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Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are chemicals formed when muscle meat is cooked using high-temperature methods, such as grilling directly over an open flame. PAHs have been found to be mutagenic-that is, they cause changes in DNA that may increase the risk of cancer. We investigated the effects of grilling procedures on the level of 4 PAHs; benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), and benzo[a]pyrene (B[a]P). PAHs were extracted and determined by gas chromatography with mass detection (GC–MS). With regard to barbecuing successive meat samples with the same batch of burning charcoal, it was observed that stable combustion contribute to reduction of PAHs. Significant reductions in the sum of the four PAHs were observed through treatments which removed meat drippings and smoke with alternative grilling apparatus. The sums of 4 PAHs were reduced 48–89% with dripping removed and 41–74% with the smoke removal treatment in grilled pork and beef meats than conventional grilling. We investigated the components of meats drippings. The major constituent of meat dripping was fat. The most important factor contributing to the production of PAHs in grilling was smoke resulting from incomplete combustion of fat dripped onto the fire.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds containing two or more fused aromatic rings derived from the incomplete combustion of organic matter, including coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat (Codex Alimentarius Commission (CX/FAC05/37/34), 2004, Rey-Salgueiro, Martinez-Carballo, Garcia-Falcon, & Simal-Gandara, 2008).

PAHs have been included in the priority pollutant lists, due to their mutagenic and carcinogenic properties, and they can be generated during the preparation of food (Jägerstad & Skog, 2005). The EU Scientific Committee on Food selected the sum of four PAHs of the 15 priority PAHs as the most suitable indicators of carcinogenic PAHs in food. These four PAHs (4 PAH) are benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), and benzo[a]pyrene (B[b]P) (European Commission (EC), 2006, Commission Regulation (EU), 2011, Wenzl, Simon, Anklam, & Kleiner, 2006). EFSA concluded that B[b]P alone was not a suitable general marker for PAHs in food, but identified a group of 4 PAHs as a better indicator based on data relating to occurrence and toxicity (EFSA, 2008; Rose et al., 2015). The predicted sources of carcinogenic PAHs contamination of foodstuffs are contaminated soils and polluted air and water (WHO, 1998, 2005).

Several researchers have investigated the presence of PAHs in food samples. Lijinsky and Shubik (1964) first studied PAHs that were present in charcoal broiled beef. Since then, studies have provided much information on the levels of carcinogens found in grilled meat products (Chen & Lin, 1997; Chung et al., 2011; Duedahl-Olesen et al., 2015; Rose et al., 2015). Grilled, smoked and roasted foods are increasingly popular both at home and in restaurants; however, based on many studies, these foods present an elevated health risk to the consumer, due to the higher levels of PAHs found in such products compared to foods prepared by other cooking methods (Kao, Chen, Huang, Chen, & Chen, 2014; Sundararajan, Ndife, Basel, & Green, 1999).

The chemical mechanism of occurrence of PAHs in grilled foods is not precisely known, PAHs can be formed from pyrolysis of

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organic matter, such as fat, at temperatures above 200 °C (Farhadian, Jinap, Faridah, & Zaidul, 2010) and smoke produced through the incomplete combustion of charcoal or open fires which can generate PAHs that adhere to the surface of foods (Bartle, 1991; Knize, Salmon, Pais, & Felton, 1999; Rey-Salgueiro, Garcia-Falcon, Martinez-Carballo, & Simal-Gandara, 2008). However, there are no studies that elucidate which grilling procedure mainly affects the occurrence of PAHs.

In this study, the most carcinogenic 4 PAHs were selected and monitored as suitable markers for PAHs in food. We investigated the effects of grilling procedures on the levels of PAHs in grilled foods by sampling meat drippings and smoke adhering to the surface of meats, and the effect of cooking with charcoal at different combustive temperatures. In this way, the obtained results can be used not only to understand PAH formation in grilled meats, but also to establish improved cooking practices.

2. Materials and methods

2.1. Chemicals and materials

The standard 4 PAH mixture and 2 deuterated internal standards were purchased from Supelco (Bellefonte, PA) and consisted of benzo[a]anthracene (B[a]A), chrysene (Chr), benzo[b]fluoranthene (B[b]F), benzo[a]pyrene (B[a]P), chrysene-d12 (Chr-d12), and benzo[a]pyrene-d12 (B[a]P-d12). Stock standard solutions of 100 μg/mL in dichloromethane were prepared and used for further dilution (10–500 μg/L).

All solvents used were of HPLC grade. Ethanol and hexane were purchased from Merck (Darmstadt, Germany) and dichloromethane was purchased from Burdick & Jackson (Muskegon, MI). Water was purified with a Milli-Q System (Millipore, Bedford, MA). Sep-pak® Vac cartridges containing 6 mL (1 g) of silica were purchased from Waters Corporation (Milford, MA).

2.2. Sample preparation

In 2014, beef and pork samples each of 6 kg were randomly purchased from various retail outlets and butcher's shops in the Republic of Korea. Beef loin, beef ribs, pork neck lean, and pork belly used in this study are meats commonly charcoal-grilled in the Republic of Korea. In particular, the fat contents of these meats are: beef loin, 31.7%; beef ribs, 24.4%; pork neck lean, 9.5%; and pork belly, 26.4% (RDA, 2011). The beef and pork were cut into circles using a Petri dish (1 cm thickness, 8.8 cm diameter).

2.3. Charcoal grilling method

A consumer-type outdoor grill (bottom width: 17 ∗ 17 cm, upper width: 34 ∗ 32 cm, and height: 8 cm) was filled with 700 g of commercial wood charcoal briquettes, and ignited by firing for 2 min (min) with a propane torch (Fig. 1). Temperature was measured with an infrared thermometer (Fluke, Everett, WA). The meat samples were grilled at an 8-cm distance from the heat source. The grilling time, when all flames had subsided, was 12 min for meats cooked until well-done, and the internal temperature reached a minimum of 80 °C. Samples were turned four times at each quarter (every 3 min) during the total cooking time. No salt or oil was applied to the samples before or after grilling.

2.3.1. Grilling with same charcoal at different combustive temperatures and times

Grilled samples were collected to evaluate if the combustive degree of charcoal influences PAH formation. Beef and pork samples were cooked for four different grilling periods: (1) for 12 min following ignition for 2 min with a gas torch, with temperature of the grill increasing from 127 °C to 320 °C, (2) for 12 min after the first period with temperature of the grill decreasing from 320 °C to 285 °C, (3) for 12 min after the second period with temperature of the grill declining from 285 °C to 250 °C, and (4) for 24 min after the third period with temperature of the grill reducing from 250 °C to 200 °C and the charcoal flames gradually extinguishing. This experiment was repeated 6 times, and samples were grilled until well-done.

2.3.2. Grilling with removing meat juices and charcoal smoke

Two apparatuses were designed for removing meat juices and charcoal smoke (Fig. 1). In the first apparatus, the grill firebox was punctured with a 9.8-cm diameter hole on the bottom for meat drippings to pass through and be collected in a beaker placed in an ice bucket. Beef and pork samples were cooked in the middle of the grill with charcoal placed around the hole during the second period (described above) and the experiment was repeated 6 times. In the second apparatus, a ventilation duct was placed between the flame and meat to remove smoke before it precipitated on the meat. These experiments were conducted during the second grilling period, and replicated 6 times.

2.4. Sample extraction and clean-up

Sample extraction and purification methods were referred to Food Code (MFDS, 2014). Grilled meat was homogenised with a blender (Mix-h03, Tongyang magic, China), and a 10 g portion of meat sample was weighed into a round-bottomed flask and saponified under reflux in a water bath at 80 °C for 3 h with 100 mL of 1 M potassium hydroxide in ethanol in the presence of the deuterated standards (4 μg/kg). After cooling, 50 mL of *n*-hexane were slowly introduced through the condenser. The mixture was transferred to a separatory funnel and shaken with 50 mL of ethanol:*n*-hexane (1:1, v/v). The hexane layer was cleaned with 50 mL of water. The aqueous layer was transferred to another separatory funnel and extracted twice with 50 mL of *n*-hexane. The pooled hexane portions were cleaned 3 times with 50 mL of water and treated with anhydrous Na_2SO_4. Then, the extracts were concentrated to approximately 2 mL using a rotary evaporator (Eyela, Tokyo Rikakikai Co. Ltd., Japan). The cartridge was previously activated with 10 mL of dichloromethane and 20 mL of *n*-hexane with a gravity flow. The concentrate was purified by passing through a cartridge; firstly eluting with 10 mL of *n*-hexane followed by 20 mL of *n*-hexane-dichloromethane (3:1, v/v). The second fraction was concentrated to dryness with a TurboVap under a nitrogen stream (40 °C, 20 psi). The dried concentrate was dissolved in 200 μL of dichloromethane and filtered through a 0.45-μm membrane filter prior to analysis. A blank sample was analysed with each series of samples.

2.5. Determination of PAHs using GC–MS

PAHs analysis was carried out using a gas chromatograph (CP-3800, Varian, USA) equipped with an MS detector (model 1200L, Varian, Santa Clara, CA) with autosampler (Combi PAL, CTC Analytics, Zwingen, Switzerland) in accordance with the Food Code method. A DB-5 ms Column (30 m length × 25 mm inner diameter × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA) was used. The oven temperature was initially held at 80 °C for 1 min, increasing to 245 °C at a rate of 4 °C/min, and finally to 270 °C at a rate of 30 °C/min and held at the final temperature for 10 min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The injector temperature was set at 320 °C, injection volume was 1 μL (splitless) and the MS detector source temperature was set at 250 °C. Mass spectrometry was acquired using

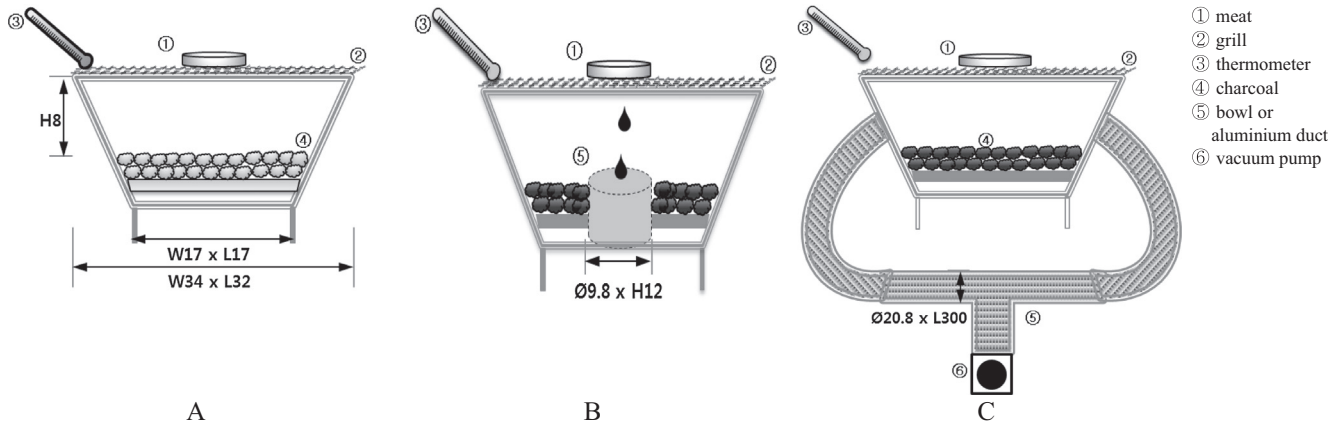

Fig. 1. Designs of grill apparatus used to study the effect of (A) conventional grilling, (B) reduced meat drippings, and (C) reduced smoke on PAHs generation.

electron ionisation (EI), at 70 eV and selective ion monitoring (SIM) mode with 0.1 s dwell time. The PAHs in samples were identified by comparing their retention times and ion mass with those of the standard PAHs. Quantitation ions were monitored for B[a]A, Chr, B[b]F, B[a]P, Chr-d12 and B[a]P-d12, and two other ions were monitored for each compound for confirmation purposes (Table 1).

2.6. Method validation

The method was validated with respect to parameters such as specificity, limit of detection (*LOD*), limit of quantification (*LOQ*), linearity, recovery, and precision following the harmonised guidelines for single laboratory validation of methods of analysis by IUPAC and AOAC (Taverniers, Loose, & Bockstaele, 2004; Thompson, Ellison, & Wood, 2002). Specificity for each compound was assessed by comparing with reference materials in blank samples and spiked samples using beef loin and pork neck. *LOD* and *LOQ* were estimated at the lowest concentration of analytes. *LOD* was calculated by multiplying standard deviation by 3 and a standard deviation of concentration was derived from measurements of 7 independent sample blanks fortified at 1 μg/kg, *LOQ* was calculated by multiplying *LOD* by 3. Based on the *LOQ* of each analyte, calibration curves were established over the range of 10–500 μg/L (six concentrations: 10, 50, 100, 200 and 500 μg/L). Linearity was evaluated by regression analysis. Calibration curves for individual PAHs were constructed by plotting average peak area against concentration and a regression equation was computed. To assay the accuracy of the method, recovery was tested. The analytical recovery experiments were performed by adding precisely measured amounts of mixed standards (2 μg/kg) and deuterated internal standards (4 μg/kg) into blank samples. The percent relative recovery of each compound was confirmed by plotting the peak area ratio of an analyte/deuterated internal standard *versus* spiked concentration. The experiment was conducted five times and the average percentage recovery and relative standard deviation (RSD) were determined. The repeatability of the method was evaluated through the RSD^r associated with the measurements of each PAH performed during the recovery tests. The reproducibility obtained by repetitive analysis of the same samples by 5 different laboratories was evaluated through the RSD^R.

Table 1
Retention time of each analyte and deuterated standards and specific ions of each compound.

Compound	Retention time (min)	Quantitative ions (*m*/*z*)	Qualitative ions (*m*/*z*)
B[a]A	40.63	228	229,226
Chr	40.81		
B[b]F	45.22	252	253,250
B[a]P	46.51		
Chr-d12	40.67	240	241,236
B[a]P-d12	46.41	264	265,260

2.7. Analysis of meat drippings

Meat drippings obtained from both beef loin and pork belly were collected in a beaker placed in an ice bucket during grilling (Fig. 1). Meats were repeatedly grilled until the amount of dripping was over 25 mL. The pooled drippings were analysed for moisture by air oven drying (AOAC Method 950.46) and for fat by the Rose-Gottlieb method (AOAC Method 905.02; AOAC, 2000, chaps. 33 and 39). The experiment was repeated 3 times. The amount of others was estimated by the 'difference method', i.e. calculated as the residue after accounting for moisture and fat.

2.8. Statistical analysis

All results are calculated as means and standard deviations. Assessment of significant differences in PAH contents between the different procedures was conducted by using the student's *t*-test (Microsoft Office Excel 2007; Microsoft Corporation, Seattle, WA).

3. Results and discussion

3.1. Analysis procedure performance

The performance of the method with spiked sample was evaluated by estimation of specificity, *LOD*, *LOQ*, linearity range, recovery, and precision (Table 2). The GC/MS chromatogram of PAHs in spiked sample is presented in Fig. 2. The correlation coefficient of the standard curves of the 4 PAHs was better than 0.99. The detection limits for B[a]A Chr, B[b]F, B[b]F and B[a]P were 0.08–0.21 μg/kg for the sample matrix. The *LOQs* ranged from 0.24 to 0.63 μg/kg. The recoveries of PAHs from beef and pork samples at a spiking level of 2.0 μg/kg were 90.2–111.0% and 83.4–110.7%, respectively. The repeatability of relative standard deviations (RSD^r) for 2 μg/kg of PAHs in meats ranged from 1.23% to 9.32% ($n = 5$). The relative standard deviation for inter-laboratory reproducibility (RSD^R) was assessed by repeated analysis of the same sample by five different laboratories and is shown in Table 2. The RSD^R ranged from 6.60% to 14.58%. All performance values were satisfied by the criteria recommended by the AOAC and IUPAC (Taverniers et al., 2004; Thompson et al., 2002).

Table 2
Linear equation, limits of detection and quantitation (*LOD* and *LOQ*), recovery, and precision obtained for PAHs.

PAHs	Linear equation	Regression coefficient	Matrix	*LOD* (μg/kg)	*LOQ* (μg/kg)	Relative recovery (%)	RSD^r (%)	RSD^R (%)
B[a]A	$y = 0.0068x - 0.0593$	0.9999	Beef	0.09	0.27	111.0	1.23	6.60
			Pork	0.15	0.45	110.7	1.91	8.08
Chr	$y = 0.0119x + 0.3210$	0.9974	Beef	0.09	0.27	101.7	1.46	9.92
			Pork	0.08	0.24	92.2	2.05	8.91
B[b]F	$y = 0.0083x - 0.0165$	0.9997	Beef	0.20	0.60	99.4	7.88	14.58
			Pork	0.18	0.54	99.9	5.45	9.21
B[a]P	$y = 0.0119x - 0.0739$	0.9999	Beef	0.18	0.54	90.2	9.32	8.11
			Pork	0.21	0.63	83.4	4.55	7.34

RSD^r: Repeatability in single laboratory.
RSD^R: Reproducibility by inter-laboratory comparison.

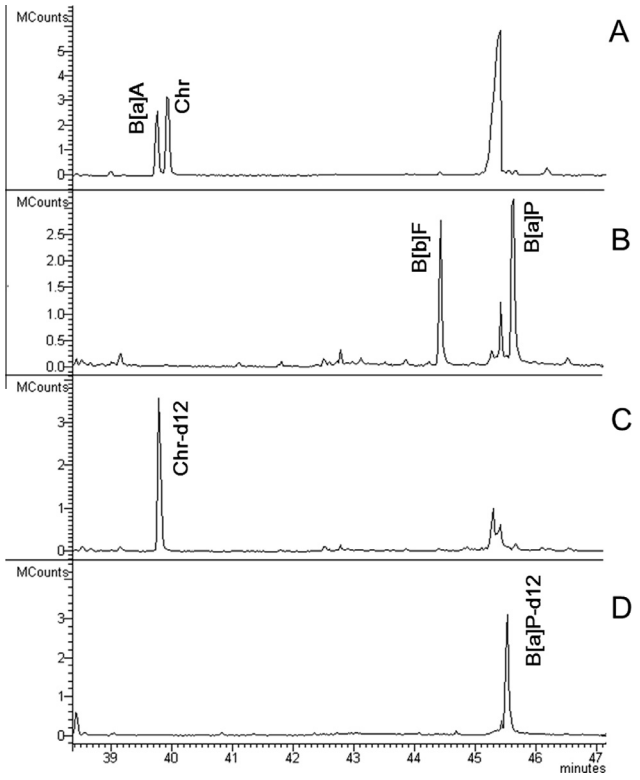

Fig. 2. GC/MS selected ion monitoring (SIM) chromatogram at (A) *m/z* 228 for B[a]A and Chr, (B) *m/z* 252 for B[b]F and B[a]P, (C) *m/z* 240 for Chr-d12, and (D) *m/z* 264 for B[a]P-d12 in spiked sample at levels of 2 μg/kg.

3.2. PAHs concentrations of meat grilled with the same charcoal at different combustive temperatures and times

The formation of the 4 PAHs in grilled samples during four different cooking periods is presented in Table 3. The mean concentrations in grilled beef rib samples were the highest level of B[a]A, Chr, B[b]F, and B[a]P during the first period. The mean value of first period samples was 23.81 μg/kg of PAH4, whereas those of other periods were 9.20–12.70 μg/kg of PAH4 in rib samples; this represented a downward trend in the sum of concentrations of 4 PAHs comparing to the first period. Similarly, in pork neck lean, each of the PAH in the first period represented the highest level; the total mean values in pork neck lean was 16.96 μg/kg in the first period, 4.38–7.63 μg/kg in the other periods.

PAH formation in the first period was much higher than for the other time periods with significant differences ($p < 0.05$) evident in beef ribs and pork neck lean. There were no significant differences ($p > 0.05$) in each sample during second, third and fourth periods for each concentrations of the 4 PAHs. This may be explained by changes in combustion of the burning charcoal. The general PAH contents were highest in grilled meats during the first period, because PAHs are generated and then deposited on the meat surface as smoke forms due to incomplete combustion at that time (Costa et al., 2009; Hassan, Magda, & Awad, 2010). During the other periods when all flames had subsided, there were no significant differences in concentrations of PAHs deposited between the later periods because of more complete combustion occurring.

In the case of beef loin, mean values of PAHs were 5.07 and 28.70 μg/kg of B[a]P and 4PAHs during the first period, 2.20–3.23 and 10.93–16.91 μg/kg in the others. The mean values of PAHs in pork belly were 5.99/33.17 μg/kg of B[a]P/4 PAHs in the first period, 2.81–5.76/11.44–21.77 μg/kg in the others. However, there were no significant differences in levels of the 4 PAHs formed during the first and second periods in beef loin and pork belly, although mean values of the 4 PAHs decreased from the first to the second period. These meats had a high fat content: 31.7% fat in beef loin and 26.4% fat in pork belly (RDA, 2011), which might cause high variations of PAH levels in grilled meat.

3.3. Effects of grilling on PAHs levels by fat content

The levels of PAHs in charcoal-grilled beef and pork samples are presented in Table 3. The samples were collected following the second grilling period, and the grilling time was 12 min. The maximum level of PAH mean values in charcoal-grilled meats was 3.62 μg/kg B[a]A in beef loin; 4.15 μg/kg Chr in pork belly; 8.77 μg/kg B[b]F in pork belly; 5.76 μg/kg B[a]P in pork belly. Among all the meats tested, charcoal-grilled pork belly had significantly higher levels of PAHs because of a high fat content of 26.4%. Our results were similar to the results reported by other researchers. The average levels of B[a]P in grilled beef and pork were 2.3–6.1 μg/kg (Larsson, Sahlberg, Eriksson, & Busk, 1983). The concentrations of B[a]P detected were 2.74 μg/kg in grilled beef and 1.75 μg/kg in grilled pork (Olatunji, Fatoki, Opeolu, & Ximba, 2014). Similarly, Rose et al. found approximately 10 μg/kg of 4 PAHs in beef barbecued over charcoal in 2015.

Differences in the levels of PAHs formed during charcoal grilling of meat might be attributed to the amount of fat originally present in the meat and not to the protein and carbohydrate content (Saito, Tanaka, Miyazaki, & Tsuzaki, 2014). This may be due to pyrolysis of fat dripping from the meat samples onto the heat source during grilling and subsequent direct deposition of PAHs from smoke produced through incomplete combustion of charcoal (Chen & Chen, 2001; Chung et al., 2011).

3.4. Effects of grilling on PAHs levels with alternative grilling apparatus

3.4.1. Collection of meat drippings

As shown in Fig. 3, B[a]A, Chr, B[b]F, and B[a]P levels in grilled beef and pork samples were reduced following treatment with

Table 3
PAH concentrations (mean ± standard deviation, $n = 6$) in beef and pork samples during different grillings with same charcoal at different combustive temperatures and times.

Meats		Period	PAH (μg/kg)				
			B[a]A	Chr	B[b]F	B[a]P	Total
Beef	Loin	1st[a]	6.96 ± 3.84	7.14 ± 4.17	9.54 ± 7.26	5.07 ± 6.27	28.70 ± 20.09
		2nd[b]	3.62 ± 1.94	3.80 ± 1.22	6.25 ± 2.09	3.23 ± 1.85	16.91 ± 5.62
		3rd[c]	2.15 ± 1.01	2.67 ± 1.20	3.91 ± 1.46	2.20 ± 1.58	10.93 ± 3.89
		4th[d]	2.68 ± 1.88	2.95 ± 1.84	4.59 ± 2.86	2.48 ± 2.62	12.70 ± 8.07
	Rib	1st[a]	7.45 ± 1.63	9.24 ± 2.53	2.31 ± 0.68	4.81 ± 1.32	23.81 ± 4.97
		2nd[b]	3.21 ± 0.96	2.27 ± 0.88	2.19 ± 1.08	3.30 ± 1.13	10.98 ± 3.69
		3rd[c]	2.76 ± 0.82	2.14 ± 0.76	1.95 ± 0.48	2.35 ± 0.86	9.20 ± 2.43
		4th[d]	2.89 ± 2.27	2.65 ± 2.58	2.81 ± 2.33	4.35 ± 4.13	12.70 ± 9.62
Pork	Neck lean	1st[a]	5.45 ± 1.64	2.80 ± 1.12	5.66 ± 3.66	3.04 ± 1.43	16.96 ± 7.61
		2nd[b]	1.75 ± 0.62	0.94 ± 0.64	1.71 ± 0.82	1.21 ± 0.49	5.60 ± 2.51
		3rd[c]	2.24 ± 0.68	0.97 ± 0.40	2.70 ± 0.84	1.73 ± 0.62	7.63 ± 2.06
		4th[d]	1.14 ± 0.30	1.30 ± 0.80	1.26 ± 0.54	0.68 ± 0.21	4.38 ± 1.04
	Belly	1st[a]	10.27 ± 5.40	7.97 ± 4.00	8.93 ± 4.18	5.99 ± 3.32	33.17 ± 15.43
		2nd[b]	3.09 ± 2.15	4.15 ± 1.93	8.77 ± 4.86	5.76 ± 3.53	21.77 ± 10.56
		3rd[c]	2.45 ± 1.35	2.16 ± 1.11	3.81 ± 1.69	3.03 ± 0.98	11.44 ± 4.42
		4th[d]	3.13 ± 3.05	2.33 ± 2.31	3.27 ± 1.66	2.81 ± 1.73	11.54 ± 8.47

[a] During 12 min after starting flames in the charcoal.
[b] During 12 min after first period.
[c] During 12 min after second period.
[d] During 24 min after third period.

removing of meat juices. The mean concentrations of PAHs in beef loin samples with treatment were 0.60, 0.27, 0.81, and 0.78 μg/kg of B[a]A, Chr, B[b]F, and B[a]P, whereas the control samples subjected to conventional grilling were 3.62, 3.80, 6.25, and 3.23 μg/kg, respectively. The sum of the four PAH compounds was reduced by about 85% from 16.91 to 2.46 μg/kg in beef loin. The concentrations of B[a]A, Chr, B[b]F, and B[a]P in beef rib were 0.63, 0.14, 0.70, and 1.13 μg/kg, respectively. The total level of the 4 PAHs was reduced by 76% compared to control samples, from 10.98 to 2.61 μg/kg in beef ribs.

The mean values of the four PAHs in pork neck lean with drippings removed were as follows: 1.05 μg/kg of B[a]A, 0.44 μg/kg of Chr, 0.89 μg/kg of B[b]F, and 0.56 μg/kg of B[a]P. In the case of the alternative treatment, the sum of the 4 PAHs decreased by 48%, from 5.60 to 2.93 μg/kg in pork neck lean. The levels of PAHs in pork belly with drippings removed were B[a]A: 0.40 μg/kg, Chr: 0.54 μg/kg, B[b]F: 0.78 μg/kg, and B[a]P: 0.66 μg/kg. The sum of the four PAH compounds was reduced from 21.77 to 2.38 μg/kg (89%) in pork belly compared to conventional grilling. Meat with higher fat content, showed greater PAHs reduction in grilled meats with drippings removed.

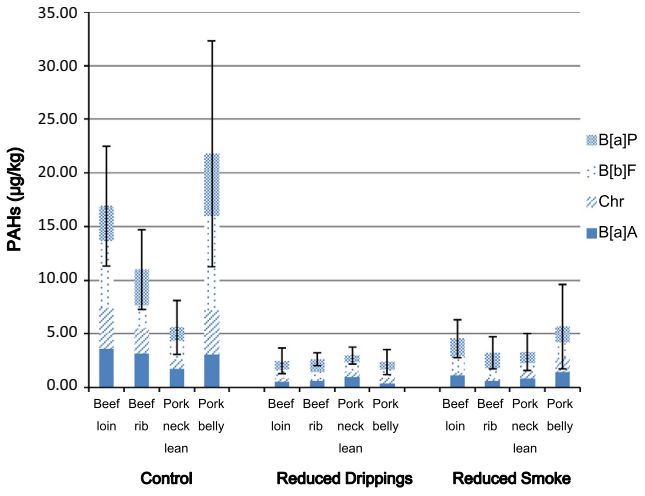

Fig. 3. Reduction of PAHs concentrations in grilled meats with removing of meat juices and removed charcoal smoke. The bars indicate the mean value and standard deviation of six replicated experiments.

In a study by Farhadian, Jinap, Hanifah, and Zaidul (2011), it was shown that samples wrapped with aluminium foil and banana leaves had total PAH levels reduced by 45.7% and 39.9%, respectively, following grilling. These reductions were to be expected as the wrapped meat was prevented from dripping fat onto the fire and was also not in direct contact to the heat source (Farhadian et al., 2011). In addition, the 'Safe Grill' (US Patent 5,331,886) included a filter placed between the fire and the meat to prevent melted fat from dripping onto the heat source, which reduces the PAHs contamination significantly. Similarly, in our results, the alternative procedure which prevents meat from dripping onto the charcoal was actually an effective way of reducing levels of PAHs, a group of carcinogenic compounds that form on grilled meat when juices interact with an open flame.

3.4.2. Reduction of smoke between charcoal and meat

In the present study, we developed a grill which included a ventilation duct placed between the fire and the meat, and during cooking, generated smoke was not deposited on the meat. As shown in Fig. 3, the grilled beef loin sample with the alternative grill contained 1.17 μg/kg of B[a]A, 0.20 μg/kg of Chr, 1.60 μg/kg of B[b]F, and 1.58 μg/kg of B[a]P. The sum of the four PAH compounds was reduced by about 73%, from 16.91 to 4.55 μg/kg in beef loin. The concentrations of B[a]A, Chr, B[b]F, and B[a]P in beef ribs with the smoke reduction treatment were 0.64, 0.32, 0.84, and 1.40 μg/kg, respectively. The total level of the 4 PAHs in beef ribs was reduced by 71% compared to the control, from 10.98 to 3.20 μg/kg.

The mean values of the four PAHs in pork neck lean following grilling with the smoke removal treatment were as follows: 0.84 μg/kg of B[a]A, 0.68 μg/kg of Chr, 0.78 μg/kg of B[b]F, and 1.00 μg/kg of B[a]P. With this alternative treatment, the sum of the 4 PAHs decreased by 41%, from 5.60 to 3.31 μg/kg in pork neck lean. The levels of total PAHs in pork belly following smokeless treatment were B[a]A: 1.48 μg/kg, Chr: 1.31 μg/kg, B[b]F: 1.42 μg/kg, and B[a]P: 1.48 μg/kg. The sum of the four PAH compounds was reduced from 21.77 to 5.69 μg/kg (74%) in pork belly compared to the control treatment.

Additionally, in a study by Viegas, Novo, Pinto, Pinho, and Ferreira (2012), the total level of PAHs was lower in salmon samples grilled with flameless and smokeless charcoal, which may be attributed to the way coconut charcoal absorbs fat that drips from cooking food. Our study has shown that using a ventilation duct

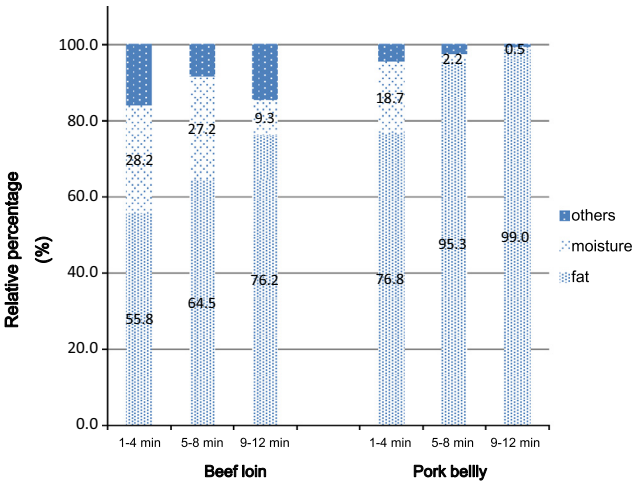

Fig. 4. Percentage of fat, moisture, and others in drippings ($n = 3$).

helps reduce levels of PAHs in grilled meats. The reduction of PAH concentrations in grilled meats was expected as the smoke contained carcinogens, which would otherwise accumulate on the meat surfaces.

3.4.3. Comparison of the two treatments

There are a number of researches to reduce the generation of PAHs in food. The concentration levels of PAHs were reduced when cooking food at a lower temperature (Lijinsky & Shubik, 1964; Chen & Lin, 1997; Farhadian et al., 2010). In addition, avoiding the direct contact of high temperature above 200 °C during grilling and cooking on direct flame was found to reduce the formation of PAHs (Farhadian et al., 2011; Chen & Lin, 1997). Even if not in direct contact, fat dripping onto the flame or heat source generates PAH compounds that are carried back onto the surface of the food. Therefore, it will help suppress the production of carcinogenic PAH within the food to prevent the dripping of melted fat (Farhadian et al., 2011). As another option for reducing PAH levels, one may consider using meats with less fat content for grilling (Lijinsky & Ross, 1967).

We investigated effects of charcoal grilling of meats contained different fat content on PAHs levels using alternative grilling apparatus. Significant reductions in the sum of the four PAHs were observed through treatments which removed meat drippings and smoke. The sums of 4 PAHs were reduced 48–89% with dripping removed and 41–74% with the smoke removal treatment in grilled pork and beef meats compared with conventional grilling. Meats higher in fat showed greater PAHs reduction in grilled meats with the two alternative grills. The reduction of the smoke using a ventilation grill caused PAHs formation of grilled food to decrease significantly, due to removing the chance to combine meats and PAHs. Although the exact mechanism of formation of PAHs in grilled foods is not precisely known, the following is considered a possible mechanism. The mechanism is the yield of direct contact of fat dripping at intense heat directly over the charcoal flame. This condition can generate volatile PAHs that in turn adhere to the surface of the meat as the smoke rises. The present study reveals that the factor of prime importance contributing to the production of PAHs in grilled meats was the incomplete combustion of fatty meat drippings.

3.5. Analysis of meat drippings

As described above, the formation of PAHs was expected as smoke containing these compounds is generated during grilling when meat juices drip onto and interact directly with the heat source. We investigated the components of meat drippings to ascertain if these drippings influence the occurrence of PAHs in grilled foods. The results are shown in Fig. 4. The fat content in meat drippings was generally higher in pork belly than in beef loin. Drippings collected from pork belly contained 0.5–18.7% moisture and 76.8–99.0% fat. In the case of beef loin, meat drippings contained 9.3–28.2%/55.8–76.2% of moisture/fat. The highest levels of fat were found in drippings from pork belly during the last grilling period. These results might be attributed to the amount of fat originally present in the meat. In our study, the major constituent of meat drippings was the fat; PAHs can be formed from pyrolysis of fat which occurs at high temperature during direct interaction with the heat source.

4. Conclusions

Grilled meat products may contribute significantly to the intake of PAHs, if such foods are a large part of the usual diet. Proper methods of PAHs reduction have received increased attention in recent years due to carcinogenic and genotoxic PAHs (Bansal & Kim, 2015). PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat duration of cooking. The results obtained from this study have shown that grilled pork belly samples contained the highest total PAHs levels (21.77 μg/kg), due to their high fat content. Based on successive grilling with the same batch of burning charcoal, it was observed that stable combustion contributes to reduction of PAHs. We recommend that meat should be laid on the grill, 12 min from starting the flame, during complete combustion. The removal of dripping fat and smoke between meat and charcoal has the effect of reducing levels of carcinogenic PAHs (B[a]A, Chr, B[b]F, and B[a]P) in beef and pork samples, because PAHs are formed when meat juices drip onto charcoal or other hot surfaces and generate smoke containing PAHs. The sums of 4 PAHs were reduced 48–89% with drippings removed and 41–74% with the smoke removal treatment in grilled pork and beef meats compared with conventional grilling. Results of the present study reveal that the most important factor contributing to the production of PAHs in grilled meats was the incomplete combustion of fatty meat drippings, except for incomplete combustion at first period. The removal of meat drippings and optimising charcoal combustion are the best procedures for reducing PAH formation in home and restaurant grills. On the basis of the results of the present study and with the intention of decreasing the intake of carcinogenic PAHs, a guideline concerning the grilling of meats at home and restaurants could be issued.

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