



Heterocyclic amines and polycyclic aromatic hydrocarbons in commercial ready-to-eat meat products on UK market



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Chemical compounds studied in this article:

2-Amino-3-methylimidazo[4,5-f]quinoline (PubChem CID: 53462)

2-Amino-3,4-dimethylimidazo[4,5-f]

quinoline (PubChem CID: 62274)

2-Amino-3,8-dimethylimidazo[4,5-f]

quinoxaline (PubChem CID: 62275)

2-Amino-3,4,8-trimethylimidazo[4,5-f]

quinoxaline (PubChem CID: 104739)

2-Amino-1-methyl-6-phenylimidazo[4,5-b]

pyridine (PubChem CID: 1530)

Benzo[a]pyrene (PubChem CID: 2336)

Benz[a]anthracene (PubChem CID: 5954)

ABSTRACT

Heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs), which are developed during meat processing, may play key roles in the imposing health risk. The consumption of ready to eat (RTE) meat products has increased dramatically in recent years due to their convenience. Therefore, it is essential to evaluate its health risk and provide dietary intake guidance to the general public. 11 RTE meat products were selected from UK market including chicken, pork and fish to investigate their health risks in concern of HCAs and PAHs levels. HCAs and PAHs were extracted by solid-phase extraction and analysed by HPLC-DAD/FLU. Chargrilled chicken contained the highest amount of HCAs (37.45 ± 4.89 ng/g) and PAHs (3.11 ± 0.49 ng/g), followed by roasted bacon (HCAs 15.24 ± 1.31 ng/g, PAHs 1.75 ± 0.17 ng/g) and honey roast salmon (HCAs 17.12 ± 5.86 ng/g, PAHs 0.38 ± 0.09 ng/g). The high dietary intake of HCAs was from chargrilled chicken and ham, which could contribute to the increase in breast cancer and colorectal adenoma. While cancer risk associated with PAHs intake from RTE meat products was relatively low according to the Lifelong Average Daily Intake of UK consumers.

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1. Introduction

The average consumption of total red meat and processed meat was 70 g per day for all adults in UK (NDNS, 2011). In processed meat products, the presence and hazard of HCAs and PAHs become a major concern for both consumers and researchers. Heterocyclic amines (HCAs) represent a class of carcinogenic compounds that were identified from protein-rich food in the 1970s (Rahman, Sahar, Khan, & Nadeem, 2014). Five of them, including 2-amino-3-methylimidazo [4,5-f]quinoline (IQ),

2-amino-3,4-dimethylimidazo[4,5-f]quinolone (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) are reasonably regarded as human carcinogens (IARC, 1993). On the other hand, PAHs are hydrocarbons that contain two or more benzene rings, which could be produced in processed meat products through incomplete combustion or pyrolysis of carbon and hydrogen. They can be accumulated in barbequed, grilled, fried and smoked food (PHE, 2008). PAH4, including benz[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF) and chrysene has recently been reported as indicator of carcinogenic potency of PAHs in food (Janoszka, 2011). In PAH4, both BaA and BaP are considered as probable carcinogens in humans (Group 2A) comparing with

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other PAHs (less carcinogenic) according to the updated IARC (2010) report. Thus, it is necessary to evaluate the dietary intake of BaA and BaP from processed meat product. Particularly, BaA largely exists in smoked meat and widely examined by researchers, BaP is one of PAHs with the highest Toxicity Equivalency Factor ($TEF_{BaP} = 1$, $TEF_{BaA} = 0.1$, $TEF_{BbF} = 0.1$ and $TEF_{BkF} = 0.1$) (Janoszka, 2011; Rozentale et al., 2015; Saito, Tanaka, Miyazaki, & Tsuzaki, 2014; Santos, Gomes, & Roseiro, 2011).

Epidemiological studies indicate that high meat intake could increase the risk of cancer, since a high level of carcinogenic compounds could be produced during high-temperature constantly cooking, such as HCAs (Egeberg et al., 2013; Janoszka, 2010; Liao, Wang, Xu, & Zhou, 2010; Oz & Kaya, 2011). González et al. (2006) carried out a cohort study and found out that there might be a close association between red and processed meat intake and gastric non cardiac cancer. Stefani, Ronco, Mendilaharsu, Guidobono, and Deneo-Pellegrini (1997) suggested that red meat intake increased the risk of breast cancer in the cohort study. Well-done meat such as beef steak and bacon contained more HCAs, which might be a factor that caused breast cancer (Zheng, Gustafson, Moore, Hong, Anderson, Kushi, et al., 1998). Exposure of PAHs has been probably associated with cause lung and skin cancer (PHE, 2008). However, these cohort studies did not provide solid evidence that increased cancer risk was caused by the amount of carcinogens in red and processed meat, in particular because of the complexity of processing conditions, meat type and composition of processed meat product. Although IARC (1993) has already classified processed meat as carcinogenic to human, the level of carcinogens in meat products varies from not detectable to 500 ng/g due to different manufacturing process and food materials (Rahman et al., 2014). With the aim of understanding the relationship of red/processed meat and health risk, it is useful to study the impact of meat processing and ingredients on the formation of carcinogens. Ready to eat (RTE) meat consumption increased nearly two-fold (115–190 g consumed per person per week) from 1975 to 2010 because of its convenience, they can be found either in packed sandwiches or meal dishes (Chalabi, 2013). Therefore, the main focus of this study was to determine the concentration of HCAs and PAHs in selected ready to eat meat products that are popular on UK's market, in order to assess the dietary intake of carcinogens that RTE meat products contributed and provide useful guideline about dietary meat intake for general public.

2. Material and methods

2.1. Meat samples

11 RTE meat products were purchased from a local supermarket (Reading, UK) including BBQ British chicken breast slices, tikka British chicken breast slices, Chargrilled British chicken breast slices, the British smoked ham slices, British ham slices, classic roasted bacon, crispy smoked streaky bacon, sliced pork sausage, Swedish meatballs, honey roast salmon flakes and sweet chilli salmon flakes. These 11 RTE meat products have been selected based on the relatively higher amount of average daily consumption (g/day) from NDNS (2015) (raw data, unpublished), with the consideration of variety of meat products, including chicken, pork and fish. All chicken products were produced in UK by using British chicken. The supplier information and ingredients information were listed in Table 1. All the samples were stored at 4 °C, and analysis were carried out within 10 days. All samples were purchased at 3 different occasions to take into account the batch effect.

2.2. Chemicals

The HCA standards IQ (2-amino-3-methyl-imidazo [4,5-f]quinoline), MeIQ (2-amino-3,4-dimethyl-imidazo [4,5-f]quinoline), MeIQx (2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-f]quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine, BaA (Benz[a]anthracene) and BaP (benzo[a]pyrene) were purchased from Toronto Research Chemicals (Toronto, Canada). Ammonium acetate, triethylamine, acetonitrile (HPLC grade), methanol (HPLC grade), ethyl acetate (>98%), hydrochloric acid, water (HPLC grade) and sodium hydroxide were purchased from Fisher Scientific (Loughborough, UK). Phosphoric acid was obtained from Sigma Aldrich (Sigma Aldrich, UK). *Extrelut NT 20* columns and diatomaceous earth refill material were purchased from Merck (Darmstadt, Germany). Bond Elut propyl-sulfonic acid (PRS) cartridges (100 mg, 10 ml), C-18 cartridges (7 ml) were purchased from VWR Inc (Lutterworth, UK).

2.3. Composition analysis

pH was measured by homogenizing 5 g sample and 5 ml distilled water (Puangsombat, Gadgil, Houser, Hunt, & Smith, 2011). The moisture content was determined by drying 3 g meat at 100 °C for 24 h. Samples were dried firstly in an oven for 4 h and analysed in Soxhlet extraction system to determine the fat level. The protein content was determined by the Kjeldahl method (Horwitz & Latimer, 2005). The creatine content was measured based on the method used by Puangsombat et al. (2011). 0.25 g well homogenized sample was stirred with 60 ml trichloroacetic acid (30 g/L in distilled water) for 5 min. The mixture was then filtered with a filter paper (No.1, Filter speed: medium fast & qualitative, 100 circles, 18.5 cm, Whatman Ltd). 10 ml diethyl ether was added to 20 ml filtrate to dissolve fat. The mixture was shaken well and held for 10 min to complete separating 2 phases. 4 ml of defat layer was added with 2 ml of diacetyl (0.2 g/L in distilled water) and 2 ml of 1-naphthol (25 g/L in 20 g/L of sodium hydroxide solution). The blend was heated for 5 min at 40 °C. The absorbance of solution was measured at 520 nm against a reagent blank in an UV spectrophotometer. The creatine content was expressed as milligram per gram of the meat sample. Standard curve was made from 5 gradient concentrations (0–20 mg/L) of creatine monohydrate (>98%, Sigma Aldrich, UK).

2.4. Sample preparation

2.4.1. Separating HCAs

HCAs extraction was based on the methods proposed by Puangsombat et al. (2011). To minimize the variation and bias due to the unevenly distribution of sauce on the surface of meat, all samples were blended well before measuring. 3 g ground meat sample was blended well with 12 ml 1M sodium hydroxide. The mixture was then transferred into an *Extrelut 20 column* with 17 g diatomaceous earth. The HCAs were eluted by 60 ml ethyl acetate in *Extrelut column*, and transferred into PRS cartridge which was pre-conditioned with 7 ml ethyl acetate. A PRS cartridge was then washed with 6 ml 0.1M HCl, 15 ml methanol/0.1M HCl (45/55, v/v) and 2 ml pure water to remove interferences from the PRS cartridge. The HCAs were then eluted by 20 ml 0.5M ammonium acetate (pH 8.5) from the PRS cartridge and transferred into a C-18 cartridge that was conditioned with 5 ml methanol and 5 ml pure water. Finally, HCAs were eluted with 1 ml methanol/ammonium hydroxide (9/1, v/v) from C-18 cartridge into 2 ml amber vial, followed by drying the mixture under nitrogen stream for 1.5 h at room temperature. The contents of the vial were dissolved with

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