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The effects of cooking on wire and stone barbecue at different cooking levels on the formation of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons in beef steak

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ABSTRACT

The effects of type of barbecue (wire and stone) and cooking levels (rare, medium, well-done and very well-done) on the formation of heterocyclic aromatic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) in beef steak were investigated. Varying levels of IQx (up to 0.29 ng/g), IQ (up to 0.93 ng/g), MeIQx (up to 0.08 ng/g), MeIQ (up to 0.75 ng/g), 7,8-DiMeIQx (up to 0.08 ng/g), 4,8-DiMeIQx (up to 4.95 ng/g), PhIP (up to 6.24 ng/g) and AαC (up to 0.20 ng/g) were determined, while MeAαC was not detected. The total HCA amounts in wire barbecued samples were higher than stone barbecued samples. Total HCA contents of the samples ranged between nd and 13.52 ng/g. In terms of PAHs, varying levels of BaA (up to 0.34 ng/g), Chry (up to 0.28 ng/g), BbF (up to 0.39 ng/g), BkF (up to 0.90 ng/g), BaP (up to 0.29 ng/g) and Bghip (up to 0.43 ng/g) were determined, while DahA and IncdP were not detected. The total PAH amounts in stone barbecued samples were higher than those of wire barbecued samples. Total PAH amounts of the samples ranged between nd and 2.63 ng/g.

1. Introduction

Epidemiological studies indicate that there is a positive relationship between various cancers (pancreas, breast, colorectal, prostate and ureter) and high consumption of meat and fish (Klassen, Lewis, Lau, & Sen, 2002). Heterocyclic aromatic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) are formed during cooking of proteinaceous foods such as meat. It was proven with epidemiological studies that most of the HCAs, determined in 1977 by Japanese scientists in grilled meat and fish (Oz & Kaya, 2011a) for the first time, were mutagenic and nearly all of them were carcinogenic (Skog, Johansson, & Jägerstad, 1998). The occurrence and amounts of HCAs depend on many factors such as type of meat, cooking conditions (temperature, duration, equipment and method), pH, water activity, carbohydrate, free amino acids and creatine (Felton et al., 1997; Oz & Kaya, 2011a; Pais, Salmon, Knize, & Felton, 1999). It has also been determined that heat and mass transfer, fats, lipid oxidation and antioxidants influence the concentration of HCAs (Oz, 2011; Oz & Kaya, 2011a).

It is declared that PAHs are the most common chemical compounds causing cancer (Chen & Chen, 2005). Approximately 660 different PAHs have been determined so far (Stolyhwo & Sikorski, 2005). PAHs may be formed during heat treatments such as

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barbecuing (Mottier, Parisod, & Turesky, 2000), roasting (Farhadian, Jinap, Abas, & Sakar, 2010), grilling (Moazzen, Ahmadkhaniha, Gorji, Yunesian, & Rastkari, 2013) and smoking (Djinovic, Popovic, & Jira, 2008; Wretling, Eriksson, Eskhult, & Larsson, 2010). Several factors are known to affect the formation of PAH during food processing and preparation, such as fuel type used (coal, gas, wood and electrical source), cooking procedures (frying, grilling and roasting), cooking temperature and duration, fat content of the food, dripping of the fat on fire, closeness of the food to heat source and direct contact (Farhadian, Jinap, Hanifah, & Zaidul, 2011). PAHs are formed once food, particularly meat, is cooked over an open fire. In case of direct contact of meat with the fire, the pyrolysis of meat fats creates a layer on the meat that contains PAHs. Even if there is no direct contact, melting fats drip over the hot coal and fire, so it causes the formation of PAHs that return to meat with smoke. The formation of PAHs with barbecuing is a result of both fat content of the meat and closeness of the food to the heat source, and it can be reduced by longer cooking duration at low cooking temperature. Some of the highest levels of PAHs reported in food were determined in barbecued samples (Phillips, 1999).

Consumption of barbecued beef steak is popular in Turkey, as it is all around the world, because of its organoleptic quality and nutritional value for consumers; however, high levels of cooking carcinogens turn the food into a risk for the society (Farhadian et al., 2011). Many researchers have emphasized that investigation of more than one group of mutagens is an urgent need in order to obtain more realistic exposure levels (Jägerstad & Skog, 2005). The formation of carcinogenic and mutagenic compounds, such as HCAs and PAHs, in barbecued meat products poses great risks compared to other food products, thus make it necessary to conduct studies on alternative cooking methods. It is an area of concern that there is not enough knowledge either about the exposure to cooking toxicants in these types of food or the strategies for decreasing them.

Barbecues generally include a wire grill. It is thought that the use of a stone grill instead of a wire grill in barbecuing of meat could prevent the formation of HCAs and/or PAHs. For this aim, in the present study, the formation of HCAs and PAHs in beef steak cooked at different levels (rare, medium, well-done and very well-done) on wire and stone barbecue was analyzed and at the same time, the effects of the types of barbecue and levels on various quality criteria of barbecued beef steak were determined.

2. Materials and methods

2.1. Beef steak

Beef M. *Longissimus dorsi* muscles were obtained from a local slaughterhouse (Et ve Süt Kurumu, Erzurum, Turkey). The M. *Longissimus dorsi* muscle was chosen because it is frequently barbecued in restaurants. The muscles were brought to laboratory under cold chain. The meat were duly prepared and used. Thereafter, meat were sliced (approximately 2 cm thickness) transverse to the longitudinal axis and used for barbecuing.

2.2. Reagents and standards

All solutions used in the study except for HPLC-grade solvents were passed through a 0.45-μm filter (Milex; Millipore, Billerica, MA). Water was from a Milli-Q water purification system (Millipore). The following HCA compounds were purchased from Toronto Research Chemicals (Downsview, Ontario, Canada): 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQx, CAS no: 108354-47-8); 2-amino-3-methylimidazo[4,5-f]quinoline (IQ, CAS no: 76180-96-6); 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx, CAS no: 77500-04-0); 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ, CAS no: 77094-11-2); 2-amino-3,7,8-trimethylimidazo[4,5-f] quinoxaline (7,8-DiMeIQx, CAS no: 92180-79-5); 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx, CAS no: 95896-78-9); 2-amino-3,4,7,8-tetramethylimidazo[4,5-f]quinoxaline (4,7,8-TriMeIQx, CAS no: 132898-07-8); 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP, CAS no: 105650-23-5); 2-amino-9H-pyrido[2,3-b]indole (AαC, CAS no: 26148-68-5) and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC, CAS no: 68006-83-7). 4,7,8-TriMeIQx was used as an internal standard. The stock standard solutions were prepared according to Oz (2011). An Oasis MCX cartridge (3 mL/60 mg, 30 mm) from Waters (Milford, MA) was used for the solid-phase extraction of HCAs. The standard PAH mixture was purchased from Supelco (Bellefonte, PA) and consisted of: 10 μg of naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Ac), fluorene (Flu), phenanthrene (Phe), anthracene (AnT), fluoranthene (FluA), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (Bghip) and indeno [1,2,3-cd]pyrene (IncdP) in 1 ml of acetonitrile. For the solid phase extraction of PAHs, Bond-Elut PRS cartridges (500 mg, 3 mL) from Agilent (Santa Clara, CA) were used.

2.3. Method

2.3.1. Barbecuing of the steak

Wire and stone barbecues (width, length and height: 25 × 40 × 20 cm) were bought from a local supermarket in Erzurum. The thickness of the stone was 2 cm and the type of the stone according to company's declaration was granite (100% natural stone). For barbecuing, charcoal was used; and after it was completely ignited, it was spread evenly in the barbecue for balanced heating. Wire and stone grills were placed onto the barbecue; after waiting for a while for the heating of grills, wire grill was cleaned with onion and stone grill with tail fat before meat was placed on the grills. Cooking levels of the samples (rare, medium, well-done and very well-done) were determined based on the results of preliminary experiments. All samples were eatable. Table 1 shows the details of cooking procedures. No salt, spices, food additives and fat or oil were used while barbecuing the samples. Surface temperatures were measured using a digital thermocouple with surface probes (Testo 926, Lenzkirch, Germany) and the surface temperature of the grill was about 200 °C. All samples were turned once every 1.5 min on the wire grill, and 2 min on the stone grill. Experiments were performed in two replications. For each replicate, two steaks were used for each condition.

After barbecuing, the samples were cooled at room temperature, homogenized (at high speed for 30 s) by using a kitchen blender (Tefal, Istanbul, Turkey) to give a uniform sample for analyses. Samples were stored at −18 °C for HCA and PAH analyses. Freezing was necessary for the samples to be stored before clean-up in order to eliminate any risk of further reactions in the beef. They were thawed in a refrigerator at 4 °C for 12–24 h prior to analysis.

2.3.2. Proximate analysis and cooking loss

Water content (hot air oven), crude fat (ether extraction) contents and pH values of the samples were determined according

Table 1
Cooking time (min) for different cooking levels of beef steak cooked with wire and stone grill barbecue.

Barbecue type	Cooking level			
	Rare	Medium	Well-done	Very well-done
Wire	6	7.5	9	10.5
Stone	8	10	12	14