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DETECTION OF POLYCYCLIC AROMATIC HYDROCARBON IN CHARCOAL – GRILLED MEAT WITH AND WITHOUT NATURAL ADDITIVES

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ABSTRACT

This study was conducted for detection of PAHs in both non marinated charcoal grilled kebab and kofta (5 of each) as a control samples and marinated charcoal grilled kebab and kofta (15 of each) using Gas liquid chromatography equipped with flam ionization detector GC/FID. Our results indicate the presence of Acenaphtene, Benzo (a) pyrene, Fluroanthene, Fluorene and Penanthrene (ppm) with a mean value of 0.027 ,0.0059 ,0.72 , 0.008 and 0.032 for kebab and 0.055, 0.023, ND, 0.04 and 0.023ppm for kofta respectively while Acenaphthylene was not detected in charcoal grilled kebab and kofta in non-marinated control samples. Moreover, after marination the mean values were 0.0041, ND, 0.068, ND and 0.0134 while in kofta they were ND, ND, 0.093, 0.0062 and ND respectively but Acenaphthylene in kofta was 0.010 ppm. However consumer not believed to be exposed to these levels so the application of this simple method by addition of natural preservatives (marination) at home and restaurants is important to reduce exposure of consumer to this group of carcinogenic agents produced during meat processing. The public health significance and economic importance of PAHs as well as recommendations for eating low PAHs meat were also discussed.

Key words: Polycyclic Aromatic Hydrocarbon – gas chromatography kebab – kofta – Benzo (a) pyrene

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbons—organic compounds containing only carbon and hydrogen—that are composed of multiple aromatic rings PAHs, are neutral non-polar and lipophilic molecules found in coal and in tar deposits. They are also produced by the incomplete combustion of organic matter (Nisbet and Lagoy, 1992).

Grilled foods are increasingly popular both at home and in restaurants; however, based on many studies, these foods present an elevated health risk to the population due to the higher levels of carcinogens found in such products compared to foods prepared by alternative cooking methods (Sundararajan *et al.*, 1999).

The US Environmental Protection Agency *(US-EPA) (2002)* proposed to use a selection of 16 PAHs which are frequenly found in environmental monitoring samples, namely, naphthalene (Na) acenaphthene (Ac), acenaphthylene (Ace), fluorene (F), anthracene (A), phenanthrene (Pa), fluoranthene (Fl), pyrene (P), Benzo (a) anthracene (BaA), chrysene (Ch), benzo (b) fluoranthene (Bbf) benzo (k) fluoranthene (BKF), benzo (a) pyrene (BaP), dibenzo (a,h) anthracene (DhA), benzo (g,h,i) perylene (BgP), indeno (1,2,3-cd) pyrene (IP).

European commission scientific committee on food selected four PAHs in the 15 priority PAHs as the most sutible indicators of carcinogenic PAHs in foods these four PAHs are benzo (a) pyrene, benzo (a) anthracene, chrysene and benzo (b) flouranththene.

The highest benzo (a) pyrene concentrations observed in barbecued meat products have been reported to be 1.5µg/kg for beef patties (Kazerouni *et al.*, 2001 and FSTA, 2007) and 0.313µg\kg for beef steaks (Aygun and Kabadayi, 2005).

It has also reported that the concentration of PAHs in barbecued food varies due to parameters such as fat content of food beside dripping of fat over the flame (Chen and Chen, 2001; FSTA, 2007), food type (Kazerouni *et al.*, 2001 and Aaslyng *et al.*, 2013), heat temperature and direct contact with heat source (Garcia-Falcon and Simal-Gandara, 2005; Reinik *et al.*, 2007; Chung *et al.*, 2011).

It is important to note that PAHs could be generated during cooking processes such as grilling, roasting, smoke curing and drying and this is the main reason

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for PAHs occurrence in food (Šimko, 2002; Chung *et al.*, 2011). According to The Scientific Committee on Food (SCF), Benzo [a] pyrene can be used as indicator for the presence of PAHs in food and their potential toxicity (European Commission, 2002) but the European food safety authority. (EFSA) stated that benzo [a] pyrene alone is not a sutible indicator for this purpose instead of other eight PAHs with potential carcinogenicity (EFSA, 2008) these eghit specifie PAHs are BaA,Ch, BbF, BkF, BjF, BaP, IP and DhA which are classified as group 2 B, probable human carcinogenics (European Commission, 2002).

Some studies try to reduce PAHs levels in charcoal grilled meat, three treatments wrapping with aluminum foil, preheating (steam and microwave) and use of marinating sauces (such as turmeric, salt, lemon,…) have been investigated. Using these pre-treatments before charcoal grilling resulted in reduced levels of carcinogenic PAHs in grilled meat samples (Farhadian *et al.*, 2011&2012). However, grilling samples of meat marinated for four hours had the powerful effect in minimizing PAHs concentration in meat dishes (Farhadian *et al.*, 2012).

PAHs are widely distributed in the environment a number of them, such as benzo [*a*] pyrene, are carcinogenic and mutagenic, and they are widely believed to make a substantial contribution to the overall burden of cancer in humans. Their presence in the environment is reflected in their presence at detectable levels in many types of uncooked food. In addition, cooking processes can generate PAHs in food, (Phillips, 1999).

Meat dishes prepared by charcoal grilling are popular and favorite food in Egypt as well as in other Arabian countries. Therefore the aim of this study was to throw the light on PAHs concentration in kebab and kofta as well as to determin the effect of addition of natural additives to meat (marination) prior to charcoal grilling as a trial to reach a most sutible methods which prevent or reduce the PAHs formation during meat processing. The present study consider a first trial to detect concentrations of PAHs in grilled kabab and kofta and effect of marinating on their concentrations.

MATERIALS AND METHODS

Forty samples of charcoal grilled meat (Kebab) and charcoal grilled kofta (20 of each) Samples were collected for detection and determination of polycyclic aromatic hydrocarbons (PAHs) compound residues, as follows:

(A) Ten samples of charcoal grilled meat (kebab) and kofta (5 of each) were collected from Mansoura city, El Dakhlia Governorate, Egypt and examined directly (non- marinated control).

(B) Thirty samples of fresh beef meat (divided into two parts one for kebab and the other used for kofta, 15 of each) were marinated and charcoal grilled then examined after marinating according to (Badry, 2010) and (Farhadin *et al.*, 2012).

Beef meat used for kebab and kofta were marinated by yoghurt, salt, turmeric, mustard, curry powder, lemon juice Cardamom, vinegar and onion, all these ingredients were mixed in a blender and added to the beef meat for 4 hours at refrigerator (4°C) until used for charcoal grilling. The samples were identified and kept frozen till the analysis was carried out

Analysis of the polycyclic aromatic hydrocarbos (PAHs) residues:

The analysis of PAHs residues was carried out extraction procedures, clean up and estimation of PAHs levels by gas chromatography were conducted in Pesticide Residue Department Central Pesticide Lab., Agriculture Research Center, Giza.

Extraction

Exactly 20 gm each of the examined samples were grinded in a mortar with anhydrous sodium sulphate (2gm). The mixture was then extracted with 60 ml of hexane-acetone (1-1) (v/v) mixture. The mixture was filtered and the tissue was extracted twice more. Organic solvent fractions were combined and filtered through filter paper with 1 gm anhydrous sodium sulphate. The extraction was evaporated to about 2 ml, then the extract was transferred to a round bottom flask and 100 ml of 10% aqueous methanolic potassium hydroxide were added, and the mixture was refluxed for 3 hours in order to saponify the lipids. At the end, the content of the round bottom flask was transferred to a separator funnel and rinsed with 150 ml of methanol-water (4:1), (v/v) mixture then extracted with hexane (80 ml) to recover the non soponified lipids. The hexane phase was concentrated down to 1.5 ml with an evaporator.

Clean up (Villeneuve *et al.*, 1999)

Clean up was achieved with a silica/alumina column. Aromatic hydrocarbons were eluted with 30 ml of a mixture of hexane and dichloromethane (90:10), (v/v).The volume of the eluted fraction was reduced to 1 ml and analyzed by a gas liquid chromatography equipped with a flame ionization detector GC/FID.

Preparation of blank solution

The same volume of solvents and anhydrous sodium sulphate, used in extraction of polycyclic aromatic hydrocarbon from the examined samples were subjected to the same procedures as for the examined

samples to detect any possible traces of the studied PAHs in the solvents or distilled water.

Gas Chromatography Analysis **(Moret and conter 2000)**

The polycyclic aromatic hydrocarbon fraction was injected into a gas liquid chromatography equipped with flam ionization detector GC/FID. Analysis was conducted on a HP-608 (Agilent, Folsom, CA) fused silica capillary column of 30 length, 0.53 mm id., and 0.5 µm film thickness. The oven temperature was programmed from an initial temperature 100 C° (2 min hold) to 260 C° at rate of 6 C°/min and was maintained at 260 C° for 15 min. Injector and detector temperature was maintained at 280 and 300 C° respectively. Nitrogen was used as a carrier at flow rate of 4 ml/min.

Determination of percentage rate of recovery:

Recovery was carried out by the addition of PAHs standards mixture at three levels of 1, 5, 10 µg. All data were corrected according to the recovery percentage values. Compounds were identified by matching retention time against those authentic Standards.

Statistical analysis

Statistical analysis was carried out by using (SPSS 18 2010).

RESULTS

Table1: Concentration of different PAHs residues (ppm) detected in non-marinated Kebab and charcoal grilled Kofta [N=10 (5 of each)].

Samples	Kebab				Kofta			
PAHs	**No**	**%**	**Rang**	**Mean±SE**	**No**	**%**	**Rang**	**Mean±SE**
Acenaphthylene	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Acenaphtene	**2**	**40**	**0-0.04**	**0.027±0.013**	**2**	**40**	**0-0.07**	**0.055*±0.0015**
Benzo (a) pyrene	**2**	**40**	**0-.0088**	**0.0059±0.0029**	**2**	**40**	**0-0.026**	**0.023*±0.003**
Fluroanthene	**4**	**80**	**0- 2.4**	**0.72±0.56**	**ND**	**ND**	**ND**	**ND**
Fluorene	**2**	**40**	**0-0.01**	**0.008±0.0015**	**2**	**40**	**0-0.06**	**0.04* ±0.002**
Penanthrene	**2**	**40**	**0-0.055**	**0.032±0.023**	**3**	**60**	**0-0.055**	**0.023±0.0015**

ND: Non Detectable (below the detection limit) P*<0.05 by using t-test.

Table 2: Concentration of different PAHs residues (ppm) detected in marinated Kebab and charcoal grilled Kofta [N=30 (15 of each)].

Samples	Kebab				Kofta			
PAHS	**No**	**%**	**Range**	**Mean± SE**	**No**	**%**	**Range**	**Mean±SE**
Acenaphthylene	**ND**	**ND**	**ND**	**ND**	**3**	**20**	**0- 0.018**	**0.010± 0.0040**
Acenaphtene	**3**	**20**	**0-0.008**	**0.0041±.0019**	**ND**	**ND**	**ND**	**ND**
Benzo (a) pyrene	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Fluroanthene	**5**	**33.3**	**0- 0.099**	**0.068±0.0030**	**2**	**13.3**	**0-0.099**	**0.093*±0.0055**
Fluorene	**ND**	**ND**	**ND**	**ND**	**4**	**26.6**	**0-0.0064**	**0.0062±0.00008**
Penanthrene	**5**	**33.3**	**0-.0040**	**0.0134±0.0006**	**ND**	**ND**	**ND**	**ND**

ND: Non Detectable (below the detection limit) P*<0.05 by using t-test.

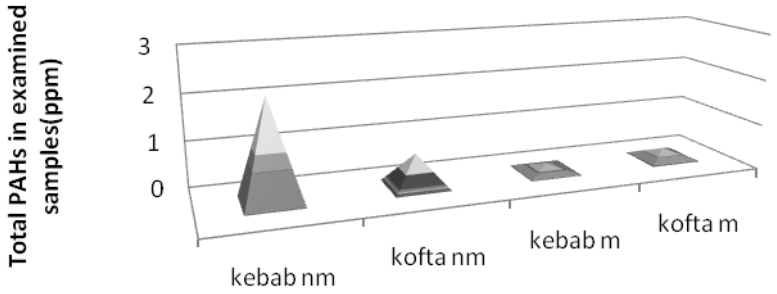
Table 3: Incidence and number of grilled non marinated and marinated kofta and kebab under and above permissible limits of Benzo (a) pyrene concentration.

BAP	Non marinated				Marinated			
	Kebab		Kofta		Kebab		Kofta	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
BAP under P.L.M	ND	ND	ND	ND	ND	ND	ND	ND
BAP above P.L.M	2	40	2	40	ND	ND	ND	ND

ND: Non Detectable (below the detection limit)

Maximum Permissible limits (P.L.M.) for Benzo (a) pyrene (BAP) in smoked meat products is 0.005 ppm (EC, 2006).

Fig (1) Comparsion between marinated and non-marinated charcoal grilled meat (kebab) and kofta samples according to total PAHs formation with mean value .



M: marinated - nm : non marinated .

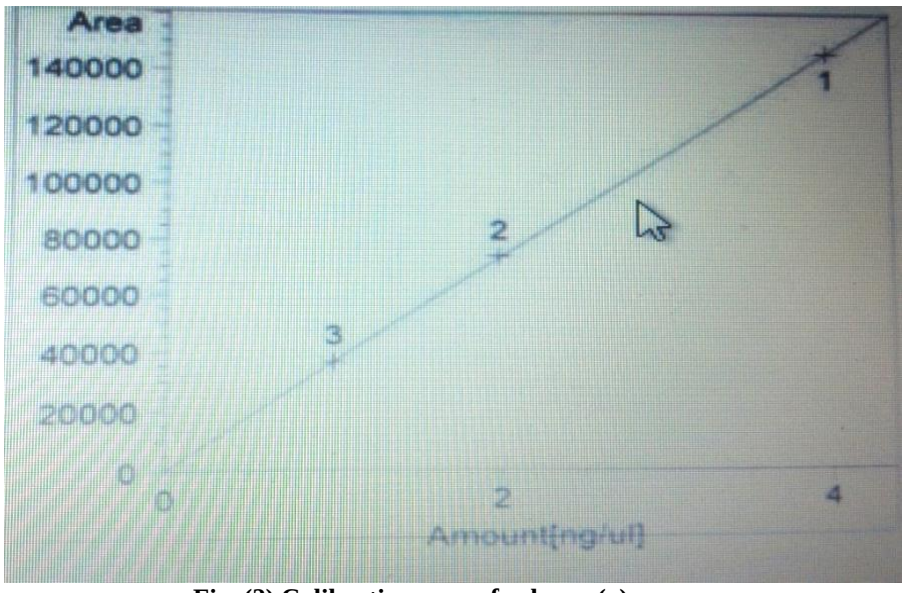


Fig. (2) Calibration curve for benzo(a) pyrene

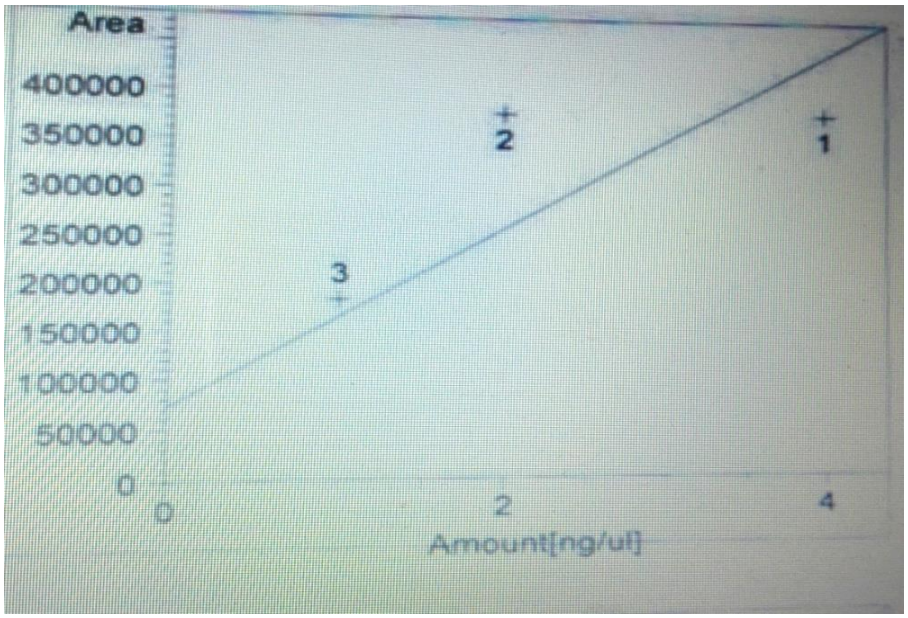


Fig. (3) Calibration curve for phenanthrene

DISCUSSION

Consumption of meat is increasing day by day due to its nutritional value and palatable flavor. PAHs, are formed when juices from meat drip onto coals or other hot surfaces and create smoke. The smoke contains these carcinogens, which are deposited onto the surface of meat as it swirls around the food.

PAHs compounds contaminate meat by grilling as it is essential step for enhancing the aroma that distinguishes the flavor of one food from another. Aroma depends upon the vapour pressure of food constituents and on the interaction of volatile compounds with non-volatile compounds (Laing and Jink, 1996).

Marinating which is commonly used method involves injection, tumbling or immersion to disperse in the muscle tissue. The marinating ingredients are used to improve color, tenderness, increase shelf life, may affect lipid oxidation and decrease PAHs as shown in Table 1. The mean concentration of Acenaphtene, Benz(a) pyrene, Fluroanthene, Flurorene and Penanthrene (ppm) (Fig. 3) are 0.027, 0.0059, 0.72, 0.008 and 0.032 for kebab and 0.055, 0.023, ND, 0.04 and 0.023 for kofta respectively while Acenaphthylene could not be detected in charcoal grilled kebab and kofta. Similar results were obtained by Mishref (2014) while higher results were obtained by (Gorji *et al.,* 2016).

The incidence of PAHs in the examined samples were 40, 40, 80, 40 and 40 % for Acenaphtene, Benz(a) pyrene, Fluroanthene, Flurorene and Penanthrene in kebab and 40, 40, ND, 40 and 60 % for kofta respectively. There were a significant increase in kofta more than kebab. The significant difference in PAHs concentration between kebab and kofta may be due to the method of grilling as kebab composed of pieces of whole meat take a long time for grilling which lead to accumulation of large amount of PAHs but kofta take low time for grilling as it composed of minced meat.

In the other hand, the addition of natural additives for kebab and kofta mainly lemon juice induces about 72% reduction of PAHs levels when applied to meat prior to charcoal grilling (Orecchioand Papuzza, 2009). On the other hands, additives have strongly antioxidant natural properites enables these foods to engulf carcinogenic compounds, these functional foods are the potent source of bioactive phenolic compounds (Hazra *et al.,* 2010).

These bioactive compounds are acidic in nature to engulf the carcinogenic compounds or free radicals by which the concentration of PAHs to be reduced.

By observing Table 2 we found that Acenaphtene, Benz(a) pyrene, Fluroanthene, Flurorene and Penanthrene concentration in kebab after marinationare 0.0041, ND, 0.068, ND and 0.0134 while in kofta they are ND, ND,0.093, 0.0062 and ND ppm respectively but the level of Acenaphthylene in koftais 0.010. (European COMMISSION REGULATION 2006) advise to continue to monitor the presence of PAHs in traditionally smoked meat and smoked meat products and shall establish programmes to implement good smoking practices where possible, within the limits of what is economically feasible and what is possible without losing typical organoleptic characteristics of those products.

From Table 3 it is evident that Benzo (a) pyrene exceeded the permissible limit before marination (Fig.2) and disappeared completely after marination which indicate the positive effect of marination.

Many researches illusterated the importance of cooking time as an important factor in production of PAHs in food.as PAHs formation is favored at a temperature range of 500-900 °C Most of these studies have concluded that the level of PAHs in food can be reduced by minimizing grilling time (Phillips, 1999; Kazerouni *et al.,* 2001; Terzi *et al.,* 2008). Fig (1) shows the positive effect of natural additives on reduction of PAHs formation.

In both samples kebab and kofta which agree with that reported by (Beata, 2011, Farhadian *et al.,* 2012 and Mishref, 2014). The reduction effect of marinating on PAHs formation may be due to presenece of onion and garlic which contain a lot of organic sulfur compounds that prevent maillard reactions (Nursten, 2005; Gibis, 2007). Carcinogenic compounds such as heterocyclic aromatic amines were inhibited by onion and garlic as reported by (Shon *et al.,* 2004).

PHAs can be reduced by following certain practices of cooking as addition of compounds with anti-oxidative properties.

CONCLUSION AND RECOMMENDATIONS

The obtained results of this study have shown that addition of natural additives (marination) of meat prior to charcoal grilling process have the maximum effects in reduction of PAHs. The levels of PAHs found in marinated kebab conducting more studies on the effects of different marinade ingredients is necessary in an effort to determine the best marinade treatments that are responsible for PAHs formation in grilled meat. On the other hand, safety procedures are important in preparing meat dishes which is popular worldwide.

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تقدير مستويات بعض المركبات الهيدروكربونيه الأروماتيه متعدده الحلقات فى اللحوم المشويه باستخدام اضافات طبيعه وبدون استخدامها

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اجريت هذه الدراسه على ٤٠ عينه من الكباب والكفته لاستبيان مدى تواجد سته من اهم الهيدروكربونات الاروماتيه متعدده الحلقات فى اللحوم والكفته المشويه على الفحم وايضا معرفه تأثير اضافه بعض المواد الطبيعيه (التتبيل) على هذه المركبات الاروماتيه وقد دلت النتائج على تواجد كل منأسينافثين، بنزو (أ) بيرين، فلورانثين، الفلورين وبينانثرين بالنسب الاتيه ٠.٠٢٧-٠.٠٠٥٩ -٠.٧٢ - ٠.٠٠٨ – ٠.٠٣٢ لعينات الكباب الغير متبله ولكن عينات الكفته الغير متبله كانت النسب هى ٠.٠٥٥ -٠.٠٢٣ – غير موجوده – ٠.٠٤ -٠.٠٠٢٣ على التوالى وكانت نسبه أسينافثيلين اقل من مستوى حساسيه الجهاز فلم يتم رصدها فى كلا من الكباب والكفته. وبعد اضافه بعض المواد الطبيعيه المستخدمه فى التتبيل لوحظ انخفاض فى مستوى المركبات الهيدروكربونيه الأروماتيه متعدده الحلقات وكانت النتائج بعد التتبيل كالاتى ٠.٠٠٤١ – غير موجود - ٠.٠٦٨ -٠ غير موجود – ٠.٠١٣٤ فى عينات الكباب لكلا من أسينافثين، بنزو (أ) بيرين، فلورانثين، الفلورين وبينانثرين ولكن فى الكفته كانت النسب غير موجود – غير موجوده – ٠.٠٩٣ – ٠.٠٠٦٢ جزء من المليون – وغير موجود وكان نسب أسينافثيلين فى الكفته ٠.٠١٠ جزء من المليون هذا وقد تمت مناقشه الاهميه الصحيه لهذه المركبات واقتراح التوصيات المناسبه للحد منها وتقليل خطرها على صحه المستهلك.