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Determination of benzo[*a*]pyrene in Turkish döner kebab samples cooked with charcoal or gas fire

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In order to investigate the levels of the potent carcinogen benzo[*a*]pyrene (B(a)P), 40 samples of döner kebab were analysed. The samples tested included 20 cooked using a charcoal fire and 20 cooked using a gas fire. A liquid chromatographic method was developed using a fluorescence detector. The mean levels of B(a)P were found to be 24.2 (s.e. 0.84) µg/kg for charcoal fire cooked meat samples and 5.7 (s.e. 3.48 µg/kg) for gas fire cooked meat samples. Sixteen samples were found to be over the maximum level recommended by FAO/WHO (10 µg/kg) and all of the samples exceeded the maximum tolerance level of the Turkish Food Codex (1 µg/kg).

Keywords: benzo[*a*]pyrene; meat

Introduction

Polycyclic aromatic hydrocarbons (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 (NRCC, 1983). B(a)P is considered here because it is the only PAH for which there is sufficient toxicological evidence to enable guidelines on allowable levels to be set.

B(a)P is one of the most potent animal carcinogens and is present in a wide variety

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of food items (IARC, 1983). It has been detected in charcoal-broiled meat, smoked-grilled foods, seafood, liquid smokes and beverages (Gomaa *et al*., 1993).

PAHs contaminate foods that have been cooked over open flames or treated with smoke condensate. When muscle foods (meat, fish, poultry) are cooked over an open flame, dripping fat is likely to be burned and PAHs formed which are carried by smoke and coat the food (charbroiling). Broiling (heat source above) can significantly reduce PAH levels (IARC, 1983). The principal sources of B(a)P in foods are the absorption and deposition of particulates during processing (smoked foods, leafy vegetables), the pyrolysis of fats and the incomplete combustion of charcoal (IARC, 1973).

Smoke production involves a process of incomplete combustion. When wood undergoes complete combustion only carbon dioxide, water and ash are produced but incomplete combustion, via thermal disintegration (pyrolysis) of higher molecular organic compounds, produces compounds of lower molecular weight. This is the case with PAHs which are volatile in the gas phase (Gilbert and Knowles, 1975; Stołyhwo and Sikorski, 2005).

Turkish döner kebab is made from fillets of meat stacked on a vertical spit and roasted on a vertical grill. "*Döner*" means "turning": the vertical spit is rotated, or turned, in front of the heat source (charcoal or gas). When the meat directly opposite the heat source is properly roasted, the spit is rotated so that the cooked meat may be sliced off with a large knife and an uncooked portion of meat exposed to the fire. Because the meat is vertical, it is self-basting and this helps to account for its rich flavour. The thin slices of meat are served in various different ways: plain on the plate, stuffed into Turkish bread *(döner sandviç)*, rolled into flat bread *(dürüm)*, or laid atop diced flat bread and topped with sauces. Traditional döner is made from milk-fed lamb but in the 1980s a heart-healthier alternative – *tavuk* (chicken) *döner* – became popular as well. Today it is not unusual to see twin döners, lamb and chicken, sizzling side by side (Anonymous, 2007).

Various methods are used for the isolation of PAHs in meat samples. The majority of methods involve the saponification of lipids by methanolic KOH solution (Phillips, 1999). KOH is strongly recommended for the transformation of phenols to a polar, non-extractable form of phenolates prior to the PAH extraction with a non-polar solvent (Simko, 2002). Lipophilic compounds such as PAHs have a tendency to diffuse not only into the non-polar part of the sample but also into tissue cells due to the existing concentration gradient. For this reason, a simple solvent extraction with non-polar solvent seems to be insufficient to achieve high recovery (Simko, 2002).

Although a number of clean-up procedures, e.g., solvent:solvent fractionation, solid phase extraction, thin-layer and column chromatography on suitable stationary phases, and size exclusion chromatography are available, probably the most efficient and selective is column chromatography (Stołyhwo and Sikorski, 2005).

B(a)P has been considered by the International Agency for Research on Cancer (IARC), which concluded that it is a probable human carcinogen (IARC, 1987). 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 (ATSDR, 1996).

This study was undertaken to determine the presence of B(a)P formed in Turkish

döner kebab samples prepared by different cooking methods (gas or charcoal fire).

Materials and Methods

Samples

In 2005, forty commercial Turkish döner kebab samples were randomly purchased from restaurants in Samsun (northern Turkey). Samples were collected from 10 different restaurants. Twenty samples each of charcoal and gas fire döner kebabs were collected. Samples weighing 100 g were collected on consecutive weeks and kept in a refrigerator at 4 °C before analysis. Twenty-five grams of meat was taken from each sample and analysed.

Reagents

B(a)P standard was purchased from Aldrich. The solvents *n*-hexane, dichloromethane, toluene and methanol were obtained from Merck. Extrelut® (20 g) extraction columns were supplied by Merck, propylsuphonic acid (PRS, 500 mg) SPE columns were supplied by Baker. Silica gel chromatography columns (70–230 mesh) were obtained from Merck.

B(a)P Standard

Stock solutions containing 100 mg/L and 100 μg/L of B(a)P were prepared in acetonitrile and stored at 4 °C 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 (R^2 = 0.99, y = 238536x–329004).

Extraction and clean-up procedure

The extraction and clean-up procedure was adapted from that of Janoszka *et al.* (2004). The 25 g sample of meat was 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, meat 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 °C 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 °C prior to HPLC analysis.

HPLC method

The HPLC system consisted of a system controller (Shimadzu, Japan, SCL-10AVP), high-pressure pump (Shimadzu, Japan, LC-10 AD), auto injector (Shimadzu, Japan, SIL-10AXL), fluorescence detector (Shimadzu, Japan, RF-10 AXL) and column oven (Shimadzu, Japan, CTO-10A). For B(a)P separation, a carbamate analysis column (150 mm × 4.6 mm, C_{18}, 5 μm) (Pickering laboratories, USA) was used. 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

Method 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

$$\frac{\text{amount of B(a)P residue in medium}}{\text{amount of B(a)P spiked in medium}} \times 100\%$$

Statistical analysis

Statistical analysis of data was carried out using SPSS 13.0 statistical package (SPSS, 2008). Differences between weeks within cooking methods were evaluated by using ANOVA and any significant differences were further evaluated using the Duncan's multiple range test. The level of significance was set at $P < 0.05$. Student's t-Test was used to evaluate the significance of differences between cooking methods.

Results and Discussion

The B(a)P concentrations of meat samples cooked by different methods are given in Table 1 with means and standard error. Each test was completed in independent triplicate. The differences between weeks were not significant ($P > 0.05$, ANOVA). The difference between charcoal and gas-fire cooked groups was significant ($P < 0.01$).

Table 1. Effect of cooking method on the benzo[*a*]pyrene concentration (B(a)P) in döner kebabs

Cooking method	*n*	Concentration (µg/kg) of B(a)P		
		Min	Max	Mean ± s.e.
Gas fire	20	2.4	14.7	5.7 ± 0.85
Charcoal fire	20	4.9	52.5	24.2 ± 0.48
t-test		–	–	*

The average recovery of B(a)P was 81% with a standard deviation of 1.5% which is much higher than the 66.5% recovery for B(a)P analysis in meat samples reported by Janoszka *et al*. (2004). Moreover, Wu *et al*. (1997) also reported a lower recovery (74%). These results show that the extraction method used in the present study was quite effective. B(a)P standard and sampling chromatographs are shown in Figure 1.

The frequency distribution of B(a)P concentrations in the döner kebab samples are given in Table 2. The results show that 12 samples (60%) cooked with a gas fire and only one sample (5%) cooked with charcoal fire were below the level of 5 µg/kg. The results also show that none of the samples cooked with a gas fire were over the level of 20 µg/kg, but 11 samples (55%) of samples cooked with charcoal were found to be above this level.

Comparison with regulatory limits

The FAO and WHO have set a maximum permissible concentration of B(a)P in food of 10 µg/kg (Joint FAO/WHO Expert Committee on Food Additives, 1987). Based on this, the acceptable limit for the Turkish Food Codex (2002) has been set as 1 µg/kg, while a level of 5 µg/kg has been set by the European Commission (2005).

All samples examined in this study were found to be over the tolerance limit (1 µg/kg) specified in the Turkish Food Codex (2002), but when they were evaluated according to the FAO/WHO limits 16 samples (40%) were over the acceptable limit.

Effect of cooking procedure

The study findings were similar to those of other research that showed the B(a)P concentration of charcoal-fire cooked

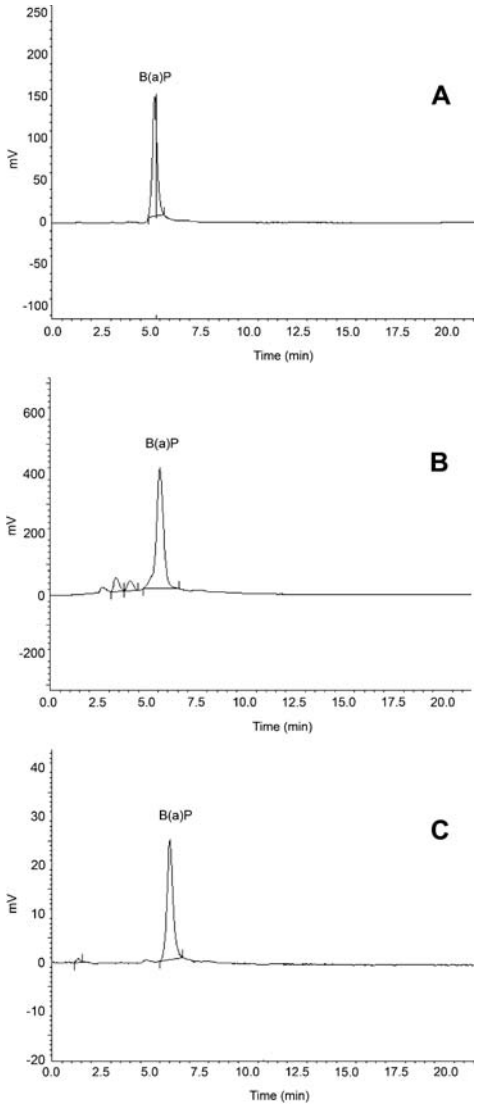

Figure 1. Chromatograms for benzo[a]pyrene (B(a)P) obtained from HPLC: (A) standard mixture; (B) fraction isolated from döner kebab cooked with charcoal fire; (C) fraction isolated from döner kebab cooked with gas fire.

meat samples was much higher than gas-fire cooked meat (Anderson *et al*., 2002; Kazerouni *et al*., 2001; Wu *et al*., 1997).

Table 2. Frequency distribution (%) of benzo[*a*]pyrene concentration in döner kebabs

Concentration (μg/kg)	Cooking method	
	Gas fire	Charcoal fire
<5	60	5
5–10	25	30
10–20	15	10
>20	0	55

However, Rivera *et al*. (1996), detected B(a)P concentrations of 4 to 19 μg/kg in charcoal grilled meat. The levels of B(a)P in smoked food, including turkey, pork, chicken, beef and fish products, were found to be between 0.15 and 3.93 μg/kg by Gomaa *et al.* (1993). Lawrence and Weber (1984) have detected levels of B(a)P in charbroiled hamburger samples (1.5 to 2.9 μg/kg) and in fried hamburger samples (0.1 μg/kg). On the other hand, Doremire *et al*. (1979) found the concentration of B(a)P in charcoal grilled beef meat samples was between 18.8 and 24.1 μg/kg. The mean level of B(a)P in different types of sausage was found to be 0.022 μg/kg (Garcia Falcon *et al.*, 1996). According to Dennis *et al.* (1984), the concentration of B(a)P detected by HPLC-FL in grilled sausage was 0.4 μg/kg, in grilled pork chops 8.2 μg/kg, smoked herring 8.5 μg/kg, smoked ham 0.2 μg/kg, and in hard grilled sausage 191 μg/kg. Binnemann (1979) found B(a)P concentrations of 0.6 to 100 μg/kg in sausages and special products.

Essentially, B(a)P formation is largely determined by the cooking method and the degree to which the meat is cooked (Kazerouni *et al*., 2001; Larsson, 1983). PAH compounds tend to form on or near the surface of the meat rather than in the interior. These compounds are generated through pyrolysis during the charbroiling of meat and when fat from the meat falls onto the hot coals, and so significant levels

of PAH will be produced during cooking directly on the meat as well as via the charcoal (Phillips, 1999; Kazerouni *et al*., 2001; Wu *et al*., 1997; Lijinsky, 1991). PAH production by cooking over charcoal is a function both of the fat concentration in the meat and the proximity of the food to the heat source, and can be reduced by cooking for longer periods at lower temperatures (Phillips, 1999).

Conclusion

The B(a)P levels of döner kebab samples cooked on either a gas fire or charcoal fire exceeded the maximum tolerance level for B(a)P of the Turkish Food Codex (2002). Therefore, an important public health risk exists and some hygiene and technique related precautions should be applied by döner kebab manufacturers.

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