IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 4 Ver. I (Apr. 2015), PP 21-27
www.iosrjournals.org

Impact of Cooking Methods on the Levels of Polycyclic Aromatic Hydrocarbons (PAHs) in Chicken Meat

Onwukeme, V. I., Obijiofor, O. C., Asomugha, R. N. and Okafor, F. A.
Pure and Industrial Chemistry Department, NnamdiAzikiwe University, Awka, Anambra State, Nigeria.

Abstract: *This study investigated the presence of polycyclic aromatic hydrocarbons (PAHs) in the different cooking methods of chicken. The levels of 16 PAHs compound were determined in raw and cooked chicken meats. The chicken was cooked with different methods, i.e. boiling, frying, barbequing and roasting. The uncooked sample served as the reference. With the aid of the 16 PAHs reference standards, the levels of the PAHs were determined using gas chromatography - flame ionization detector (GC-FID) after extracting with methylene chloride by soxhlet extraction.Column chromatographic clean – up was employed for the PAHs extraction, packed with anhydrous sodium sulphate and silica gel with amixture of pentane and methylene chloride as the eluting solvents.The obtained data showed the total PAHs as 0.0521,01408, 10.8374, 0.2008 and 0.1817 and total carcinogenic PAHs as 0.0516, 0.0933, 7.4868, 0.1343 and 0.0.3610 µg/kg in the control, boiled, fried, barbecued and roasted chicken samples respectively.*

Keywords**:** *Carcinogenic PAHs clean – up, cooking methods of chicken, GC – FID,soxhletextraction.*

I. Introduction

Chicken is one of the world's favourite foods, according to USDA, chicken is being cooked in many different ways, either ordinarily or combined with other foods such as grains, vegetables or fruits. It can be used in appetizers, soups, salads, sandwiches and main dishes. Deep-fat frying, grilling, broiling, roasting, baking, stir-frying and braising are the more common cooking methods. Among these, deep fat frying and grilling are probably the most popular dry-heat cooking methods [1].

Cooking and food processing at high temperature have been shown to generate various kinds of genotoxic substances or cooking toxicants, including PAHs. Grilling (broiling) meat, fish or other foods with intense heat over a direct flame results in fat dripping on the hot fire and yielding flame containing a number of PAHs [2]. These chemicals adhere to the surface of the food. The more intense the heat, the more the PAHs is present [3].

Polycyclic aromatic hydrocarbons (PAHs) are a class of high lipophilic compounds that comprise a class of chemical compounds known to be potent carcinogens. PAHs are present in the environment; in water, air, soil and traces of these substances have been found in various food products. Food can become contaminated during thermal treatments that occur in processes of food preparation and manufacture (drying and smoking) and cooking (roasting, baking, and frying) [4]. Most PAHs are chemically inert, hydrophobic, andsoluble in organic solvents. PAHs are ubiquitous environmental pollutants, resulting from the incomplete combustion or pyrolysis of organic matter during industrial processing and various human activities. They originate from diverse sources such as tobacco smoke, engine exhausts, petroleum distillates, and coal-derived products, with combustion sources predominating [5]. Due to their carcinogenic activity, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Human exposure to PAHs occurs in three ways, inhalation, dermal contact and consumption of contaminated foods. Diet is the major source of human exposure to PAHs as it accounts for 88 to 98% of suchcontamination [6]. Processing of food at high temperatures (grilling, roasting, frying and smoking) are major sources generating PAHs. Levels as high as 200 µg/kg have been found for individual PAH in smoked fish and meat samples. For instance, in barbecued meat, 130 µg/kg has been reported whereas the average background values are usually in the range of 0.01 to 1 µg/kg in uncooked foods [7].

PAHs in food samples have been analysed by HighPerformance Liquid Chromatography (HPLC) with Ultraviolet (UV) or Fluorescence detection (FLD), Gas Chromatography- Flame Ionization Detection (GC - FID), Gas Chromatography–Mass Spectrometry (GC–MS) and GC– MS–MS. Most of these methods, however, require sample preparation steps, such as extraction, concentration, and isolation, to enhance the sensitivity and selectivity of their detection. For example, liquid– liquid extraction with several organic solvents, pressurized liquid extraction gel permeation or open column chromatography and solid-phase extraction (SPE) have been used as cleanup procedures [4]. These contemporary analytical procedures make it possible to determine individual PAH in smoked foods at concentrations of the order of 0.1µg/kg or even 0.01 µg/kg [8].

II. Materials and Method

Sampling and Preparation

The fattened broiler chickens used in this study were obtained from a local market at Eke-Awka, Anambra State, Nigeria. The cooking condiments which comprised of garlic cloves, ginger, curry powder, thyme, common salt (sodium chloride) and seasoning cubes were also gotten from the same local market.

The chickens were slaughtered and the feathers were removed by inserting in hot water and subsequently plucked with the hand. After cleaning, the chicken meats were made into five portions, four of which were later mixed together, seasoned and boiled. After boiling, the four portions initially mixed together were re-established. One portion was fried, one was roasted, one was barbecued and the last portion was left without further treatment.

All five portions of the chicken meat were separately dried to a constant weight in the oven at 60°C and allowed to cool in a desiccator.

The cooled samples were pulverised using a ball grinder, further sifted through a 0.5mm mesh to a fine particle size for exhaustive extraction. 10g was weighed for each sample, mixed with 10g of anhydrous sodium sulphate (Na_2SO_4).

PAHs Extraction

The blended sample was transferred into a soxhlet extractor thimble. Approximately 200 mL of the extraction solvent (methylene chloride) was measured into a 500-mL round bottom flask containing two clean boiling chips. The flask was attached to the extractor and the sample was extracted for 6 hours at 4 - 6 cycles/hour. The extract was allowed to cool after the extraction was complete [9].

Sample Pre-concentration and Clean-up

The methylene chloride extract was made to dry up on a water bath set at 50°C and exchanged with 4ml Cyclohexane for clean-up. A column chromatographic technique was employed for the clean-up. 10g of previously activated 100/200 mesh silica gel at 130°C for 16 hours was weighed into a 50ml beaker with sufficient volume of methylene chloride, stirred with a glass stirring rod until an even slurry was made. The slurry was transferred into a previously cleaned and oven dried 10mm ID chromatographic column. The column was tapped to settle the silica gel and eluted the methylene chloride. 1-2 cm of anhydrous sodium sulphate was added to the top of the silica gel [10].

The column was pre-eluted with 40 mL of pentane. The rate for all elution was about 2 mL/min. The eluate was discarded and just prior to exposure of the sodium sulphate layer to the air, 2 mL cyclohexane sample extract was transferred onto the column using an additional 2 mL cyclohexane to complete the transfer. Just prior to exposure of the sodium sulphate layer to the air, 25 mL of pentane was added and the elution of the column continued. Pentane eluate was discarded [10].

Next, the column was eluted with 25 mL of methylene chloride/pentane (2:3) (V/V) into a 50 mL K-D flask equipped with a 10 mL concentrator tube. The collected fraction was further concentrated to dryness and finally reconstituted in 1 mL n-hexane for GC/FID analysis [10].

Stock Standard Solutions

A stock standard solution previously prepared at a concentration of 1.00ug/uL by dissolving 0.0100 g of assayed reference material in n-hexane and diluting to volume in a 10-mL volumetric flask. The stock standard solution was transferred into Teflon-sealed screw cap bottle. Store at 4°C and protected from light.

Sample Analysis

Calibration standards: Calibration standards of five concentration levels (0.1, 0.5, 1.0, 1.5 and 2.0 µg/ml)were prepared through dilution of the stock standards(1000µg /mL)with n-hexane. One of the concentration levels was at a concentration near, but above, the method detection limit. The remaining concentration levels corresponded to the expected range of concentrations found in real samples or as defined by the working range of the GC/FID.

III. Results and Discussions

Table 1: Levels of PAHs in the control and other four differently cooked chicken samples

	Raw Chicken (control) (µg/kg)	Boiled Chicken (µg/kg)	Fried Chicken (µg/kg)	Barbecued Chicken (µg/kg)	Roasted Chicken (µg/kg)
Naphthalene	ND	ND	0.0030	ND	ND
Acenaphthene	ND	ND	ND	ND	ND
Acenaphthylene	ND	0.0475	ND	0.0073	ND
Fluorene	ND	ND	0.0355	0.0094	ND
Phenanthrene	0.0005	ND	0.0095	0.0044	0.0008
Anthracene	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	0.0079	0.0006	0.0016
Pyrene	ND	ND	3.2947	0.0448	ND
1, 2 Benzoanthracene*	ND	ND	ND	ND	ND
Chrysene**	ND	ND	ND	0.0612	ND
Benzo[b]flouranthene**	0.0117	ND	ND	ND	ND
Benzo[k]flouranthene**	ND	ND	2.2733	0.0379	ND
Benzo[a]pyrene*	0.0399	0.0933	1.8249	0.0352	0.1793
Indeno[1,2,3-cd]pyrene**	ND	ND	3.3886	ND	ND
Dibenzo[a,h]anthracene*	ND	ND	ND	ND	ND
Benzo[g,h,i]perylene	ND	ND	ND	ND	ND
Total PAHS	0.0521	0.1408	10.8374	0.2008	0.1817
Total Carcinogenic PAHs	0.0516	0.0933	7.4868	0.1343	0.3610

(ND): Not detectable. (*): IARC Group 2a: probably carcinogenic to human [11]. (**): IARC Group 2b: possibly carcinogenic to human [11]. (* and **): classified as carcinogenic to human [12, 13, 14].

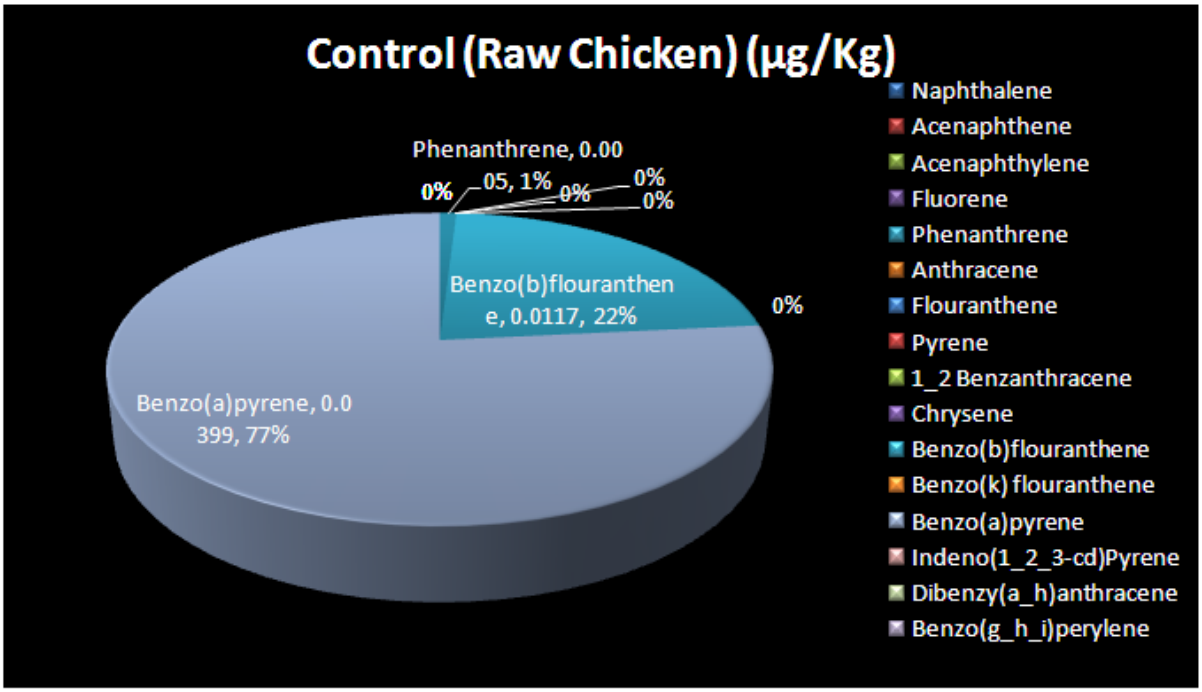

Fig. 1: Chart showing the levels and percentages of the 16 PAHs in the control chicken sample.

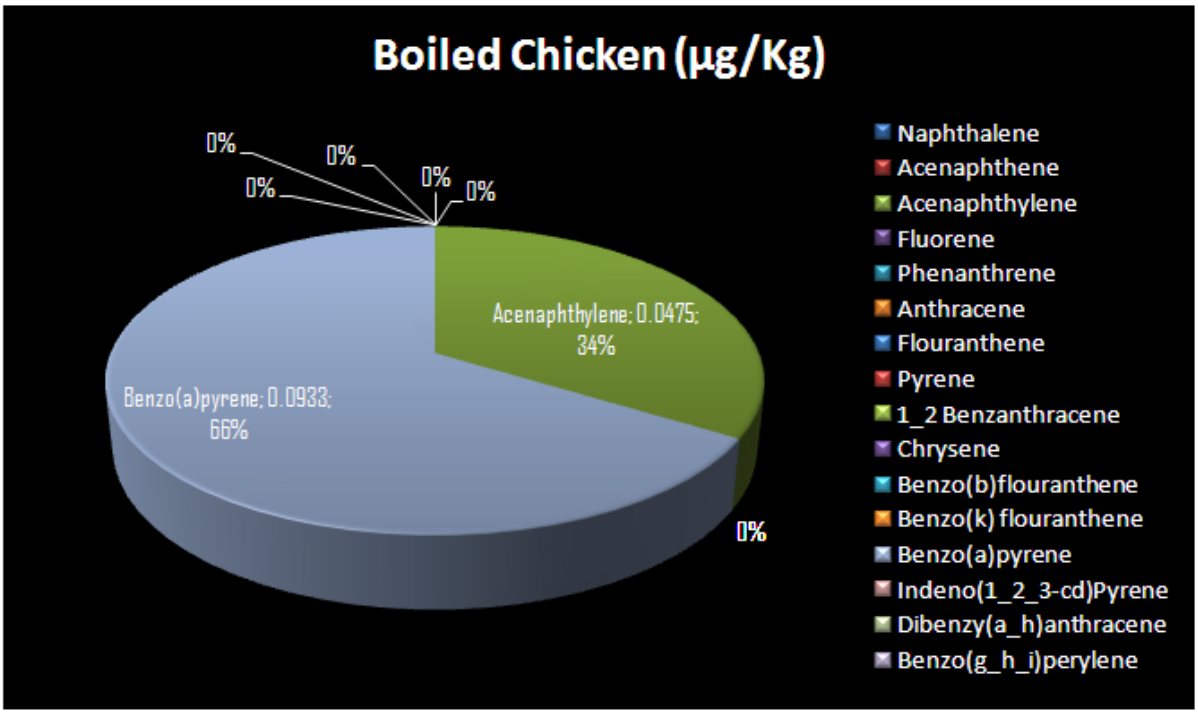

Fig. 2: Chart showing the levels and percentages of the 16 PAHs in the boiled chicken sample.

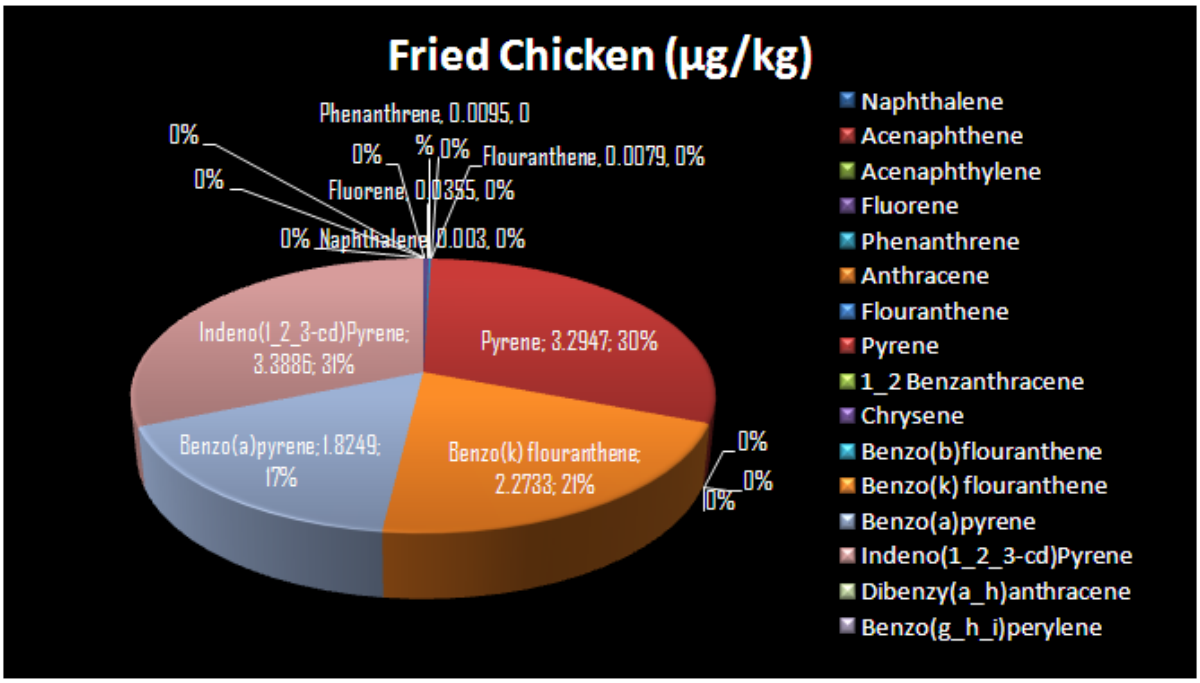

Fig.3: Chart showing the levels and percentages of the 16 PAHs in the fried chicken sample.

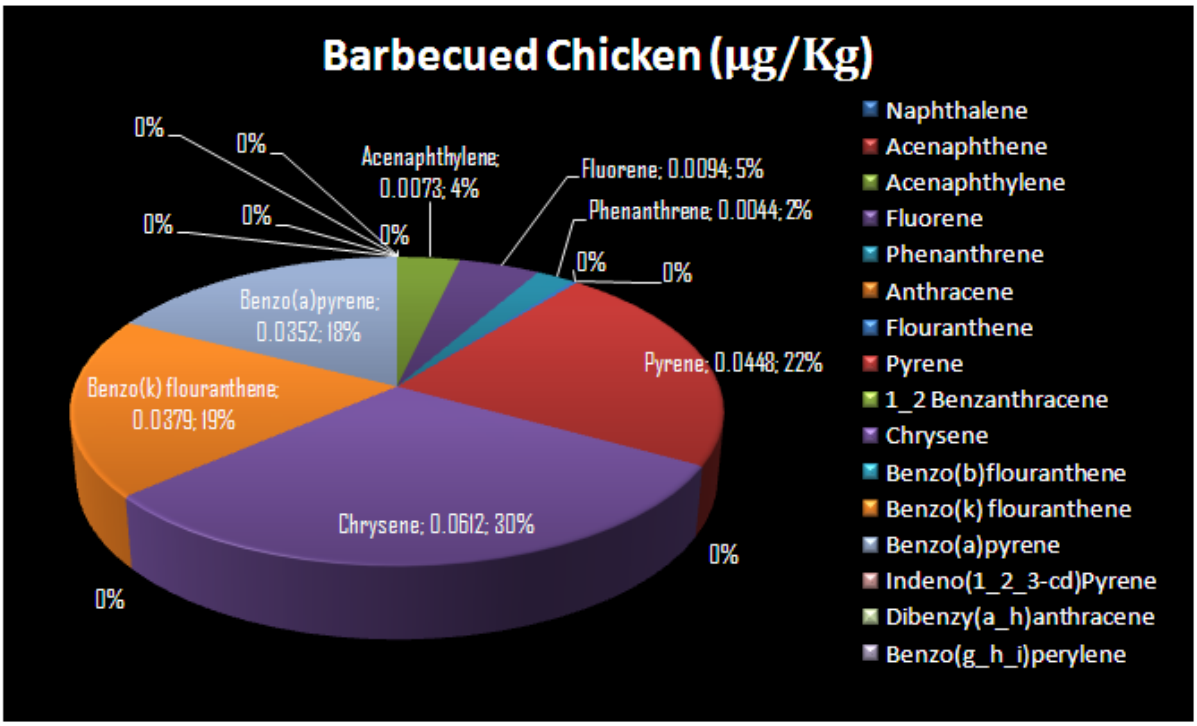

Fig. 4: Chart showing the levels and percentages of the 16 PAHs in the barbecued chicken sample.

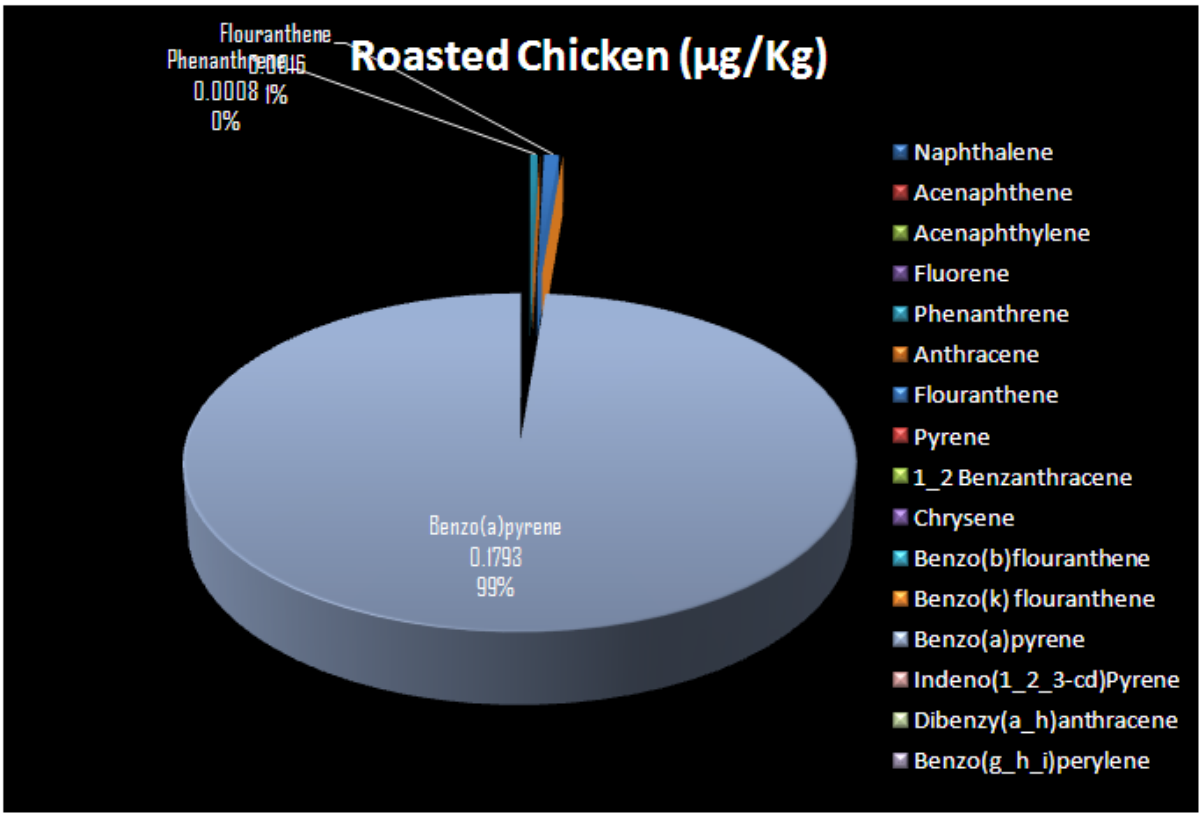

Fig.5: Chart showing the levels and percentages of the 16 PAHs in the roasted chicken sample.

The levels of 16 Polycyclic Aromatic Hydrocarbons (PAHs) compounds were determined in the raw and cooked chicken meat. The chicken meat was processed by different methods of cooking i.e. boiling, frying, barbequing and roasting. The uncooked sample served as the control. The levels of individual PAHs in the control, boiled, fried, barbequed, and roasted chicken meat are shown in table 1.

The data revealed that the control containedphenanthrene, benzo[b]fluoranthene and benzo[a]pyrene in the concentrations of 0.0005µg/kg,0.0117µg/kg and0.0399 µg/kg respectively. It could only be seen that only 2 PAHs, acenaphthylene and benzo[a]pyrene were contained in the boiled sample in the concentrations of 0.0475 µg/kg and 0.0933µg/kg respectively. The roasted sample only contained phenanthrene (0.0008µg/kg), fluoranthene (0.0016µg/kg) and benzo[a]pyrene (0.1793µg/kg). The fried and barbequed samples were the most heavily loaded with the PAHs. The highest number of the PAHs compound found in the two samples were in line with ElBadry, 2010, that food processing or cooking steps such as roasting, grilling, frying, generate PAHs and increase the level of PAHs in the food being cooked [1]. Also according to Ujowundu et al, 2014, cooking processes especially the high temperature ones are known to induce the production of potential carcinogens and also increase the levels of PAHs in the food being prepared [15]. Data indicated that the PAHs, in the samples varied, with the fried sample containing naphthalene (0.0030µg/kg) flourene (0.0355µg/kg),phenanthrene (0.0095µg/kg), fluoranthene (0.0079µg/kg), pyrene (3.294µg/kg), Benzo[k]fluoranthene (2.2733µg/kg), Benzo[a]pyrene (1.8249µg/kg) and indeno[1,2,3-cd]pyrene (3.3886µg/kg).

The result also gave the following PAHs and their concentrations in the barbequed sample as acenaphthylene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, benzo[k]fluoranthene and benzo[a]pyrene in the concentrations of 0.0073µg/kg, 0.0094µg/kg, 0.0044µg/kg, 0.0006µg/kg, 0.0448µg/kg,0.0612µg/kg,0.037µg/kg and 0.0352µg/kg respectively.

Among the PAHs found in the fried sample, three of them were among the PAHs which has been declared carcinogen by the IARC. These include benzo[k]fluoranthene, benzo[a]pyrene and indeno[1,2,3-cd]pyrene. Indeno[1,2,3-cd]pyrene has the highest concentration(3.3886µg/kg) followed by benzo[k]fluoranthene(2.2733µg/kg) then benzo[a]pyrene(1.8249µg/kg). The fried sample contained the highest concentration of the carcinogenic PAHs. This shows that the PAHs in both samples were as a result of pyrolitic processes.

Acenaphthylene was not detected in the other samples but in the barbequed and boiled chicken. Chrysene was only found in the barbequed chicken sample.

However, indeno[1,2,3-cd]pyrene was detected in the fried chicken sample only and was the highest in concentration. The study also showed that naphthalene, a low molecular weight PAH was only found in the fried chicken sample at a concentration of 0.0030µg/kg.

Acenaphthene, 1, 2- benzanthracene, dibenzo[a,h]anthracene and benzo[g,h,i]perylene were not detected in any of the samples.

The obtained data proved that total carcinogenic PAHs were 0.0516, 0.0933, 7.4868, 0.1343 and 0.0.3610 µg/kg in the control, boiled, fried, barbecued and roasted chicken samples respectively. They showed that PAHs are incorporated in fats of chickens owing to their lipophilic nature.

In France, PAHs were found in differently cooked chickens at total levels of 37 and 27 ng/g fat [16, 17] indicated that the parent compounds of PAHs did not detect, but found the hydroxy-metabolites from phenanthrene and pyrene in the barbecued chicken which was chronically exposed to PAHs through pyrolysis of fat. They also concluded that it is likely that low molecular mass PAHs with less than 5 rings are transferred to the meat as native compound after oral exposure in addition; evidence from literature suggests that even more PAHs are transferred as metabolites, possibly including those of the high molecular mass PAHs.

IV. Conclusion and Recommendation

The levels of the PAHs were strongly affected by the cooking methods, as boiling of all the four cooking methods had it safest. Though, the levels of PAHs in the samples were below the tolerance limit by the European regulations.

The amount of PAHs formed during cooking or processing of food depends markedly on the conditions used. Simple practices are known to result in a significantly reduced contamination of foods by PAHs [18, 19, 20] as well as by other undesirable contaminants. This may include selecting preferentially lean meat and fishes, avoiding contact of foods with flames for barbecuing, using less fat for grilling, and, in general, cooking at lower temperature for a longer time. Broiling (heat source above) instead of grilling can significantly reduce the levels of PAH.

Actually the fat should not drip down onto an open flame sending up a column of smoke that coats the food with PAHs. The use of medium to low heat, and placement of the meat further from the heat source, can greatly reduce formation of PAHs. The intensity of flavour is not necessarily associated with the depth of the brown colour of grilled foods. It is therefore needless to overcook the food to get the flavour. However, cooking must always remain effective as regards inactivation of any possible contaminating bacteria or endogenous toxins.

References

[1]. **El-Badry, N.** (2010). Effect of household cooking methods and some food additives on polycyclic aromatic hydrocarbons (PAHs) formation in chicken meat. J. of World Applied Sci. 9(9): 963-974.

[2]. **Agerstad, M. J., & Skog, K.** (2005). Review genotoxicity of heat-processed foods. Mutation Research, 574, 156–172.

[3]. **Larsson, B. K.** (1986). Polycyclic aromatic hydrocarbons in Swedish foods.Aspects on analysis, occurrence and intake.Doctoral thesis, Swedish University of Agricultural Sciences.

[4]. **Ishizaki, A., Saitoa, K., Haniokab, N., Narimatsub, S. &Kataokaa, H.** (2010).Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection.J. Chromatogr., 1217: 5555-5563.

[5]. **Simko, P.** (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. B: Analytical Technologies in the Biomedical and Life Sciences, J. Chromatogra., 770: 3-18.

[6]. **Farhadian, A., Jinap, S., Hanifah, H.N. &Zaidul, I. S.** (2011).Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. Food Chem., pp. 141-146.

[7]. **Gómez-Guillén, M. C., Gómez-Estaca, J., Giménez, B. & Montero, P.** (2009). Alternative fish species for cold-smoking process. Int. J. Food Sci. Technol., 44: 1525-1535.

[8]. **Stołyhwo, A. and Sikorski, Z.** (2005). Polycyclic aromatic hydrocarbons in smoked fish - a critical review. Food Chem., 91: 303-311.

[9]. United **States Environmental Protection Agency,** US EPA Method – 3540c. Soxhlet extraction.

[10]. **United States Environmental Protection Agency,** US EPA Method – 3630c. Silica gel clean- up.

[11]. **IARC – International Agency Research on Cancer** (2006). Monographs on the evaluation of carcinogenic risk of chemicals to humans, polynuclear aromatic compounds. Part 1: Chemical environmental and experimental data. Lyon, France. International Agency for Research on Cancer (IARC).

[12]. **U. S. EPA**, **United States Environmental Protection Agency** (2002). Polycyclic Organic Matter.Environmental Protection Agency, Washington, DC. Available at: http://www.epa.gov/ttn/atw/hlthef/polycycl.html

[13]. **WHO/IPCS**, **World Health Organization / International Programme on Chemical Safety** (1998). Environmental Health Criteria 202: Selected Non-Heterocyclic Polycyclic Aromatic Hydrocarbon. International Program on Chemical Safety, United Nations Environmental Program, World Health Organization. Geneva.

[14]. **Larsen, J.C. and Larsen, P. B.** (1998). Chemical carcinogens. In: R.E. Hester and R.M. Harrison, eds. Air pollution and health. Cambridge, UK. Royal Society of Chemistry, pp.: 33-56.

[15]. **Ujowundu, C. O., Ihekweazu, K. L., Alisi, C. S., Ujowundu, F. N. &Igwe, C. U.** (2014). Procarcinogens: Polycyclic aromatic hydrocarbons and heavy metal content in some locally processed foods in South – Eastern, Nigeria. British journal of applied science and technology, 4: 249 – 260.

[16]. **Grova, N., Feidt, C., Crepineau, C., Laurent, C., Lafargue, P.E., Hachimi, A. &Rychen, G.** (2002). Detection of polycyclic aromatic hydrocarbon levels in milk collected near potential contamination sources. J. Agric. Food Chem., 50, 4640- 4642.

[17]. **Lutz, S., Feidt, C., Monteau, F., Rychen, G., Le Bizec, B. and Jurjanz, S**. (2005). Transfer of polycyclic aromatic hydrocarbons and their principle metabolites to milk after chronic exposure of dairy cows to contaminated soil. Abstract from Symposium New Methods for Assessing Human Exposure to PAHs Toronto.

[18]. **Lijinsky, W. and Ross, A.E.**(1967). Production of carcinogenic polynuclear hydrocarbons in the cooking of food.Food Cosmet.Toxicol. 5: 343-347.

[19]. **Lijinsky, W.** (1991). The formation and Occurrence of polynuclear aromatic hydrocarbons associated with Foods. J. Mutt. Res. 259(3-4):251-261.

[20]. **Knize, M. G., Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P. &Levander, O. A.** (1995). Heterocyclic amine content in fast-food meat products. Food and Chemical Toxicology, 33(7), 545–551.