

**DETERMINATION OF POLYCYCLIC AROMATIC OF HYDROCARBONS (PAHs) BENZO [a] PYRENE LEVEL IN HEAT TREATED FOOD FROM EGYPTIAN MARKET BY GC AND HPLC-FLUORESCENCES DETECTION**



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**ABSTRACT**

This study was aimed to measure benzo(a)pyrene (BaP) as the most important one of polycyclic aromatic hydrocarbons (PAHs) in many kind of foods by GC and HPLC-FD.

The detection was carried out for the presence of benzo(a)pyrene in more than 90 food samples collected from retail stores in Egyptian markets (including vegetable oil, meat, cheese, fish, coffee..etc.) in order to investigate the effect of processing treatments such as hot and cold pressing of prepared oils, strength of roasting process of coffee, smoking treatments and direct exposure of food to temperature on the formation of benzo(a)pyrene.

The results showed that Benzo(a)pyrene content in smoked samples were varied from high level in smoked herring fish to low in canned tuna samples. Also; it was notable that smoked processed beef, sausages and salmon had a low BaP values. Around 30% of the smoked samples examined in this study had BaP levels exceeded than the acceptable limit as specified by the European Commission (E.C, 2005).

The amount of BaP detected in more than 24 coffee, tea and cocoa were higher than the permissible limit (1ppb) set by European Commission(E.C, 2005). In eighteen coffee samples, fourteen samples had upper values for the exceeding. The content of BaP in strong roasting coffee samples (dark) was higher than moderate or lighter roasting process. The highest value in this category was in flavored green tea; while in the cocoa powder there was a negligible amount of BaP.

The levels of BaP in 28 oil samples showed that the highest value was reported in roasted sesame oil samples from local market while the least was reported in cold press olive oil samples from local market. In butter, vegetable oil associated with pickles and cheese there were no detectable level of BaP; In cold press olive oil, flax seed oil and sesame levels of BaP were significantly lower as compared to the thermal press oils samples. Olive-pomace oil contained high levels of BaP.

Analyzed 10 vegetable oil associated with canned fish; the smoked sardine and tuna contained the highest levels of BaP. In four samples (tuna, mackerel, sardines and anchovies) showed a BaP level less than 1 µg/kg.

Among twelve charcoal barbecued samples analyzed for BaP and 50% of the samples were exceeding the level set by European Commission regulations. BaP charcoal barbecued lamb meat had around three fold the BaP level of beef meat sample. Charcoal barbecued (sausages and lamb) samples contained high levels of BaP. Liver and shrimp samples showed moderate level of BaP; while the minced meat (kofta) sample gave high ratio of BaP.

BaP contents 10 times lower in fish sample than fish skin sample; also, the chicken skin contained BaP three times higher than chicken meat.

Fifteen samples include nuts, dried fruits, cereals and chocolates, infant formula showed content of BaP comply with the EU regulations. While in dark soya sauce sample, the BaP was relatively higher than the limit set by EU regulations.

**Keywords:**Vegetable oil, coffee, Polycyclic aromatic hydrocarbon ,HPLC-FD,GC-FID ,Benzo(a)Pyrene, Coffee powder, Roasting, Smoked , Solid phase extraction( SPE) , Charcoal barbecued .

## INTRODUCTION

Hydrocarbons of polycyclic aromatic compounds (PAH's) are a group of aromatic rings. The five fused rings are known as Benzo-[a]-Pyrene. Some of PAHs are: benz[a]anthracene, benzo[a]pyrene, benzo[b] fluoranthene, benzo[k] fluoranthene, chrysene, dibenz[a,h] anthracene, and indeno (Howard *et al.*, 1996) pyrene (García-Falcón *et al.*, 1999 ). Nevertheless, benzo[a]pyrene (BaP) is usually taken as marker substance for PAH exposure in general because of its extremely high carcinogenic potential (Laffon *et al.*, 1997 ) BaP is a member of polycyclic aromatic hydrocarbons (PAHs) which are a large group of organic compounds with mutagenic and carcinogenic properties (Ramalhosa *et al.*, 2009). Benzo[a]pyrene is the only polycyclic aromatic hydrocarbon with sufficient toxicological evidence to allow the setting of a guideline (Moret *et al.*, 2005 and Yusty and Davina, 2005).

Contamination of foodstuffs by PAHs can occur at source through atmospheric deposition on crops (Culotta *et al.*, 2002), or from preservation of food by drying and cooking procedures. This contamination is enhanced during smoking and intense thermal processing (Chen and Chen, 2001). During intense thermal processing the contamination occurs by direct pyrolysis of food nutrients (Orecchio and Papuzza, 2008).

Animal studies have shown that dietary intake of BaP causes increased levels of tumors at several sites, particularly in the upper gastrointestinal tract. Humans can be exposed to BaP through different routes. For the general population non-smokers, the major routes of exposure are from food consumption. Most individuals are predominantly exposed to PAHs from dietary sources (Bordajandi *et al.*, 2008).

Food can be contaminated from environmental sources, industrial food processing and from certain home food preparation.

SCF (Scientific Committee on Food),( 2002) estimated a maximum daily intake of benzo[a]pyrene from food of approximately 6-8 ng/kg b.w. per day for a person weighing 70 kg but 5 µg / kg(EU regulations).

After eating BaP, our body attempts to rid of the foreign substance by converting it into a water-soluble compound for metabolism, a diol epoxide. This compound, in turn, reacts with and binds to cellular DNA. Consequently, diol epoxide alters DNA and mutates genes (Hecht *et al.*), (2010). BaP has been shown to affect both the development and response of T and B cells in the immune system (Chan *et al.*, 2011). It can decrease epididymis sperm quality, possibly by disturbing testosterone levels, and StAR may be a major steroidogenic protein that is targeted by B[a]P or other PAHs. Research has also shown that PAHs could be transferred from their mothers to newborns and young infants, and cause brain and behavior toxicity (Leadon *et al.*, 1988 and ATSDR., 1996).

The International Agency for Research on Cancer (IARC) 1987, has upgraded its overall evaluation of B[a]P from group 2B (possibly carcinogenic to humans) to 1 (carcinogenic to humans).

The presence of PAHs in coffee has also been reported and it has been suspected to be due to either a contamination of green coffee beans during the drying step or an endogenous formation in the coffee beans during the roasting process (Houessou *et al.*, 2005 and Houessou *et al.*, 2008).

Therefore, this study has been carried out to evaluate the level of B[a]P in 90 selected foods to supply new information on B[a]P levels in a variety of foods from Egypt.

## **MATERIALS AND METHODS**

### **MATERIALS:**

**Reagents:** N-Hexane (HPLC grade), Dichloromethane (HPLC grade), Acetonitrile (HPLC grad); Benzo (a)pyrene (100mg/ml); PTFE syringe filter (0.22µm); SPE cartridge.

**Instruments:** HPLC-FD dionex ultimate 3000, GC clarius 600 perkin elmer, Rotary evaporator , Ultrasound instrument, 24 Position Vacuum manifold SPE instrument, centrifuge .

**Chemicals:** Benzo[a]pyrene standard from Sigma-Aldrich Chemie (Steinheim, Germany) had a minimum of 97% purity. A solutions stock containing 100 mg and 100 mg of BaP were prepared in hexane, ACN and DMSO dimethyl sulfoxide then stored at 4°C in volumetric flasks (with glass stoppers) wrapped in aluminum foil to avoid possible light degradation. The BaP standard was prepared by appropriate dilutions of these stock solutions.

In this study a detection was carried out for the presence of benzo(a)pyrene BaP in more than 90 samples collected from retail stores in Egyptian markets include olive oil prepared by hot and cold pressing and many types of vegetable oil such as corn oil, sunflower oil, sesame oil, coconut oil, palm oil, soya oil, canola oil, , grape seed oil, toasted sesame oil, and mixed vegetable oil, (oil associated with canned fish) as well as soya sauce (imported and from local production available on the market). Cold and heat press olive and sesame oil samples were examined to compare the two processes. Barbequed food samples (meat, chicken, shrimp, bread, corn and fish ) were also investigated.

Also, BaP were determined in selected samples of tea, cocoa and roasted coffee powder; in order to evaluate the effect of high temperature of roasting processes on benzo (a) pyrene content in comparison to low roasting temperature and green coffee seeds.

Smoked salmon, herring, processed meat, cheese, dried fruits, chocolates, and cocoa powder also were analyzed to benzo (a) pyrene detection.

The values set in the result tables is average of the measured concentrations of BaP in replicate samples (three times in separate runs).

**METHODS:**

The present study was carried out to measure BaP which is the most important one of PAHs in many kind of foods by GC and HPLC-FD. The analysis of PAHs at these low concentration levels is a major challenge to analytics, especially due to the laborious removal of the complex lipid matrix for chromatographic detection. To extract hydrophobic Benzo(a)pyrene from oil matrix which is also hydrophobic, hexane was chosen as the best solvent to dissolve samples after investigation. Liquid-liquid extraction was applied to extract benzo (a) pyrene (BaP) and two methods were generally applied for PAH extraction from samples. The first of which is a combination of liquid-liquid extraction (LLE), typically using Acetonitrile(ACN) or DMSO dimethyl sulfoxide followed by back-extraction with hexane with a solid phase extraction (SPE) step, for which normal-phase as well as reversed-phase (RP) solid phases can be applied (Balenovic, et.al 1995; Van Stijn, et. al 1996 and Pupin,1996) Extraction of BaP was achieved using C18 solid phase extraction (SPE) prior to analysis by gas chromatography and HPLC-FD.

**Sample treatment**

**Extraction and clean-up:** Sample was weighed (3g to 5g) depending on the particle size of sample was weighed in a centrifuge tube with sealing cap and extracted with three portions of 10 ml Acetonitrile ACN, shaking vigorously for 3 min. After each extraction, the sample was centrifuged at 2500 rpm for 10 min to separate the layers, drawing off upper layer with pipette. The combined ACN extracts (30 ml) were concentrated to dryness in rotary evaporator, and the residue was dissolved in 5 ml of hexane; which was then passed onto a SPE cartridge and eluted with 10 ml of hexane to wash the cartridge and 5ml dichloromethane to elute the benzo(a)pyrene out. The hexane elute was evaporated to dryness and the residue was dissolved in 1 ml of acetonitrile to reconstitute the sample. In addition, if some samples kind contained more impurities which was need to be treated with SPE again; In the case of heavy matrix sample may use silica gel post- extraction cleanup; which the concentrated extracts were purified by column chromatography on silica gel, as described by Hossain et al. (2009). then filtered through 0.22 mm pore-size PTFE filters. The detection was carried out on GC with FID detection; Using standard capillary column and quantitation was done. The applied extraction method allowed handling over 90 samples using GC perkin elmer clarius 600 with a programmable FID detector to determine the content of the heavy polycyclic aromatic hydrocarbons (PAHs); benzo(a)pyrene, also detection on the HPLC-FD Dionex 3000 Ultimate carried to confirm results using HPLC column C18 5u hypersil 15cm on exc:254 nm and the emission 406 nm ; the mobile phase was hexane 99.5% to dichloromethane 0.5% on flow rate 0.6 ml/min. there was no significant differences between the results of the two instruments.

After 10s ultrasound treatment and filtration by 0.22µm PTFE Syringe filter, the sample was ready for GC or HPLC Injection.

## RESULTS AND DISCUSSION

With the current legislation prescribing benzo[a]pyrene to be used as an indicator for general PAH contamination; the relevance of such use was tested by calculating concentrations of the other PAHs in the absence of benzo[a]pyrene. The European Union Commission Regulation (EC) No 2008/2005 set maximum limits of PAHs in food and forces the producers to a more strict monitoring of PAH as there is the limitation of benzo(a)pyrene in all types of food (5( $\mu\text{g}/\text{kg}$ )).

**Table: (1) The BaP concentrations in experimental kinds of different types of food samples**

Category of smoked samples	Samples tested	Concentration of BaP ( $\mu\text{g}/\text{kg}$ )	
		Minimum	Maximum
Smoked cheese	2	ND	0.16
Smoked cooked cheese	3	ND	8.72
Smoked processed sausages	2	0.2	1.52
Smoked turkey	2	0.5	3.6
Smoked salmon	2	1.22	5.2
Smoked tuna	2	0.3	10.86
Local smoked herring	1	-	17.6
Imported smoked herring	2	1.2	4.9
Smoked processed beef	2	ND	0.76

Benzo[A]pyrene concentration in smoked samples ranged from 0.16 to 17.6  $\mu\text{g}/\text{kg}$  among the samples as shown in Table(1). The least BaP concentration was recorded in smoked imported cheese sample while the highest was recorded in smoked herring fish sample obtained from local market. Also, the smoked canned tuna had a high level of BaP (10.86  $\mu\text{g}/\text{kg}$ ). It was notable that smoked processed beef, sausages and salmon had a low BaP values of (0.76,0.2 and 1.22 respectively).

Around 50% the smoked samples examined in this study, had BaP levels which exceeded the acceptable limit of 5  $\mu\text{g}/\text{kg}$  as specified by the European Commission (E.C, 2005).

Smoked fish constitute an important and significant part of the human diet. Nutritionally this is due to their desirable sensory properties, high nutritional value and abundance of lipids rich in n-3 fatty acid residues. The findings from the present study indicated that the wood smoke used in smoking process of fish contributed to contamination of the fish with BaP. The variation in the levels of BaP observed among sampled smoked fish could be attributed to the different processing, differences in the type of wood used for smoking process or even differences in construction of smoking kilns. BaP in smoked fish come from the wood smoke findings to work done elsewhere (Stolyhwo and Sikorski, (2005).

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Traditional commercial smoking techniques, in which smoke from incomplete wood burning came into direct contact with the product, can lead to its considerable contamination with various PAHs, if the process is not adequately controlled. Critical treatments include temperature, time, humidity, types of control and smoke used (natural or generated), and the design and types of smokehouses or kilns effected on the PAHs contamination.

Visciano *et al.* (2006) studied the levels of PAHs in fresh and cold-smoked Atlantic salmon fillets and found no differences between raw and smoked samples in the concentrations of several carcinogenic PAHs, but significant differences for some low weight molecular PAHs, which confirmed that PAH concentrations in smoked fish are the result of both sea pollution and the smoking process.

Guillén and Sopelana (2004) investigated the possible presence of PAHs in 7 commercial types of cheese smoked by traditional techniques. In order to provide an indication of the PAH contamination in the cheeses that are commonly consumed. The samples were manufactured with milk from cows, sheep, goats, or a mixture of them, and purchased in local supermarkets. The results revealed the presence of several PAHs in the exterior zone of the samples, some of the PAHs having methyl groups. In all cases, the concentrations of PAH compounds of low molecular weight were much higher than those of high molecular weight.

**Table (2): The BaP concentration in different kinds of coffee and tea samples**

Category of (coffee and tea samples)	Concentration of BaP
	( $\mu\text{g}/\text{kg}$ )
Turkish coffee (regular)	2.33 -7
Turkish coffee with cardamom	2.4
Dark Turkish coffee	6.8
Dark Turkish coffee With herbal	6.2
Medium Turkish coffee with herbal	5.1
Light Turkish coffee	1.7
Light Turkish coffee with herbal	0.9
American coffee Dark	4.3
American coffee Medium	3.7
American coffee Light	2.1
Instant coffee	2.9
Nescafe	2.2
Espresso dark	2.6
Espresso light	1.5
Green coffee	ND -0.08
Green coffee with cardamom	0.02
Green tea with jasmine flavor	11
Tea powder	0.5-7.9
Dark tea powder	1.46-9.2
Cocoa powder	0.035-0.05

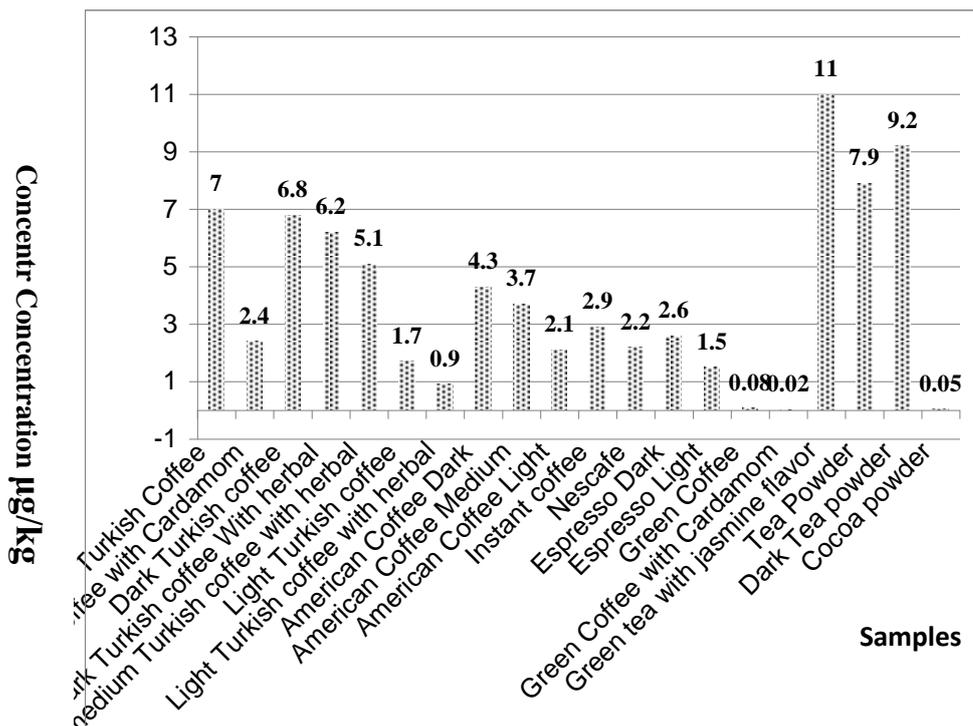


Fig (1): The BaP concentrations in experimental kinds of coffee and tea samples .

Roasting is a critical process in coffee production as it enables the development of flavor and aroma. At the same time, roasting may lead to the formation of no desirable compounds, such as polycyclic aromatic hydrocarbons (PAHs) Contamination of PAHs by intense thermal processing occurred due to generation by direct pyrolysis of food nutrients. Also due to direct deposition of PAHs from smoke of different thermal agents (Luch, A., 2005).

According to existing regulations by European Commission (2005) the content of BaP in some foods and baby foods should not be exceed 1 ppb.

Benzo (a) pyrene was detected in more than 95.8% of coffee, tea and cocoa 24 tested samples which amounted in range of 0.02 to 11 ppb (Table 1). BaP detected in most samples are higher than the permissible limit (1 ppb) set by European Commission(2005). In eighteen coffee samples ,fourteen samples had upper values for the exceeding 1µg/kg.

The content of BaP in strong roasting coffee samples (dark) was higher than moderate or lighter roasting process with the highest average values of 7,6.8 and 6.2µg/kg, respectively. This might be because high temperatures (400–500°C) and long heating times were used for roasting.

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The highest value in this category was in jasmine flavored green tea with value of 11µg/kg . In the cocoa powder there was a negligible amount of BaP.

**Table: (3)** The BaP concentrations in different oil and fat samples

Category of (oil and fat samples)	Concentration of BaP
	(µg/kg)
Imported extra virgin olive oil	1.1
Imported virgin olive oil	0.99
Refined olive oil	0.97
Crude pomace oil	2.38
Local extra virgin oil	1.2
Local virgin olive oil	1.4
Local ordinary olive oil	0.69
Cold press olive oil	ND -0.4
Mix oils (palm, soya – olien)	3.3
Corn oil	1.2
Palm oil	2.1
Sesame oil thermal extract	49
Sesame oil extract on cold	ND - 2.6
Canola oil	0.92
Sun flower oil	2.1
Flax seed oil	3.6
Flax seed oil on cold press	1.0
Coconut oil	6.7
Cocoa butter	0.1-1.7
Butter	ND
Veg oil from (sardines cans)	0.4
Veg oil from (Anchovies)	ND - 0.66
Veg oil from (Tuna)	ND - 0.12
Veg oil from (Mackerel)	0.16
Veg oil from (Smoked Tuna)	1.89
Veg oil from (Smoked Sardines)	4
Veg oil from (Cheese)	ND
Veg oil from (Pickles)	ND

**Veg = vegetable**

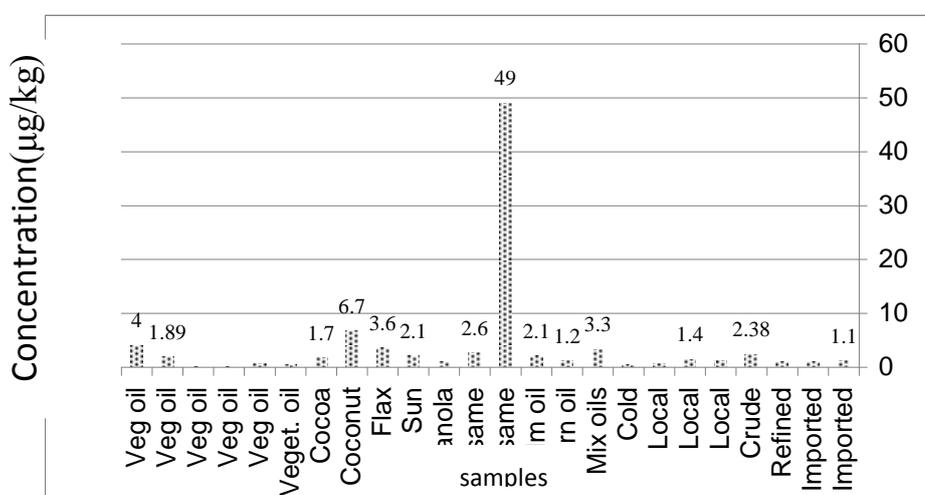
However, since benzo[a]pyrene is considered a carcinogenic compound, monitoring the amount of this compound in a popular drink such as coffee is crucial. So; the assessing of the dietary exposure of consumers to carcinogens is a significant.

Studies showed that the PAHs concentration in the roasted coffee beans was dependent on roasting temperature and roasting time. The content of low molecular PAHs, such as phenanthrene, fluoranthene, and pyrene was increased during the roasting process under elevated temperatures above 220°C. Strong roasting conditions (260°C) led to significant levels of pyrene, chrysene and benzo[a]anthracene. While the

presence of PAHs in coffee has been attributed to their formation during the roasting step. The transfer of PAHs from coffee powder to coffee brew during coffee making has been reported to be relatively low; (García Falcón *et al.*, 2005). This may be due to the low solubility of PAHs in water. ( 1% or less) of benzo[a]pyrene initially present in the solid coffee.

Since infusion strength influenced the transferred benzo[a]pyrene concentrations, higher transfer percentages up to 26% with the mean value of 5% were found by Maier (1991). More recently Houessou *et al.* (2007) reported a transfer percentage to vary from 0.6 to 30.9% for benz[a]anthracene, chrysene and benzo[b] fluoranthene depending on the PAH compound, which was slightly lower extractability for dark-roasted coffee as compared to light-roasted coffee.

More than 5 dried tealeaf samples had BaP contents varied between 0.5 and 11 µg/kg in which 80% of the samples exceeding 1 µg/kg. However, preparation of the tea will dilute the level found in the beverage before consumption(Maier,1991).



**Fig(2): The BaP concentrations in different oil and fat samples**

The concentrations of BaP in more than 30 oil samples ranged from 0.4 to 49 µg/kg. The highest value was reported in roasted sesame oil samples also from local market while the least was reported in cold press olive oil samples from local market. In butter, vegetable oil associated with pickles and cheeses there were no detectable level of BaP;

In cold press olive oil and sesame levels of BaP were significantly lower 0.4 and 2.6 as compared to the thermal press oils samples as presented in Table ( 3 ) .

The levels of BaP in virgin olive oil and olive oil was between 0.4 and 1.4 µg/kg; but olive-pomace oil contained high level of BaP ( 2.38 µg/kg).

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Benzo(a)pyrene concentrations were below the allowed limit of the European Union (2 µg/kg) in 68 % of the oil samples; These values are in agreement with most literature data for European oils (Preedy, 2010; Moret,2000 ;Conte, 2000 and Ballesteros , 2006).

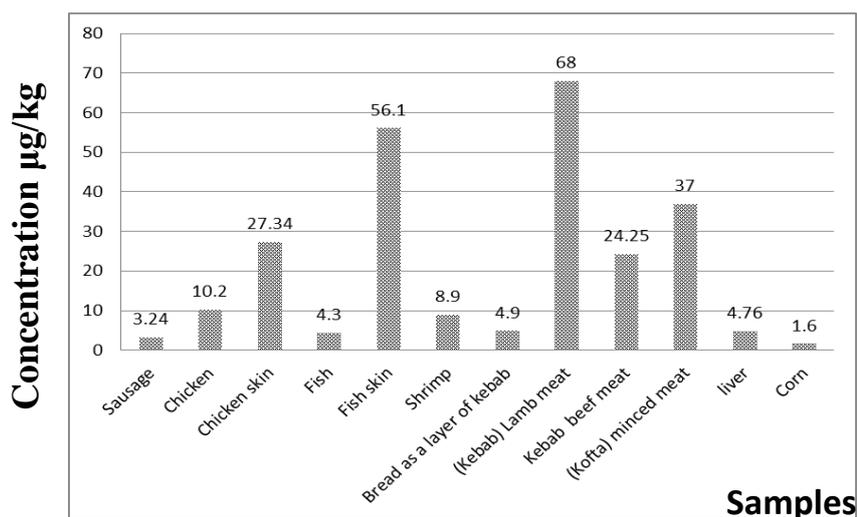
Similar values of BaP were found for treated and non-treated oils, because the refined process eliminate the light PAHs (2–3 benzene rings), while the heavy PAHs (4–5 benzene rings, e.g. BaP), remain in the oil (Moret and Conte, 2000).

The benzo (a) pyrene content in aqueous enzymatic extracted oil and cold pressing oil can meet the safety need without refining. The major cause of forming excessive benzo (a) pyrene in traditional solvent extracted oil may be due to high temperature, rather than solvent. Neutralizing could lead to obvious increase in benzo (a) pyrene content in oil, and deodorizing lead to slight increase. But the processes of bleaching and winterization could reduce benzo (a) pyrene content in the oil to a safety range.

For the more than 30 vegetable oils analyzed samples in (vegetable oil associated with canned fish) the smoked sardine and tuna contained the highest levels of BaP (4 and 1.89 µg/kg respectively) . It is possible that these high levels in the oil might result from leaching the lipophilic BaP from the smoked fish products. This behavior was also observed by Lawrence and Weber (1984) when they analyzed smoked mussels and oysters in vegetable oil. Although, in general the BaP values were low. Four samples (tuna, mackerel, sardines and anchovies) showed a BaP level less than 1 µg/kg, the maximum that the EU intends to set for foods.

**Table (4): The BaP concentrations in charcoal barbecued food samples**

<b>Category of charcoal barbecued samples</b>	<b>Concentrations of BaP (µg/kg)</b>
Sausage	3.24
Chicken	10.2
Chicken skin	27.34
Fish	4.3
Fish skin	56.1
Shrimp	8.9
Bread as a layer of kebab	4.9
(Kebab) Lamb meat	68
(Kebab ) Beef meat	24.2
(Kofta) Minced meat	37
Liver	4.76
Corn	1.6



**Fig(3):The BaP concentrations in different charcoal barbecued samples**

The BaP contents in charcoal barbecued samples exceeding the EU limit are illustrated in Figure (3). Seen from this figure and table (4) the BaP contents those higher than 10 µg/kg were observed in six samples, including, kebab, kofta, and charcoal grilled chicken, with contents of 68, 37 and 10.2 µg/kg, respectively.

Twelves charcoal barbecued samples analyzed for BaP and 50% of the samples were exceeding the level set by European Commission regulations (10µg/kg). Barbecuing with charcoal gave the highest benzo[a]pyrene levels in each type of food.

The strong influence of the cooking method and the type of heat source affected on the PAH levels in the processed food as demonstrated by Larsson *et al.* (1983).

BaP of charcoal barbecued lamb meat had around three folds the BaP level of beef meat sample prepared by the same process. The results indicated that BaP was mainly present in heat-treated animal source foods, which was considerably heavy BaP. From the results of the present study, it can be inferred that fat content might be an impacting factor for the generation of BaP in foods during food processing. Charcoal barbecued samples content of BaP ranged from 3.24 µg/kg in sausage sample to 68 µg/kg in lamb meat.

The influence of the fat content of the meat upon the amount of PAHs formed during charcoal grilling was also demonstrated by Mottier *et al.* (2000) who found significantly higher levels of carcinogenic PAHs in high fat containing lamb sausages compared to other meat products with lower fat content.

In traditional charcoal barbecued corn had BaP content of 1.6 µg/kg; while the topped kebab charcoal barbecued bread had value of BaP 4.9 µg/kg. This trend is conflict with what was reported in a previous study, where Rey-Salgueiro *et al.*(2008) reported that BaP did not generate until bread was heated to 300°C, and the contents were lower than 0.5 µg/kg.

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Liver and shrimp samples showed moderate level of BaP which were 4.76 and 8.9 respectively; while the minced meat (kofta ) sample gave high ratio of BaP 37µg/kg.

On the other hand, benzo[a]pyrene contents were 10 times lower in fish than in fish skin; also, the chicken skin contents of BaP were three time higher than chicken (table 4 and fig. 3).

Kebab is made from fillets of meat stacked on a horizontal (charcoal) grill close to the heat source and this helps to account for its rich flavor and it became popular as well. This indicated that a certain carcinogenic risk of BaP for public health comes from charcoal-grilled meat(EFSA, 2008) .

In a recent study, White *et al.*(2008) investigated the effects of frying, grilling, barbecuing, toasting and roasting on the formation of PAHs in foods prepared in the home and from catering outlets , barbecuing with charcoal plus wood chips gave the highest benzo[a]pyrene levels in each type of food, In general PAH levels were increased when the food was cooked closer to the heat source.( EFSA, 2008)

Smoked sausages cased in cellophane casings. The levels of benzo[a]pyrene in the latter samples were lower than those found in the chorizo samples(Gillbert and Knowles, 1975). The authors explained the difference by the hydrophilic nature of cellophane, which renders it essentially impermeable to the PAHs. In contrast, the natural casings, being primarily protein and lipid, are more likely to absorb such hydrophobic compounds, thus allowing diffusion and hence penetration to take place.

Lijinsky and Ross (1967) studied the effect of variation in methods of cooking meat on the content of benzo[a]pyrene and other PAHs in the prepared food. They showed that PAHs arised from the pyrolysis of fat which drips to the heat source. The amount produced increased paralleled with increased fat content, longer exposure of the food to the flames and closeness to the heat source.

The grilling of frankfurters in the flames of a log fire resulted in extremely high PAH contents, up to 212 µg/kg benzo[a]pyrene.

**Table (5) : The BaP concentrations in experimental kinds of different types of food samples**

Category of (miscellaneous) samples	Samples tested	concentrations of BaP (µg/kg)	
		Minimum	Maximum
Dried fruit(fig, raisin, prunes & cherries)	4	0.13	0.29
Spices(mixed)	2	ND	3.1
Nuts(kajo, almond)	2	0.15	0.26
Chocolates	1	0.1	
Cereals (cornflakes)	2	ND	0.34
Processed fruits( cocktail )	1	ND	
Light soya sause	1	1.6	
Dark Soya sauce	2	1.6	2.6
Infant food	1	ND	

Among 16 different samples of nuts, spices, soya sauce, cereals, chocolates and infant formula tested appeared to contain low levels of BaP ranged from zero in four samples to 0.34 µg/kg in one kind of cornflakes which comply with the EU regulations compared to the dark soya sauce samples which were relatively higher (equal to 1.6 to 2.6 µg/kg, respectively), these contents were found to be lower than the EU limit.

Finally, a wide variation was observed for BaP contents of the heat-treated foods between different food types. As seen from our results, there were more than 80% out of analyzed samples exceeding the level set by EU regulations, the minimum contents of BP were found in cocoa powder 0.02 µg/kg in which the highest contents were found in charcoal lamb meat 68 µg/kg.

There were no significant differences between the results of the two instruments (GC and HPLC-FD).

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## تقدير البنزو (أ) بيرين كأحد الهيدروكربونات العطرية متعددة الحلقات الناتجة من المعاملة الحرارية لبعض المواد الغذائية في الأسواق المصرية باستخدام جهاز HPLC و GC- منال عبد المطلع عطوه المركز الاقليمي للاغذية والاعلاف – مركز البحوث الزراعية

وتهدف هذه الدراسة إلى قياس واحد من أهم الهيدروكربونات العطرية متعددة الحلقات و هو البنزو (أ) بيرين باعتباره واحدة من أكثر المركبات المسرطنة والتي هي معروفة بانها تتكون من الحلقات العطرية و هي من النواتج التي تتكون خلال الاحتراق ومنها : البنزو أنتراسين، بنزو بيرين، بنزو فلورانتين، و بنزو فلورانتين، كريسين، داينيز أنتراسين، واندينو بيرين.

في هذه الدراسة تم إجراء الكشف عن وجود البنزو (أ) بيرين في أكثر من 90 عينة من العينات التي تم جمعها من السوق المصرية وتشمل العديد من أنواع الزيوت النباتية (المستوردة، والمحلية المتاحة في السوق المصرية) ومنها (زيت النخلة و زيت الكتان وزيت عباد الشمس وزيت السمسم، و الكتان وزيت جوز الهند وزيت النخيل و زيت الصويا، زيت الكانولا، زيت بذور العنب، زيت السمسم المحمص، والزيت النباتي المخلط) وصلصة الصويا. وقد تم تحليل أيضا عينات الاغذية المشوية على الفحم (لحم، دجاج، الجمبري، والخبز والسمنك) وكذلك عينات لحوم واسماك وجبن مدخنة. كما انه ايضا، تم تحديد عينات مختارة من الشاي والكافو ومسحوق البن المحمص؛ من أجل تقييم تأثير درجة التحميص على تكون البنزو (أ) بيرين بالمقارنة مع العمليات الأقل من التحميص وكذلك بذور القهوة الخضراء.

و قد تفاوت تركيز البنزين (أ) بيرين في العينات المدخنة. وسجلت اقل تركيز في عينة الجبن المستوردة المدخنة بينما سجلت أعلى المعدلات في عينة الاسماك الرنجة المدخنة من السوق المحلية. أيضا، كان محتوى التونة المعلبة المدخن عالي من البنزو (أ) بيرين. و كان من الملاحظ أن لحم البقر المصنعة المدخن، والنقانق، و سمك السلمون المدخن يحتوى على قيم منخفضة من البنزو (أ) بيرين.

و بالبحث ثبت ان حوالي 30% من عينات الاغذية المدخنة في هذه الدراسة لديها مستويات من مادة البنزو (أ) بيرين تجاوزت الحد المقبول من 5 ميكروجرام / كج على النحو المحدد من قبل المفوضية الأوروبية (EC، 2005).

و بتحليل أكثر من (24) عينة من البن والشاي والكافو، تم الكشف عن البنزو (أ) بيرين في 95.8% من العينات وكانت النسب في معظم العينات أعلى من الحد المسموح به (1 جزء في البليون) التي وضعتها المفوضية الأوروبية. و قد تجاوزت نسبت البنزو (أ) بيرين 1 ميكرو/ كج في أربعة عشر عينة من اصل ثمانية عشر عينة مختلفة من القهوة، وكانت القيم الاعلى من محتوى البنزو (أ) بيرين في القهوة الناتجة من عمليات تحميص القهوة القوية (البن الغامق) بينما تقل النسب في منتجات عمليات التحميص المعتدلة أو الخفيفة. كما اشارت النتائج ان أعلى قيمة من البنزو (أ) بيرين في هذه الفئة تحققت في عينة الشاي الأخضر المنكه معطبا قيمة 11ميكرو / كج. بينما في عينة مسحوق الكافو كان هناك كمية لا تذكر من البنزو (أ) بيرين.

وفي أكثر من 5 عينات اوراق الشاي الجافة تجاوزت نسبة البنزو (أ) بيرين في 80% من العينات 1 ميكروجرام / كج. ويتقدير مستويات البنزو (أ) بيرين في 28 عينة من عينات الزيت حققت عينة من عينات زيت السمسم المحمص أعلى قيمة من مركب البنزو (أ) بيرين بينما كانت اقل قيمة. في الزبدة بينما في الزيت المرتبطة بالمخللات والجبن لم تكن هناك مستويات تذكر من البنزو (أ) بيرين.

من ضمن أكثر من 30 عينة من عينات الزيوت النباتية تم تحليل 10 من الزيوت النباتية المرتبطة بالاسماك المعلبة (السردين المدخن والتونة و الماكريل ) واعطت النتائج أعلى مستويات من مركب البنزو بيرين في السردين المدخن والتونة). ولكنه بوجه عام كانت قيم البنزو بيرين منخفضة، فقد أظهرت أربع عينات الزيوت المتعلقة (بالتونة و الماكريل و السردين و الأنتوجه) على مستوى أقل من 1 ميكروجرام / كج، وهو الحد الأقصى لتشريعات الاتحاد الأوروبي في هذه الفئة من الاغذية.

في استخلاص الزيوت على البارد كانت مستويات البنزو (أ) بيرين في زيت الكتان و الزيتون وزيت السمسم أقل بكثير بالمقارنة مع الزيوت المستخلصة علي درجات حرارة مرتفعة.

وكانت المستويات في "زيت الزيتون البكر" و "زيت الزيتون" متوسطة بينما تضمن زيت ثقل الزيتون مستويات عالية من البنزو (أ) بيرين.

في عينات المكسرات والفواكه المجففة، الحبوب، الشوكولاته و حليب الأطفال تراوح محتوى البنزو بيرين من السالب إلى المنخفض في نوع واحد من رقائق النخلة مطابقة للائحة الاتحاد الأوروبي. بينما في عينة صلصة الصويا الداكنة كانت نسبة البنزو بيرين أعلى نسبيا.

واكدت النتائج على ان 50% من العينات من الاغذية المشوية على الفحم قد تجاوزت المستوى المحدد من البنزوبيرين . وكذلك فقد تعدت نسب البنزو (أ) بيرين في عينات لحم الضأن المشوية على الفحم نحو ثلاثة اضعاف مستوى البنزو (أ) بيرين من عينة اللحم البقرى مسجلة اعلى قيمة في جميع العينات التي تم تحليلها.

وأظهرت عينات الكبد و الجمبري المشوي على الفحم مستوى معتدل من البنزو (أ) بيرين في حين أعطت نتائج تحليل اللحم المفروم (الكفتة) قيمة مرتفعة نسبيا من البنزو (أ) بيرين

من ناحية أخرى، اكدت النتائج انخفاض نسب البنزو (أ) بيرين 10 مرات أقل في عينة لحم الاسماك عنه في عينة جلد السمك المشوي. وكذلك، ارتفاع محتوى جلد الدجاج من البنزو (أ) بيرين ثلاث اضعاف أعلى من لحوم الدواجن.