



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.

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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[a]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 Na₂SO₄. 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

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