Original Article

# Determination of benzo (a) pyrene in Iraqi Chicken, doner kebab and fish samples cooked with charcoal or gas fire

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***Abstract:***

**Background:** Polycyclic aromatic hydrocarbons (PAHs) have been found in protein-rich food products where they are generated during certain food processing procedures. Benzo[a]pyrene (B(a)P) is a member of a class of PAHs in which the molecular structure includes two or more fused aromatic rings with adjacent rings sharing two or more carbon atoms with the formula C20H12. Dietary intake of these compounds via a formation of B(a)P on processing or cooking lead to metastasis of tumors at several sites, particularly in the upper gastrointestinal tract.

**Objective:** We aimed to determinate B(a)P in charcoal and gas broiled chicken, doner kebab and fish meats taken from some restaurants in Baghdad.

**Methods:** Prospective study was done in Baghdad from july to decemper 2015 in (Palestine st and alsadria). 120 samples of chicken, doner kebab and fish were collected from some resturants in Baghdad area randomly.A 20 cooked samples using a charcoal fire and gas fire for each kind of meat mentioned above to determinate B(a)P in these samples.HPLC technique was used for determination.

**Results:** Mean levels of B(a)P were found to be (11.6±11.08 µg/kg) ,(10.0±9.4 µg/kg) and (8.4±7.8 µg/kg) for a charcoal fire broiled chicken, doner kebab and fish respectively while mean levels of B(a)P were found to be (2.5±1.9 µg/kg) ,(2.3±1.7 µg/kg) and (2.0±1.4 µg/kg) for a gas fire respectively.

**Conclusion:** The B(a)P levels of three samples cooked on a charcoal fire exceeded the maximum tolerance level for B(a)P of the European Union establishment (2005). The highest levels of B(a)P were found in charcoal-grilled samples.Therefore,present study provides important information on B(a)P in charcoal grilled meats in Iraq. This might help to avoid the contamination in food processing to secure food safety and to protect iraqi citizens.

**Keywords:** Benzo (a) pyrene, meat ,chicken,doner kebab ,fish.

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## Introduction:

Harmful compounds in food come from various sources, such as environmental pollution, food processing and illegal additives(1-5). Besides coming from environmental pollution, polycyclic aromatic hydrocarbons (PAHs) have been found in protein-rich food products where they are generated during certain food processing procedures(6-8). (PAHs) are a group of chemicals that are formed during the incomplete burning of coal, oil, gas, wood, garbage or other organic material, such as tobacco and charbroiled meat. There are more than 100 different PAHs. Benzo[a]pyrene (B(a)P) is a member of a class of PAHs in which the molecular structure includes two or more fused aromatic rings with adjacent rings sharing two or more carbon atoms with the formula C20H12.(9-12) Dietary intake of processed meat containing benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), causes an increase metastasis of tumors at several sites, particularly in the upper gastrointestinal tract and is present in awide variety in food items . It has been detected in charcoal broiled meat, smoked-grilled foods, seafood, liquid smokes and beverages . PAHs contaminate foods that have been cooked over open flames or treated with smoke condensate.(13-14) Cooking muscle meats (beef, pork, fish, and poultry) at high temperatures can lead to the formation of polycyclic aromatic hydrocarbons (PAHs), which are carried by smoke and coat the food (charbroiling). (15) (B(a)P) also found in coal tar,in automobile exhaust fumes (especially from diesel engines), in all smoke resulting from the combustion of organic materials (including cigarette smoke) and in charbroiled food. Cooked meat products,regular consumption of which has been epidemiologically associated with increased levels of colon cancer(16-17).

## Materials and Methods:

Twenty samples of each of commercial chicken, doner kebab and fish were randomly purchased from 10 different restaurants in Baghdad (capitol of Iraq) ,every above twenty samples were broiled (10 by charcoal fire and other 10 by gas fire). Samples weighting 100 g were collected on consecutive weeks

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and kept in a refrigerator at 40C before analysis. Twenty-five grams of each samples were taken and analysed. B (a) P Standard was purchased from Aldrich. The solvents; sodium hydroxid, toluene, acetonitrile, dichloromethane, methanol and n-hexane were obtained from merck. Carbamate analysis column(150 mm×4.6 mm,C18, 5µm) was obtained from (Pickering laboratories, USA). HPLC System consisted of a high-pressure pump, fluorescence detector , column oven, auto injector and system controller were obtained from shimadzu, Japan CTO-10A. Propylsulphonic acid(PRS,500mg) SPE columns were supplied by Baker. Silica gel chromatography columns (70-230 mesh) were obtained from Merck. (20 g) extraction columns were supplied by Merk.

Stock solutions containing 100 mg/L and 100 µg/L of B(a)P were prepared in acetonitrile and stored at 4 C0 in volumetric glass flasks wrapped in aluminium foil to avoid possible light degradation. B(a)P standards were prepared by appropriate dilutions of these stock solutions. The signal due to B(a)P was identified by comparison of sample chromatograms with the chromatogram of the B(a)P standard. Quantification was done by the external standard method. The calibration line was constructed by regressing mean (n=3) peak area on standard concentration (0.001 to 10 µg/L in acetonitrile). The response was highly linear (R2=0.99,y=238536-329004.(9)

The extraction and clean-up procedure was of Janoszka et al.(2004) (18). The 25 g of samples were homogenised with 75 mL cold 1M NaOH solution (1 min) and 20 g was taken from this mix and added to extrelut refill material (approx. 20 g). Liquid solution 95:5 (v/v) dichloromethane :toluene (60 mL) was included in the extrelut column. A PRS SPE column was preconditioned with 4 mL dichloromethene. After filling, samples extract was added to PRS SPE (500 mg). The dichloromethene extract was evaporated to dryness and the residue re-dissolved in n-hexane (1 mL). Silica gel was activated at 200 oC for 12 h. A glass column including silica gel was eluted with 25 mL n-hexane. Extract was added to the top of a glass column packed with deactivated silica gel (10 g) and 60:40 (v/v) n-hexane-dichloromethene (60 mL) was added to the column. PAH extract including B(a)P was evaporated and the residue dissolved in acetonitrile (250 µL). The extract was stored in a brown vial at −20 oC prior to HPLC analysis. An aliquot (20 µL) of the acetonitrile solution was injected into the HPLC system and eluted with acetonitrile: water (80:20 v/v) at a constant flow rate of 2.0 mL/min. To quantify the B(a) P, the detector was set at excitation wavelength 290 nm and emission wavelength 430 nm .

Recovery was determined by applying the full procedure to three replicate samples of meat spiked with B(a)P (4 µg/kg). Recovery of B(a)P was calculated as Amount of B(a)P residue in medium(µg/kg)/ amount of B(a)P spiked in medium(µg/kg) × 100%.

**Statistical analysis ;** Statistical analysis of data was obtained by using the Statictical Package for Social Science (SPSS) version (10) and Microsoft Excel (2007) software .descriptive statistics for all data of each set were expressed as mean ± SD, student's t- test was used to evaluate the significance of differences between cooking methods, student t-test and correlation regression,it would be significant if $P \leq 0.05$.

**Results:**

The B(a)P concentrations of samples cooked by different methods are given in Table (1) & figure (1) with means and standard deviation. Each test was completed in independent triplicate. The differences between weeks were not significant. The differences between the means ±SD of concentrations of B(a) P in charcoal cooking method and gas cooking method for three types of meats were significant, the estimated mean value of B(a)P concentration was in majority at the chicken meat which cooked with charcoal fire (11.6±11.08), while the lowest estimated mean value of B(a)P concentration was in fish meat cooked by gas fire(2.0±1.4).

**Table (1). Discriptive analysis including (Means±SD) and Pvalues of B(a)P in three types meats(chicken-doner kebab-fish) under two cooking methods effects.**

| Cooking methods | Concentration of B (a) P µg/ Kg meat | | |
|---|---|---|---|
| | Chicken | Doner Kebab | Fish |
| | Mean±SD µg/kg | Mean±SD µg/kg | Mean±SD µg/kg |
| Charcoal fire | 11.6±11.08 | 10.0±9.4 | 8.4±7.8 |
| Gas fire | 2.5±1.9 | 2.3±1.7 | 2.0±1.4 |
| Pvalues | Pvalue | Pvalue | Pvalue |
| | P<0.05 | P<0.05 | P<0.05 |

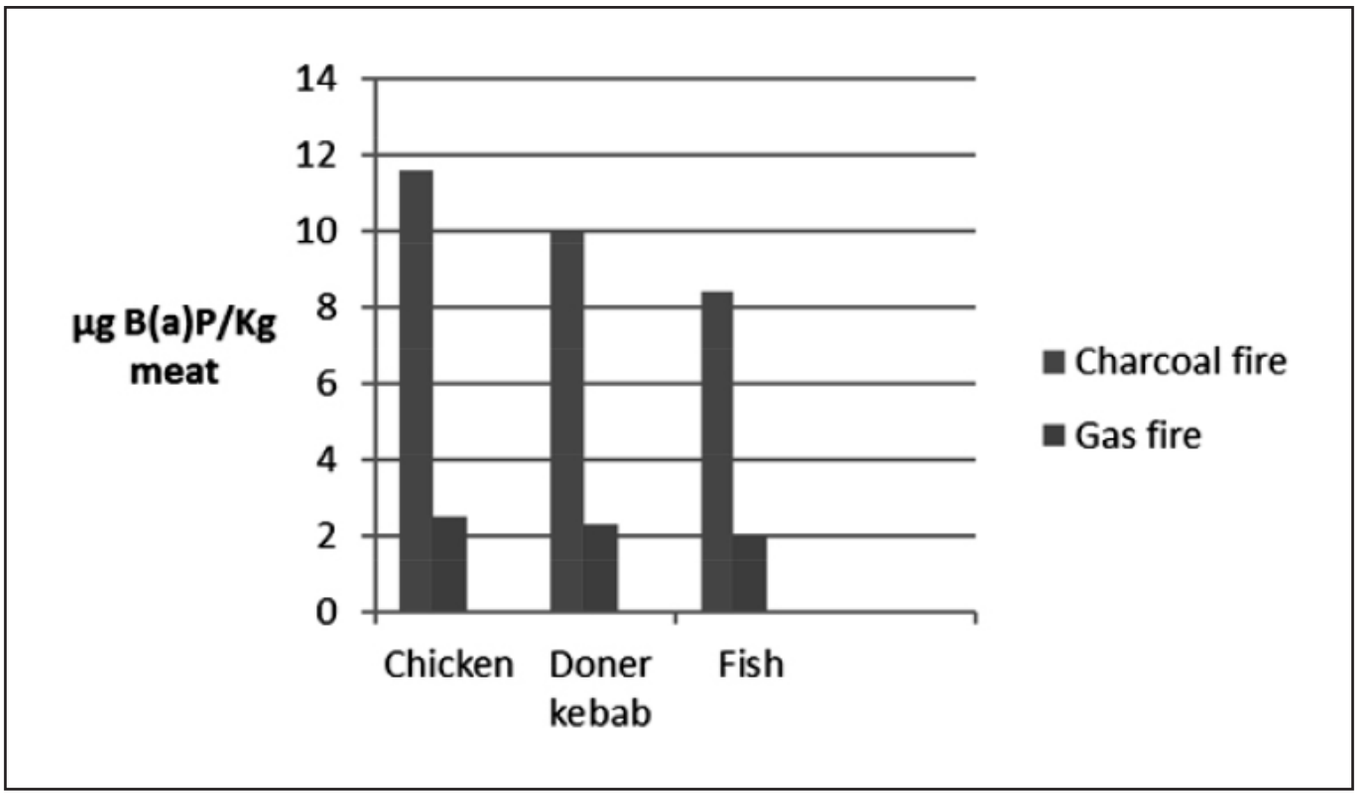


**Figure (1),levels of B(a)P concentrations due to effect of cooking methods on three types of meats (chicken,doner kebab,fish)**

**Discussion:**

From the results of this study ,it has been noticed that charcoal fire produced gas might be an impacting factor for the generation of B(a)P in foods during food processing. Some studies have examined the effect of using the wood as fuel in cooking methods of B(a)P formation in food samples such as G. Terzi , T.H. Çelik and C. Nisbet,2008, Yong-Hong Chen et al,2012 and others (19-26). We found that all charred (grilled) samples which collected from local restaurant contain B(a)P more than 1µg/kg (A limit value of 1µg/kg was established by European Union).The results showed that the smoke produced by the charcoal during the heating procedures rises and penetrates into the meat, this reason can explain why the charcoal broiled fish,donar kebab and chicken meats contained the highest levels of B(a)P ranging from 8.4 to 11.6 µg/kg in the present study. In addition,the effect of cooking method on the generation of B(a)P in the present study showed awidly difference ,that the charcoal broiling of three types chicken, donar kebab and fish meats showed highly levels of B(a)P as compared to the gas broiling of same three types of meats. The results showed that charcoal broiled meat might induce a nearly 4-5 times higher content of B(a)P compared to the gas broiled samples. There are no significant differences in means of concentrations of B(a)P in samples of three types of meats that broiled by charcoal cooking method, also there are no significant differences in means of concentrations of B(a)P in samples of same three types of meats which broiled by gas cooking method. B(a)P has been considered by the International Agency for Research on Cancer (IARC) ,1983(27), which concluded that it is a probable human carcinogen . Some other PAHs have also been identified as carcinogens with possible genotoxic properties. Some people who have been exposed by inhalation, or touch,to mixtures of PAHs and other chemicals for long periods of time have developed cancer(28) .The present results indicated that the local public of Baghdad city was exposed to heavy carcinogenic risk from B(a)P due to eating chicken,donar kebab and fish meats that broiled by charcoal broiling method for many years.This study supplied new data on B(a)P levels of three types of meats broiled with charcoal and gas fires in some restaurants of Baghdad city,these levels are too high when compared with specific concentration limite of B(a)P according to European Union establishment(29) which should be very helpful for the citizens to avoid the exposure to high levels of the carcinogenic hydrocarbons which produsedthrough the cooking process.

**Conclusion;**

The B(a)P levels of chicken, döner kebab and fish samples obtained from local restaurants of Baghdad, cooked on a charcoal fire exceeded the maximum tolerance level for B(a)P

of the European Union establishment (2005). The highest B[a] P levels were found in charcoal-grilled samples,chicken,doner kebab and fish while trace levels of B(a)P were present in gas grilled same samples.Therefore,present study provides important information on B(a)P in charcoal grilled meats in Iraq. This might help to avoid the contamination in food to secure food safety and to protect iraqi citizens .

**Auther's contributions:**

Dr.Khalid Nsaief Jasim designed the study ,acquired the data and reviewed the study

M.Sc.Samir Laybi Shkhier designed the study ,analyzed the data ,wrote the article and reviewed the study

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