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Content of heterocyclic amines and polycyclic aromatic hydrocarbons in pork, beef and chicken barbecued at home by Danish consumers

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ABSTRACT

It is a well-known fact that, when meat is barbecued, several harmful components, including heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH), may be formed. The aim of this study was to determine the HCA and PAH content in meat (pork, chicken and beef) when barbecued at home by Danish consumers according to their normal practice. With regard to HCA, beef contained the highest concentrations of 9*H*-pyrido[3,4-*b*]indole (norharman) and 2-methyl-β-carboline (harman), while chicken contained more 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) than pork and beef. The analysis of PAH showed a markedly higher concentration of PAH in beef compared with pork and chicken. In general, a correlation between the HCA content and the surface colour of the meat was found, the darker the colour the higher the HCA concentrations.

1. Introduction

It is well known that, when meat is heat-treated using traditional procedures such as frying, barbecuing and smoking, several harmful components, including various mutagens and carcinogens, may be formed (Jägerstad & Skog, 2005). Epidemiological studies show an increased risk of cancer in the intestine, breast, bladder, prostate and pancreas after high consumption of well-done, fried and barbecued meat, in particular red meat (Knutsen, Binderup, Vikse, & Øvrebø, 2007; Lin et al., 2010; Norat, Bingham, & Ferrari, 2005). Red meat is defined as pork, beef, goat and lamb. In this context, it is important to note that all meat types, and not just red meat, may produce heterocyclic aromatic amines (HCA) when cooked at high temperatures. It is also important to bear in mind that the risk of cancer is complex, and, besides diet, it also includes general lifestyle habits (WCRF & AICR, 2007) and individual genetic make-up (Alaejos, Pino, & Afonso, 2008; King, Kadlubar, & Turesky, 2000).

The use of barbecues or outdoor grills for cooking food has increased considerably in Denmark and a recent survey performed by Weber-Stephen Nordic showed that 83% of the participating Danes (age 25–65 years of age) own a barbecue. During barbecuing, especially two groups of harmful chemical components may be formed: heterocyclic aromatic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) (Jägerstad & Skog, 2005). HCAs are generated as a reaction between precursors in the meat during high temperature treatment (Felton et al., 1997; Thomson, Lake, Cressey, & Knize, 1996). PAHs are components in the smoke and are formed either from the charcoal itself or from lipids dripping on the hot charcoal (Jägerstad & Skog, 2005). Even though both HCAs and PAHs are associated with serious health risks, there are only few reports concerning the general intake of these components. A worst case scenario with 30 barbecues per year in Norway estimated that 60% of the PAH intake in the adult Norwegian population was associated with barbecued food (Knutsen et al., 2007). However, this estimation was based on a small amount of data. Maximum levels for the content of some of the PAHs in processed meat products have been established (European Commission, 2006, 2011), but there are so far no maximum levels for HCAs. In order to evaluate the potential health risk of consuming these components, it is of outmost importance to collect valid data on the actual amount consumed.

It is a well-known fact that the formation of HCAs is closely related to temperature during cooking and that the formation of HCAs primarily occurs when cooking methods involving contact heating, such as pan-frying, barbecuing and deep-frying, are used. An increase in temperature generally enhances the formation of HCAs (Jägerstad, Skog, Arvidsson, & Solyakov, 1998; Knize, Dolbeare, Carroll, Moore, & Felton, 1994; Skog, Steineck, Augustsson, & Jägerstad, 1995), and from approximately 200 °C the formation of some HCAs is accelerated, which is seen very clearly especially for 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Knize et al., 1994; Persson, Sjöholm, & Skog, 2002; Skog et al., 1995; Solyakov & Skog, 2002). Besides temperature, the formation of HCAs depends on the presence of precursors, including creatinine and amino acids and, in some

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cases, also carbohydrates (Jägerstad et al., 1998; Pfau, Rosenvold, & Young, 2006). Precursor concentrations vary both between and within animal species, but, even so, it is not believed that the precursor concentrations are the limiting factor for HCA formation (Felton, Jägerstad, Knize, Skog, & Wakabayashi, 2000; Jägerstad et al., 1998; Pfau et al., 2006; Skog & Jägerstad, 2006). Among the known HCAs, some are not directly mutagenic but are so-called co-mutagens, e.g. 9*H*-pyrido[3,4-*b*]indole (norharman) and 2-methyl-β-carboline (harman), which means that these two compounds enhance the mutagenicity of other HCAs (Jägerstad et al., 1998; Skog & Solyakov, 2002; Sugimura, Wakabayashi, Nakagama, & Nagao, 2004).

Of the known PAHs (now more than 250), 15 have been shown to be mutagenic or genotoxic and carcinogenic (EFSA, 2008; SCF, 2002). Of the known 15 genotoxic and carcinogenic PAHs, benzo[*a*]pyrene (BaP) is the most commonly studied, and this component has shown various toxicological effects in experimental animal studies (SCF, 2002; Schneider, Roller, Kalberlah, & Schumacher-Wolz, 2002). Since 2002, BaP has been used as a marker of carcinogenic PAHs in food. BaP concentrations were measured in some barbecued meat products: 1.5 μg/kg in hamburgers (FSTA, 2007; Kazerouni, Sinha, Hsu, Greenberg, & Rothmann, 2001), up to 1.8 μg/kg in pork (Reinik et al., 2007), up to 4.9 μg/kg in steaks (Kazerouni et al., 2001) and 9.2 μg/kg in duck with skin (Chen & Lin, 1997). However, BaP is not always detectable in foods containing PAHs, and in 2008 EFSA suggested using the sum of four carcinogenic PAHs, called PAH4, including benzo[*a*]anthracen, benzo[*b*]fluoranthene, BaP and chrysene, as markers for PAH concentration (EFSA, 2008). This has recently been implemented in an EU regulation (European Commission, 2011).

There is a general lack of knowledge concerning the HCA and PAH content in home-cooked meat, since most studies on the formation of mutagenic compounds in meat have been carried out as model studies or as highly controlled frying experiments (e.g. Arvidsson, van Boekel, Skog, & Jägerstad, 1998; Pais, Salmon, Knize, & Felton, 1999; Persson, Oroszvari, Tornberg, Sjöholm, & Skog, 2008) or have concerned smoked foods with BaP levels of up to 1 μg/kg in smoked ham, bacon and sausages (Duedahl-Olesen, White, & Binderup, 2006; Jira, 2004; Kazerouni et al., 2001). Therefore, in order to contribute data to this field of knowledge, this survey on barbecuing pork chops and beef steaks (50 consumers) or pork chops and chicken fillets (50 consumers) includes HCA and PAH analysis. Methods for assessing the meat surface colour and core temperature were included to evaluate correlations between the degree of cooking and the concentrations of HCA. At the same time data on the Danish consumers' actual barbecuing methods and barbecuing habits in general were collected.

2. Materials and methods

2.1. Meat

Boneless pork loins (intramuscular fat content of 1.4%–2.0%, with an average of 1.9% fat) with approximately 3 mm fat were sliced into 14 pieces of 2 cm-thick chops per loin (n = 100). Beef strip loins (intramuscular fat content of 2.7%–5.9%, with an average of 4.6% fat) were sliced into 10 pieces of 2 cm steaks per strip loin (n = 50). Chicken breast fillets (n = 50, intramuscular fat content of 0.6%–1.0%, with an average of 0.9% fat) were used as one muscle per sample. All pieces of meat were individually vacuum-packed.

2.2. Consumers

Consumers (n = 100) were recruited from the Roskilde area. Roskilde was chosen because it represents an average medium size town in Denmark and consumers were recruited from two large firms within the city. They were given one pork chop and either one chicken fillet (n = 50) or one beef steak (n = 50) for research purposes (analysis of HCA and PAH). All consumers were also given additional meat for the dinner of the family. The meat was distributed on a Friday, and the consumers were instructed to barbecue the research samples together with the "family dinner meat" according to their normal barbecue practice. The barbecued "research meat" samples were stored in the refrigerator according to the instruction to the consumers. On the following Monday, these meat samples were returned to the Danish Meat Research Institute (DMRI). A total of 94 pork chops, 47 chicken fillets and 48 beef steaks were returned.

2.3. Colour assessment of the barbecued meat

To estimate an approximate core temperature of the consumer samples, the internal colour was assessed on a visual five-point scale made from similar meat samples fried to internal temperatures of between 60 °C and 80 °C with 5-degree intervals (beef steaks between 40 °C and 80 °C with 10-degree intervals).

The surface colour was assessed on a five-point visual scale constructed from consumer samples in the investigation by choosing the lightest and the darkest samples and three samples in between. To document the surface colour scales, photographs were taken of the meat samples (Fig. 1).

The internal colour and surface colour of all the samples were assessed by one person experienced in assessing meat colour. The classification was performed by a comparison between the samples and the photographs. The method was not further validated as it represented a rough classification of the degree of cooking.

2.4. Sample preparation for chemical analysis

The barbecued meat samples were weighed (pork, n = 94; beef, n = 48; chicken, n = 47), and ten samples of each species were selected for analysis of PAH. These samples were selected in order to cover the spectrum of variation in surface colour. The ten samples selected for both PAH and HCA analyses were divided into two halves, and the half part for PAH was frozen until analysis. For the HCA analysis, the crust of the meat (depth of 5 mm) was carefully sliced using a kitchen slicer. The crust was weighed, and subsequently used for chemical analysis of HCA.

2.5. Analysis of HCA content

2.5.1. Reagents and standards

2-Amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 2-methyl-β-carboline (harman), harman-d_3, 9*H*-pyrido[3,4-*b*]indole (norharman), norharman-d_7, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and 2-amino-1-(trideuteromethyl)-6-phenylimidazo[4,5-*b*]pyridine (PhIP-d_3) were supplied by Toronto Research Chemicals Inc. (Toronto, Canada). High performance liquid chromatography (HPLC)-grade acetonitrile was supplied by JT BAKER (Phillipsburg, USA). Sodium hydroxide, methanol, ammonium formate (NH_4HCO_2), 1-octanol and acetone, HPLC-grade, were obtained from Th. Geyer (Roskilde, Denmark). Formic acid was obtained from FLUKA/Sigma-Aldrich (Broendby, Denmark). Sulphuric acid was obtained from Merck (Glostrup, Denmark). All aqueous solutions were prepared using reagent water purified by a Milli-Q Gradient system (Millipore, Copenhagen, Denmark). Solutions of the HCA standards were prepared in methanol/water (1:1) with a concentration of 100 mg/l. All the solutions were kept refrigerated at 5 °C. Diluted standard solutions were prepared with reagent water in the range of 0.05–50 μg/l and stored at 5 °C for a maximum of one week. Solutions of the HCA standards were prepared in methanol/water (1:1) with a concentration of 100 mg/l. All the solutions were kept refrigerated at 5 °C. Diluted standard solutions were prepared