885±0.08
Long processed pork	17.57±0.44	<0.001	85.00±0.25	0.305±0.01	2.135±0.03

Values = mean ± SD of triplicate determinations