Food and Chemical Toxicology 50 (2012) 2128–2134

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods

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ARTICLE INFO

Article history:
Received 25 January 2012
Accepted 12 March 2012
Available online 20 March 2012

Keywords:
Grilled muscle foods
Coconut shell charcoal
Heterocyclic aromatic amines
Polycyclic aromatic hydrocarbons

ABSTRACT

Grilling muscle foods involves high temperatures that lead to production of cooking toxicants, such as heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs). To obtain realistic exposure levels of these two groups of mutagens analyses of the same samples using similar separation/detection techniques were performed. HAs and PAHs were quantified in well-done meat and fish samples grilled with wood and coconut shell charcoal at 200 °C. Quantitative HAs and PAHs profiles were different for beef and salmon using the same type of charcoal. Higher levels of HAs and PAHs were found in salmon samples. No significant differences were observed for HAs and PAHs in beef samples grilled with both charcoal types, whereas salmon grilled with coconut shell charcoal presented significantly lower amounts of HAs and PAHs than salmon grilled with usual wood charcoal. Continuous barbecuing with the same charcoal shown that combustion of fat that dropped along the grilling period contributed to higher formation of HAs and PAHs. Special attention must be given to the intake of barbecued foods since high amounts of HAs and PAHs can be taken in a single meal.

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1. Introduction

Diet contains various carcinogens: naturally occurring chemicals, synthetic compounds and compounds produced during cooking (Doll and Peto, 1981; Domingo, 2011; Nagao and Sugimura, 1993). The cooking toxicants have been receiving special attention in the last decades concerning their formation, occurrence, mitigation, and the impact of these substances on human health (Jägerstad and Skog, 2005; Skog et al., 1998). The risk of exposure to these compounds depends on the kind of diet, eating habits and cooking practices, which often result from regional traditions (Kobayashi et al., 2002; Melo et al., 2008; Gasperlin et al., 2009).

Cooking toxicants (HAs) and (PAHs) were significantly produced through meat grilling process at high temperature (Ferguson, 2010; Jägerstad and Skog, 2005).

Since their discovery 30 years ago, more than 25 HAs have been isolated and identified in cooked foods (Alaejos and Afonso, 2011; Murkovic, 2007). They can be divided in two main families: amino-imidazo-azaarenes or "thermic HAs" and amino-carbolines or "pyrolytic HAs". Formation of thermic HAs is the result of complex reactions that involve creatine/creatinine, free amino acids and sugars through the Maillard reaction at temperatures between 150 and 250 °C (Jägerstad et al., 1998; Nagao et al., 1977). Concerning pyrolytic HAs, a pathway for the formation was not so clear than thermic HAs, however it was suggested that may be produced from pyrolysis of proteins or amino acids heated at higher temperatures (>250 °C), and are not dependent of creatine (Matsumoto et al., 1981).

PAHs can be formed from a variety of combustion and pyrolysis processes and thus their natural or anthropogenic sources are numerous, however food seems to be the major route of exposition. High PAHs concentration in food is usually found in charcoal grilled/barbecued foods through the pyrolysis of fat and smoke from heat source (EFSA, 2008; Phillips, 1999). PAHs comprise fused aromatic rings, those containing two to four benzene rings are called "light PAHs", and those containing more than four, more stable and toxic, are called "heavy PAHs". The EU selected the sum of eight of the 15 priority PAHs as the most suitable indicators of carcinogenic PAHs in food, this PAH8 are the eight high molecular weight/carcinogenic from US-EPA PAHs list (Wenzl et al., 2006).

Competent authorities in most Western countries recommended minimizing the occurrence of cooking toxicants (Jägerstad and Skog, 2005). The IARC (1993) considered eight of the HAs tested to date, including the most abundant 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Viegas et al., 2012a), as *possible human carcinogens* (*Group 2B*) and one, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) as a *probable human carcinogens* (*Group 2A*),

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http://dx.doi.org/10.1016/j.fct.2012.03.051

and recommends a reduced exposure to these compounds. Concerning PAHs, recently IARC (2010) reviewed PAHs carcinogenicity, and classified benzo[*a*]pyrene (B*a*P) as *carcinogenic to humans* (*Group 1*), and some of other PAHs as *probably carcinogenic* or *possibly carcinogenic*.

Several researchers have highlighted an urgent need of to study more than one group of mutagens at the same time, to obtain more realistic exposure levels (Ferguson, 2010; Jägerstad and Skog, 2005; Sugimura, 2000). Furthermore, these compounds occur in mixtures, information about combined effect of compounds from the same group or interactions with carcinogens from different groups, but concomitant, especially if some metabolizing pathways are common, as in the case of HAs and PAHs (Dumont et al., 2010; Tarantini et al., 2011), need to be taken into account in considering their risk.

Grilled foods are increasingly popular both at home and in restaurants; however the higher levels of cooking carcinogens, make these foods a risk to the population (Farhadian et al., 2011). Thus, adequate information about exposition to both cooking toxicants, and mitigation strategies in this type of foods is a matter of concern.

The main objective of this work was to study the influence of charcoal type on HAs and PAHs formation and select the safer charcoal to grill muscle foods. With this propose HAs and PAHs were quantified in meat and fish samples grilled with two different types of charcoal under standard temperature conditions. Another goal of this work was evaluate if the continuous barbecuing with the same charcoal influences the HAs and PAHs formation. Contribution of barbecued muscle foods to the diet exposure of these hazard compounds will also be highlighted.

2. Materials and methods

2.1. Reagents and standards

All the solvents used were of HPLC grade (Merck, Darmstadt, Germany). Water was purified with a Milli-Q System (Millipore, Bedford, MA, USA). The chemicals used for HAs extraction [sodium hydroxide, hydrochloric acid, ammonium acetate, ammonia solution 25% (v/v)] and mobile phase triethylamine were of analytical grade and were purchased from Merck.

Concerning HAs standards, all individual, IQ, MeIQx, PhIP, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-*b*]indole (MeAαC), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), were purchased from Toronto Research Chemicals (Toronto North York, ON, Canada). Stock standard solutions of 100 μg/mL in methanol were prepared and used for further dilution.

The standard PAHs mixture was purchased from Supelco (Bellefonte, PA, USA) and consisted of: 10 μg/ml of naphthalene (Na), acenaphthene (Ac), acenaphthylene (Ace), fluorene (F), anthracene (A), phenanthrene (Pa), fluoranthene (Fl), pyrene (P), benzo[*a*]anthracene (BaA), chrysene (Ch), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), BaP, indeno [1,2,3-*cd*]pyrene (IP), benzo[*g*,*h*,*i*]perylene (BgP), dibenzo[*a*,*h*]anthracene (DhA) (16 US-EPA PAHs) in 1 ml of acetonitrile.

A combined pH glass electrode connected to a pH-meter (MicropH 2001, Crison, Barcelona, Spain) and a Magna membrane nylon 0.22 μm were used.

2.2. Samples and grilling conditions

2.2.1. Effect of two different types of charcoal

Beef and salmon samples were used to evaluate the effect of two different types of charcoal in HAs and PAHs formation. The beef samples used in this study were obtained from the *Longissimus dorsi* muscle of middle-aged bovine carcasses. The meat was obtained from a major butchery in Porto, Portugal. The beef samples (six steaks with 2.5 cm of thick, and weighing 399.4 g (±22.4 g) were chilled overnight in a cooling room (5 ± 1 °C). Following the chilling process, all trimmable fat and connective tissue (epimysium) were removed from the *Longissimus dorsi* muscle.

Samples of fresh salmon from Atlantic were obtained in a fish market in the same city. Six fillets of salmon with 2 cm of thick and weighting 216.2 (±19.4 g) were prepared.

Two similar garden-type grills (35 cm width, 52 cm length, and 15 cm height) were fuelled with two different types of charcoal: the traditional wood charcoal and another called "ecological charcoal" from 100% coconut shell. Aiming to keep the temperature next to the grid at 200 °C the distance to the heat source was selected depending on the type of charcoal. Temperature was measured by using a digital thermocouple with a surface probe (Testo 926, Lenzkirch, Germany). For wood charcoal the samples were grilled at 18 cm distance to the heat source, and for coconut shells charcoal the samples were grilled closer to the heat source, 8 cm. The grilling time was 18 min for beef and 23 min for salmon until well-done cooked, golden color for salmon and moderately browned for beef. Samples were turned once during grilling at half the total cooking time. Internal temperature reached the minimum 75 °C in all our experiments. No salt or oil was applied to the samples before or after grilling.

2.2.2. Effect of continuous barbecuing with the same charcoal

Barbecued chicken samples were collected to evaluate if the continuous barbecuing with the same charcoal influences the HAs and PAHs formation. Chicken samples were collected in one of the traditional restaurant for chicken charcoal grilled (*Frango de Churrasco)*, at lunch time, in Porto city. Raw chickens were open in the breast and cooked during 30 min and turned randomly during cooking period. Salt or other seasonings were not added. The "*churrasco* grill" (horizontal apparatus) was 92 cm width, 190 cm length, and 62 cm height. Operators ignited the fire and when all flames had subsided, the grilling procedure starts. Temperature next to the grid ranged between 230–300 °C (measured by using a digital thermocouple with a surface probe). The final product was well-done cooked, with a white color in the inner and a golden color in the skin (little beat charred).

Chicken collection was performed in two different periods of barbecuing; three samples were collected in each period. The first at the beginning of charcoal combustion when all flames had subsided, corresponding to the first chickens grilled in the charcoal, and the second period (one and a half hour later), was the last chickens grilled before the addition of new charcoal to the processing bed. Between the two periods of collection several chickens were cooked and commercialized.

2.2.3. Samples treatment after cooking procedures

Samples were weighed in all steps (uncooked, after grill, edible and no edible parts. Non-edible parts were removed, such as remaining fat or connective tissue in beef, bones and skin in fish, and in chicken samples, thighs were selected for further analysis and bones were removed.

Each sample was mixed in a kitchen blander (Moulinex, France) to produce a uniform sample. In the end, the homogenized samples were properly identified and frozen at −20 °C until the analysis of contaminants. Samples for PAHs analysis were protected from light and plastic adsorption, wrapping in aluminum foil before placing it in plastic containers (EC, 2011).

2.3. Analysis of HAs

Extraction and purification of HAs were performed using the reference method in interlaboratorial exercises (Santos et al., 2004), developed by Gross (1990) and further modified by Galceran et al. (1996).

Separation and quantification of HAs were performed by liquid chromatography with diode array fluorescence detection (HPLC-DAD/FLD) according by Gross (1990). HAs separation was carried out in an analytical HPLC unit equipped with one HPLC pump PU-1580, a fluorescence detector Jasco FP-920 coupled to a Multi-wavelength detector MD 910 and an auto sampler AS-950 equipped with a 20 μL loop (all from Jasco, Japan). The column was a TSK gel ODS80 (Toyo Soda, Japan) (5 μm; 250 mm length; 4.6 mm internal diameter). The Borwin PDA Controller Software was also used.

Three solvents were used for mobile phase: 0.01 M triethylamine adjusted with phosphoric acid to pH 3.2 (A), 0.01 M triethylamine adjusted with phosphoric acid to pH 3.6 (B) and acetonitrile (C) with a flow rate 1 ml/min. The linear gradient program was: 0–10 min, 5–15% C in A, 10–10.1 min exchange of A with B; 10.1–20 min, 15–25% C in B; 20–30 min, 25–55% C in B; 30–55 min, column rinse and re-equilibration. Separations were carried out at room temperature. DAD was set at 263 nm and FLD at excitation 307 nm and emission 370 nm.

Peak identification in food samples was carried out by comparing retention times and spectra of unknown peaks with reference standards, as well as co-chromatography with added standards and peak purity. Quantification of PhIP, MeAαC, and AαC was based on fluorescence peak area. Standard addition method was used for quantification of HAs using the non-spiked sample and two fortified levels (25 and 50 ng of thermic HAs; 50 and 100 ng of pyrolytic HAs and PhIP) before extraction procedure.

2.4. Analysis of PAHs

Extraction and clean up procedures were performed according by Viegas et al. (2012b) for grilled muscle foods, based on the method of Moret and Conte, applied for vegetable oils (Moret and Conte, 2002) and smoked meat (Purcaro et al., 2009).

The glassware, mostly amber, was carefully washed and rinsed with distilled solvent (acetone and hexane) before use (EC, 2011).

Separation and quantification of PAHs were performed by liquid chromatography with fluorescence detection (HPLC-FLD) according with Viegas et al. (2012b) conditions.

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