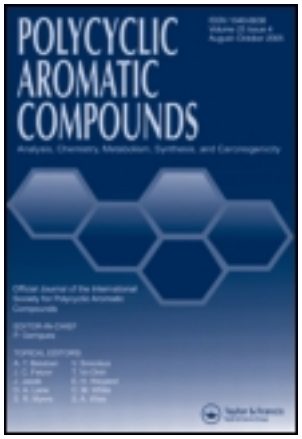

Polycyclic Aromatic Compounds
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpol20

The Effect of Local Cooking Methods on Polycyclic Aromatic Hydrocarbons (PAHs) Contents in Beef, Goat Meat, and Pork as Potential Sources of Human Exposure in Kisumu City, Kenya

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Version of record first published: 13 Nov 2012.

To cite this article: ALICE ANYANGO ONYANGO, JOSEPH O. LALAH & SHEM O. WANDIGA (2012): The Effect of Local Cooking Methods on Polycyclic Aromatic Hydrocarbons (PAHs) Contents in Beef, Goat Meat, and Pork as Potential Sources of Human Exposure in Kisumu City, Kenya, Polycyclic Aromatic Compounds, 32:5, 656-668

To link to this article: http://dx.doi.org/10.1080/10406638.2012.725195

Polycyclic Aromatic Compounds, 32:656–668, 2012

ISSN: 1040-6638 print / 1563-5333 online
DOI: 10.1080/10406638.2012.725195

The Effect of Local Cooking Methods on Polycyclic Aromatic Hydrocarbons (PAHs) Contents in Beef, Goat Meat, and Pork as Potential Sources of Human Exposure in Kisumu City, Kenya

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Roasted meat is known to be a major source of human exposure to PAHs. The contribution of direct-heat charcoal-roasted, electric- oven grilled, and shallow-pan fried meat to human exposure in Kisumu City was not known although the three modes of cooking meat are very prevalent. This study analyzed the concentrations of the PAHs in raw beef, goat meat, and pork, investigated the effect of direct-heat charcoal roasting, electric-oven grilling, and shallow-pan frying on these concentrations, and compared their concentration levels with international standards for foods in order to assess the potential risks to consumers. Samples were taken from three popular meat-roasting hotels within Kisumu City, Kenya. Extraction of PAHs was done using liquid-liquid partition after saponification with alcoholic potassium hydroxide followed by clean-up on a silica gel column and final analysis by gas chromatography-mass spectrometry (GC-MS). Roasting and shallow-pan frying introduced new PAHs and significantly

Received 15 February 2012; accepted 25 August 2012.
This research was partly supported by a student from the School of Graduate Studies (SGS), Maseno University. The authors also wish to express their gratitude to the Alexander von Humboldt Foundation of Germany for donating the reference standards.
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($P \leq 0.05$) increased the concentrations of those existing in raw meat. Direct-heat charcoal roast beef had 5 new PAHs and a total mean PAH content of 17.88 μg/kg, compared with a mean of 1.39 μg/kg for raw beef, with the potent dibenz(a,h)anthracene also being detected. Direct-heat charcoal roasted goat meat had three new PAHs and a total mean PAH content of 4.77 μg/kg, compared with a mean of 2.13 μg/kg in raw meat, with the potent benzo(a)pyrene concentration being 8.84% of the total mean PAH. Fried pork had 7 new PAHs and a total mean PAH content of 3.47 μg/kg, compared with a mean total of 0.17 μg/kg, detected in the raw meat. Roast beef had the highest individual PAH concentration (5.03 μg/kg) and highest total PAHs concentration (17.88 μg/kg), both being higher than acceptable EU limits. The PAHs from local raw and cooked meat were characterized and quantified for the first time in Kisumu City and the study therefore provided the needed baseline data on PAHs in raw and cooked meat.

Key Words: frying, Kenya, Kisumu city, meat, polycyclic aromatic hydrocarbons, roasting

INTRODUCTION

PAHs are of great concern in environmental monitoring because they are known or suspected carcinogens and/or toxicants. The United States Environmental Protection Agency (USEPA) lists 16 PAHs as priority pollutants that need to be periodically monitored in the environment because of their known carcinogenicity. The USEPA (1) developed an oral "virtually safe dose" (VSD) for benzo(a)pyrene of 0.14 ng/kg body weight/day to monitor the carcinogenic risk it poses. Cancer incidence in Kenya is increasing (data from Nairobi Cancer Registry and verbal reports from practicing physicians; Kakamega Hospice Registry) as reported by Lisouza et al. (2) and now numbers among the top 10 causes of mortality (2, 3). However, the cause of the increase in cancer incidence and prevalence is not known. The population may be exposing themselves to carcinogens through food by eating contaminated fish (4–6) and roasted, grilled, and fried meat (7–12). Human exposure may also be through smoke (smoking tobacco, smoke from burning of wood, and petroleum fuel (13)) and emissions from creosote treated wood products (14), foods from soils contaminated with PAHs, and industrial workplace exposure (15).

Other potential sources of PAHs in the environment exist in Kisumu City with the major point sources including petroleum fuel spillages, Kenya Pipeline Company depot runoff, car wash activities at the shore, oil spills from vessels at the Pier and Yatch Club, mechanical workshops (*Jua Kali* sheds), and petrol-station runoff (16). Mobile sources include motor vehicle exhaust (17) and consumer products waste dumped into the lake. Diffuse sources include asphalt roads and road tar, fires of all types (municipal garbage incineration and burning of sugar cane from the surrounding sugar belt) and biomass energy combustion (2, 4, 18), agricultural runoff, and natural alteration of organic matter (19). It is not known how much these sources contribute to the PAH exposure as their levels are neither known nor periodically monitored in

meat products which are main components of diet in the area. Recent studies have indicated that relatively high levels of PAHs are present in sediment and water in Winam Gulf of Lake Victoria where the city gets its water from (16).

The aim of this study was to analyze the concentration levels of PAHs in raw and cooked meat from selected hotels in Kisumu City in order to provide baseline data and knowledge on potential exposure in humans. Various cooking methods are used in Kisumu City for meat preparation. The choice of a particular method depends on the type of establishment, income levels of the inhabitants, the available cooking equipment or apparatus, and the type of fuel used. The cooking methods targeted in this study were open-air direct-heat charcoal oven roasting and electric-oven grilling (for beef and goat meat) and shallow-pan frying (for pork). These are the local meat cooking methods used in the hotels that were selected for the study. Pork is not served roasted and, as such, roasting pork would not provide a true potential source for PAH exposure in humans in our case. The pork roasting method was therefore not included in the sampling design in the study.

MATERIALS AND METHODS

General Purpose Grade (GPR) methanol and diethyl ether from Kobian (K) Ltd, Nairobi, were distilled in the laboratory prior to use for extraction. Analytical HPLC grade n-hexane, 95% pure, was obtained from Kobian (K) Ltd, Nairobi. Potassium hydroxide, sodium sulphide ($Na_2S.9H_2O$), anhydrous sodium sulphate (Na_2SO_4), and silica gel (230–400 mesh) were pure analytical grade and were obtained from Kobian (K) Ltd, Nairobi. Pure nitrogen and helium for gas chromatography-mass spectrometric (GC-MS) analysis were obtained from East African Oxygen Company, Kisumu, Kenya.

Sampling

Samples of raw, open-air direct-heat charcoal- and electric-oven grill roasted and shallow-pan fried meat were taken in triplicates from various meat-roasting places in Kisumu City (0°6′S, 34°45′E) located on the North tip of Winam Gulf, Lake Victoria, Kenya (Figure 1). Kisumu City has a population of 504,000 and the most common sources of protein for the local population include fish, beef, mutton, goat meat, and pork. The specific sampling sites were Highway Inn (on Kisumu-Kakamega Highway) for goat meat and beef, Apok Inn (on Kisumu-Nairobi Highway) for beef, and Kisumu Hotel (in the city center) for pork. A sample of raw meat (1 kg) was taken and divided into halves. The first half was packed for laboratory analysis while raw and the second half was open-air charcoal roasted using direct heat (Highway Inn), electric-oven grilled (Apok Inn), or shallow-pan fried (Kisumu Hotel) as necessary before

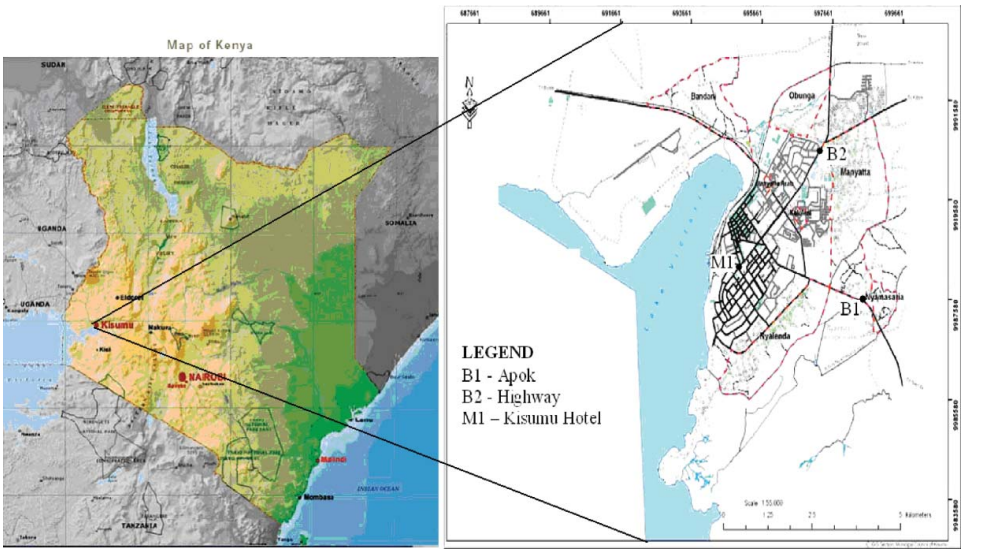

Figure 1: Map of Kenya showing Kisumu City and Bay (0°6′S, 34°45′E) with sampling locations (color figure available online).

packing. The samples were wrapped in pieces of pre-cleaned aluminum foil and placed in an ice-cold container for transport to the laboratory.

Extraction and GC-MS Analysis of PAHs

Extraction of PAHs from raw meat followed a modified method described by Takatsuki et al. (4) where samples of meat were cut into small pieces and homogenized in a blender; 50 g of the homogenized sample was saponified using a mixture of methanol (200 mL), 50% aqueous potassium hydroxide (35 mL) and sodium sulphide (2 g) and then refluxed on a water bath for 2 h. The mixture was cooled to 40°C and 150 mL of n-hexane added in small portions, with occasional swirling. The mixture was poured into 150 mL de-ionized water in a 500-mL glass separation funnel, the flask rinsed twice with 10 mL methanol, and the rinses added to the mixture. The separation funnel was gently and adequately shaken and then set to separate. The aqueous layer was extracted twice with 150 mL and 100 mL portions of n-hexane, respectively. All the n-hexane extracts were combined, washed with 100 mL de-ionized water, dried over anhydrous sodium sulphate, transferred to a rotary evaporator, and then concentrated to about 3–5 mL.

For clean-up and separation, a chromatographic glass column (1 cm diameter) was packed with 8 g silica gel (in a slurry) and 3 g anhydrous Na_2SO_4, added on top. The column was covered with aluminum foil to protect it from light and washed with 30 mL n-hexane; the solvent was then drained to just above the slurry. The concentrated extract was transferred to the column.

Clean up and elution of PAHs was done slowly under gravity with 150 mL volume of 10% diethyl ether in n-hexane. The extract was evaporated to 1–2 mL, in a rotary evaporator, then under a mild stream of nitrogen to dryness with gentle warming and the residue was taken up in 1 mL pentane (Sigma, St. Louis, MO) and stored in sealed vials for analysis.

The samples were analyzed by GC-MS, a 7890A stand-alone GC (Agilent Technologies, Inc., Beijing, China) and a 5975 C MSD (Agilent Technologies, Inc., Santa Clara, CA). The conditions were: inlet temp 270°C, transfer line temp of 280°C, and column oven temperature programmed from 35–285°C initially for 5 min then 10°C/min to 280°C for 10.5 min and then at 5°C/min to 285°C for 29.9 min. The GC had HP-5 MS low bleed capillary column (30 m $\times$ 0.25 mm i.d., 0.25-μm) (Restek, Bellefonte, PA). Helium was the carrier gas (flow rate 1.25 mL/min). The MSD ion source temperature was 250°C and quadruple temperature of 180°C. Electron impact mass spectra were obtained at an acceleration energy of 70 eV. Fragment ions were analyzed over 40–550 m/z mass range in the full scan mode. A 1.0 μL aliquot of extract was automatically injected in the split/splitless mode using an auto sampler 7683 (Agilent Technologies, Inc., Beijing, China). The filament delay time was set as 3.3 min. Library –MS searches were done using NIST/EPA/NIH Mass Spectral Library (NIST 05) and NIST Mass Spectral Search Program Version 2.0d.

External PAH standards (PAH Mix 9; Dr. Ehrenstorfer mbH, Augsburg, Germany) were used in different dilutions to come up with calibration curves which were used to relate the various concentrations to peak areas. Calibration standards of 5 concentration levels—0.1 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L—were prepared in HPLC-grade pentane. This was used to give the peak retention times for identification and the calibration curves for quantification. To evaluate the recovery of PAH and to account for matrix effects in the GC–MS chromatograms, spiked control samples were analyzed through the same procedure. The results obtained were then corrected based on percentage recovery for each PAH (Table 1).

Statistical Analysis

The results were analyzed using a factorial two design with PAH type as the main treatment and meat type (raw and cooked) as a sub-treatment. The results obtained were then processed for ANOVA using INSTAT software. For beef the sub-treatments were meat type and location.

RESULTS AND DISCUSSION

It should be noted that these results are based on a method that is not very recent (4). However, there are more recent and more sensitive methods for

Table 1: PAHs recovery in meat samples

PAH type	% recovery ± standard deviation
Naphthalene	83.5 ± 11.5
Acenaphthylene	85.0 ± 7.9
Acenaphthene	80.0 ± 12.3
Fluorene	86.3 ± 6.9
Phenanthrene	92.5 ± 1.5
Anthracene	96.5 ± 1.4
Fluoranthene	91.6 ± 11.4
Pyrene	92.4 ± 2.5
Chrysene	76.2 ± 12.1
Benz(a)anthracene	72.7 ± 18.0
Benzo(b)fluoranthene	98.3 ± 2.1
Benzo(k)fluoranthene	87.0 ± 19.1
Dibenz(a,h)anthracene	87.9 ± 12.7
Benzo(a)pyrene	94.4 ± 5.6
Indeno(1,2,3-cd)pyrene	91.7 ± 2.9
Benzo(g,h,i)perylene	76.8 ± 2.5

determination of PAHs, for example, Gosetti et al. method (21). However, the use of GC-MS and the quality control checks done during the analysis support the accuracy of our method.

PAHs in Raw Meat

Raw beef was found to contain 7 of the 16 priority PAHs, as shown in Table 2. The sample from The Highway Inn contained 7 of the 16 priority PAHs while beef from The Apok Inn exhibited three of the priority PAHs. Average background values are usually in the range of 0.01–1 μg/kg in uncooked foods (20, 24) and the values obtained at Highway are above that range.

Apok and Highway obtain their beef from the same slaughterhouse. At Highway, the beef is kept in the open in close proximity to the roasting place prior to cooking. This could expose the raw beef to ambient PAHs originating from the roasting place (2) which could get adsorbed onto the surface of the meat. However, at Apok, the meat is kept at a butchery which is located in a different room from the kitchen where roasting is done.

Raw goat meat was found to contain 9 of the 16 priority PAHs (Table 2). The PAHs were within the average background values for uncooked foods (20) with the exception of anthracene which had the highest mean PAH concentration recorded. However, the total mean PAH concentration was well above the average background value in uncooked foods (20). Raw pork contained 2 of the 16 priority PAHs (Table 2). The values are within the average background values for uncooked foods (20).

Table 2: The mean PAH concentration in raw meat (± standard deviation in μg/kg ww)

	BEEF			GOAT MEAT	PORK	MEAN PAH
PAH	Apok	Highway	Mean	Highway	Kisumu Hotel	All sites
Naphthalene	0.087 ± 0.003	0.118 ± 0.015	0.102 ± 0.019	0.105 ± 0.007	nd	0.077
Acenaphthylene	0.070 ± 0.001	0.124 ± 0.004	0.097 ± 0.030	0.031 ± 0.004	0.097 ± 0.005	0.081
Acenaphthene	0.053 ± 0.001	0.086 ± 0.002	0.069 ± 0.018	0.074 ± 0.004	0.072 ± 0.003	0.071
Fluorene	nd	0.310 ± 0.009	0.155 ± 0.170	0.229 ± 0.020	nd	0.337
Anthracene	nd	0.870 ± 0.001	0.435 ± 0.477	1.033 ± 0.045	nd	0.476
Pyrene	nd	0.934 ± 0.010	0.467 ± 0.512	nd	nd	0.234
Benz(k)fluoranthene	nd	nd	nd	0.332 ± 0.009	nd	0.083
Indeno(1,2,3)-cd)P	nd	0.097 ± 0.002	0.067 ± 0.073	0.097 ± 0.003	nd	0.058
Db(a,h)A	nd	nd	nd	0.102 ± 0.022	nd	0.025
Benzo(g,h,i)P	nd	nd	nd	0.127 ± 0.011	nd	0.032
Total mean PAH	**0.210**	**2.539**	**1.392**	**2.130**	**0.169**	**1.474**

nd = not detected/below detect limit of the machine (detection limit = 10exp-12g) and assumed to be zero in statistics; Indeno(1,2,3-cd)P = Indeno(1,2,3-cd)pyrene; Db(a,h)A = Dibenz(a,h)anthracene; Benzo(g,h,i)P = Benzo(g,h,i)perylene

Effect of Direct-Heat Charcoal Roasting on PAH Concentrations in Beef

Roasting increased the concentrations of all the PAHs and also introduced new PAHs (Table 3). At Apok, the new PAHs introduced included fluorene, phenanthrene, anthracene, pyrene, benzo(k)fluoranthene, and indeno(1,2,3-cd)pyrene while the new ones at Highway included dibenz(a,h)anthracene, benzo(k)fluoranthene, and benzo(ghi)perylene. Therefore, it is a dynamic process where some PAHs are burnt out during roasting while new ones are generated (Table 3). This is comparable to what is found in literature. Janoszka et al. (10) who studied roast meats in Poland found that the total PAH content was within the range 2.43–16.10 ng/g meat while Reinik et al. (12) found 16 μg/kg in smoked meat and ham as part of Estonian Food Safety Monitoring. Martonell *et al.* (11), however, reported higher levels at 33.99 μg/kg in meat and meat products in a population exposure study of Catalonia, Spain. In this study, the concentrations of individual PAHs were also significantly different with respect to location with Highway showing higher levels than Apok ($P \leq 0.05$). This could be due to the fact that Apok used an indoor electric-oven grill to prepare their meat, which would yield less PAHs, while Highway used open-air charcoal stove which could perpetuate production of more PAHs (2, 22).

Table 3: Effect of roasting on the PAH concentration in beef (in μg/kg ww)

PAH	Apok	Highway	Roast Mean	Raw Mean	LSD (P = 0.05)	CV(%)
Naphthalene	0.113	0.233	0.173	0.102	0.016	19.70
Acenaphthylene	0.087	0.256	0.172	0.097	0.019	24.80
Acenaphthene	0.065	0.193	0.129	0.069	0.016	27.40
Fluorene	0.255	1.604	0.930	0.155	0.173	55.30
Phenanthrene	0.223	nd	0.112	nd	0.037	115.80
Anthracene	0.832	4.804	2.818	0.435	0.540	57.80
Fluoranthene	nd	1.270	0.635	nd	0.212	115.60
Pyrene	0.395	3.648	2.021	0.467	0.387	53.80
B(k)F	0.143	0.202	0.172	nd	0.010	20.10
Indeno(1,2,3-cd)P	0.097	0.253	0.175	0.067	0.006	9.10
Db(a,h)A	nd	5.032	2.516	nd	0.839	115.50
Benz(g,h,i)P	nd	0.385	0.193	nd	0.064	115.50
Total mean PAH	**2.21**	**17.88**	**10.046**	**1.392**		

nd = not detected/below detect limit of the machine (detection limit = 10exp-12g) and assumed to be zero in statistics; CV: Coefficient of variation; LSD: Least square deviation; B(k)F = Benzo(k)fluoranthene; Indeno(1,2,3-cd)P = Indeno(1,2,3-cd)pyrene; Db(a,h)A = Dibenz(a,h)anthracene; Benzo(g,h,i)P = Benzo(g,h,i)perylene; Note: Apok: electric-oven grill used, Highway: charcoal stove used.

Effect of Direct-Heat Charcoal Roasting on PAH Concentrations in Goat Meat

Acenaphthene, anthracene, indeno(1,2,3-cd)pyrene, and dibenz(a,h) anthracene concentrations were found to be significantly higher ($P \leq 0.05$) in roasted than in raw goat meat (Table 4). Twelve of the 16 priority PAHs were found in roasted goat meat as compared to the nine found in raw goat meat. The new PAHs generated included phenanthrene, fluoranthene and the potent benzo(a)pyrene whose percentage in comparison to total PAH concentration was 8.84% (Table 4). This is relatively low as compared to the range 2.43–16.10 ng/g found by Janoszka et al. (10) in roast meats in Poland; 16 μg/kg in smoked meat and ham found by Reinik et al. (12), as part of Estonian Food Safety Monitoring, and 33.99 μg/kg in meat and meat products found by Martonell et al. (11) in a population exposure study of Catalonia, Spain. This shows that the local roasting methods are safer than these smoking methods reported in literature. However, the type of meat roasted influences the PAHs concentrations as higher total PAH concentrations were recorded in direct-heat charcoal roast beef than in direct-heat charcoal roasted goat meat.

Effect of Shallow-Pan Frying on PAH Concentrations in Pork

Frying increased the number of PAHs detected in raw pork from two to nine. However, the concentration of the PAHs existing in raw pork were not significantly ($P \leq 0.05$) increased by frying (Table 5). The new

Table 4: Effect of charcoal roasting on the PAH concentrations in goat meat (μg/kg ww)

PAH	Roast Mean	Raw Mean	LSD (P = 0.05)	CV (%)
Naphthalene	0.308	0.105	0.026	3.55
Acenaphthylene	0.126	0.031	0.007	2.56
Acenaphthene	0.092	0.074	0.022	7.53
Fluorene	0.892	0.229	0.073	3.50
Phenanthrene	0.207	nd	0.020	5.54
anthracene	1.670	1.033	1.305	31.35
Fluoranthene	0.251	nd	0.035	7.86
Benz(k)fluoranthene	0.410	0.332	0.024	1.86
Benzo(a)pyrene	0.422	nd	0.011	1.44
Indeno(1,2,3-cd)pyrene	0.121	0.097	0.100	2.54
Dibenz(a,h)anthracene	0.113	0.102	0.069	18.24
Benz(g,h,i)perylene	0.164	0.127	0.020	3.99
Total mean PAH	**4.774**	**2.130**		

nd = not detected/below detect limit of the machine (detection limit = 10exp-12g) and assumed to be zero in statistics

Table 5: Effect of shallow-pan frying on PAH concentrations in pork (μg/kg)

PAH	Fried Mean	Raw Mean	LSD (P = 0.05)	CV (%)
Naphthalene	0.074	nd	0.011	8.44
Acenaphthylene	0.325	0.097	0.025	3.37
Acenaphthene	0.367	0.072	0.055	7.07
Fluoree	0.341	nd	0.039	6.51
Anthracene	0.543	nd	0.047	4.98
Fluoranthene	0.491	nd	0.020	2.26
Benzo(a)pyrene	0.701	nd	1.295	105.10
Benz(k)fluoranthene	0.237	nd	0.063	15.06
Indeno(1,2,3-cd)P	0.386	nd	0.069	10.17
Total mean PAH	**3.465**	**0.169**		

nd = not detected/below detect limit of the machine (detection limit = 10exp-12g) and assumed to be zero in statistics; Indeno(1,2,3-cd)P = Indeno(1,2,3-cd)pyrene

PAHs introduced included fluorene, anthracene, fluoranthene, benzo(a)pyrene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene. The concentration levels found in this study were much lower than what Janoszka et al.,(10) found showing that shallow-pan frying of pork generated less PAHs.

Comparison of the Concentration Levels of PAHs with the Standards Allowed Internationally in These Foods

The values of individual PAHs in raw beef, goat meat, and pork are lower than the maximum limits set by Commission Regulation (23) of 5 μg/kg for benzo[a]pyrene (Table 6). For the roast/fried foods studied, Highway beef has a greater potential source of exposure (total 16 USEPA PAHs) to humans followed by goat meat and pork and, the least potential source of exposure to humans after cooking is Apok beef. Highway used open-air, direct-heat, charcoal stove roasting and Apok used electric-oven grilling method. Benzo[a]pyrene was reported only in goat meat at 0.422 μg/kg which is below the maximum

Table 6: Comparison of maxima of PAH concentration range in tested foods with maximum limits set by European Commission Regulation (23) (in μg/kg)

Sample	Beef Individual	Beef Total	Goat Meat Individual	Goat Meat Total	Pork Individual	Pork Total	ECR (2006)
	PAH	PAH	PAH	PAH	PAH	PAH	
Raw	0.934	2.105	1.033	2.201	0.097	0.169	2
Cooked[a]	5.032	17.875	1.670	4.781	0.701	3.464	5

[a] roasted for beef and goat meat; fried for pork

limit set by the European Commission Regulation (23). Data on meat consumption by Kisumu population does not exist. However, data on Nairobi population's meat consumption exists (25). Gamba et al. (25) found that households in Nairobi consume a mean per adult equivalent of 15.81 kg of beef and 11.65 kg goat meat annually. Assuming that meat consumption in Kisumu follows the same pattern, then the daily consumption of goat meat is 0.032 kg per person (based on 365 days in a year) and the daily benzo(a)pyrene exposure is 13.50 ng. This implies that based on the USEPA VSD (1), one should weigh at least 96.46 kg to be safe from this level of exposure.

CONCLUSION

The PAH concentration in raw meats, both individual and total, were in the μg/kg range. Open-air charcoal-roasting increased the PAH concentration in beef and goat meat significantly ($P \leq 0.05$) in all cases. It also introduced new PAHs in these foods. Shallow-pan frying did not increase the PAH concentration in pork significantly ($P \leq 0.05$) but introduced new PAHs. The figures for benzo(a)pyrene concentration show levels higher than USEPA virtually safe dose (VSD) to persons below 96.46 kg body weight which are indicative of significant potential human exposure to total USEPA 16 PAHs. Open-air direct-heat charcoal-roasted beef posed the highest potential for exposure followed by open-air direct-heat charcoal roasted goat meat while shallow-pan fried pork was the least potential source of exposure. There is need to minimize poor handling practices such as keeping raw meat in close proximity to roasting places/kitchens from where external and ambient sources may introduce PAHs in stored raw meat as shown in this study. Electric-oven grilling is a better method of roasting than open-air direct-heat charcoal stove roasting.

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