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Effect of cooking methods on the formation of heterocyclic aromatic amines in chicken and duck breast

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ABSTRACT

Heterocyclic aromatic amines (HAAs), potent mutagens/carcinogens, are pyrolysis formed during the cooking of meat and fish. In the present study, the effects of various cooking methods, pan-frying, deep-frying, charcoal grilling and roasting on the formation of HAAs in chicken breast and duck breast were studied. The various HAAs formed during cooking were isolated by solid-phase extraction and analyzed by high-performance liquid chromatography (HPLC). Results showed that chicken breast cooked by charcoal grilling contained the highest content of total HAAs, as high as 112 ng/g, followed by pan-fried duck breast (53.3 ng/g), charcoal grilled duck breast (32 ng/g), pan-fried chicken breast (27.4 ng/g), deep-fried chicken breast (21.3 ng/g), deep-fried duck breast (14 ng/g), roasted duck breast (7 ng/g) and roasted chicken breast (4 ng/g). For individual HAA, the most abundant HAA was 9H-pyrido-[4,3-b]indole (Norharman), which was detected in charcoal grilled chicken breast at content as high as 32.2 ng/g, followed by 1-methyl-9H-pyrido[4,3-b] indole (Harman) and 2-amino-1-methyl-6-phenylimidazo[4,5-f]pyridine(PhIP) at 32 and 31.1 ng/g in charcoal grilled chicken breast, respectively. The content of PhIP in pan-fried duck and chicken breast were 22 and 18.3 ng/g, respectively. Generally, the type and content of HAAs in cooked poultry meat varies with cooking method and cooking conditions.

1. Introduction

Diet plays an important role in cancer development. Several epidemiological studies suggest that the consumption of fried, broiled or roasted meat is associated with the development of cancer, while other studies have found no reliable correlation (for review see Knize & Felton, 2005). It has been proposed that heterocyclic aromatic amines (HAAs), potent mutagens present at ng/kg levels in cooked foods, play an important role in the aetiology of human cancer (Sugimura, 2000). The presence of HAAs in cooked foods has become a major concern for consumers.

Since first discovered by Sugimura et al. (1977), more than 20 different HAAs have been identified in cooked foods (Felton, Jägerstad, Knize, Skog, & Wakabayshi, 2000). Several animal studies have shown that HAAs are potent carcinogens and induce tumours in various organs (Nagao, 1999; Nagao & Sugimura, 2000; Sugimura, 1997).The International Agency for Research on Cancer (IARC, 1993) regards eight of the HAAs tested to date (2-amino-3-4-dimethyl-imidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx), PhIP, 2-amino-9H-pyrido [2,3-b]indole (AaC), 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAaC), 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) and 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1)) as possible human carcinogens (class 2B) and one (2-amino-3-methylimidazo[4,5-f] quinoline (IQ)) as a probable human carcinogen (class 2A), and recommends a reduced exposure to these compounds. 1-methyl-9H-pyrido[4,3-b] indole (Harman) and 9H-pyrido- [4,3-b]indole (Norharman) are often referred to as co-mutagens, because they are not mutagenic in the Ames/*Salmonella* test, but enhance the mutagenic activity of other compounds; for example, Norharman enhances the mutagenic effects of Trp-P-1 and Trp-P-2 (Nagao, Yahagi, & Sugimura, 1978; Sugimura, Nagao, & Wakabayashi, 1982). Furthermore, Harman and Norharman have been discussed in relation to neurotoxins and enzyme inhibitors (de Meester, 1995; Kuhn, Muller, Grosse, & Rommelspacher, 1996). Thus, the presence of Harman and Norharman in cooked foods should not be ignored. HAAs are formed in meat during preparation of food using heat. Formation pathway for HAAs is complex. The major mechanism can be attributed to heating of four naturally occurring substances present in meat, free amino acids, creatine, creatinine and sugars, or to pyrolysis of amino acids and proteins (Felton & Knize, 1990; Jägerstad et al., 1983). The variety and content of HAAs in cooked meat products can be dependent on many factors, such as food type, processing methods, cooking

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duration, cooking temperature, pH, water activity, precursor content, and lipid oxidation, etc. The processing methods and conditions are the most important parameters in the formation of these compounds (Knize, Dolbeare, Carroll, Moore, & Felton, 1994). Many reports have demonstrated that frying and roasting are the major processing methods that cause formation of high content of HAAs (Abdulkarim & Smith, 1998; Salmon, Knize, Felton, Zhao, & Seow, 2006; Solyakov & Skog, 2002).

Poultry such as chicken and duck meat is an important food commodity in China, and the production and consumption of poultry have increased steadily in recent years. The major processed poultry products in China include charcoal grilled duck, roasted duck, deep-frying chicken and roasted chicken. Due to the fact that the HAAs formation can be correlated well to the processing methods, the formation of HAAs profiles as affected by various processing methods has to be investigated. To date, more than 20 reports on HAAs content in poultry, mainly chicken, have been published (for review see Skog & Solyakov, 2002). However, no information is available as to the variety and content of HAAs in cooked duck meat by various cooking methods. The objective of the present study was to investigate the effects of thermally treated meat methods (pan-frying, deep-frying, charcoal grilling and roasting) on the HAAs formation in chicken and duck breast. The information derived from this study can provide clues to understanding the factors that affect HAAs formation, it also can be used to estimate the intake of HAAs from poultry meat and can indicate means of reducing or eliminating these compounds.

2. Materials and methods

2.1. Materials

All chemicals including hydrochloric acid, sodium hydroxide, ammonium acetate, a-naphthol, diacetyl, trichloroacetic acid, Sigma Kit GAHK-20 were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). The HPLC grade solvents, including methanol, methylene chloride and acetonitrile were from Tedia Co. (Fairfield, OH, USA), acetonitrile was degassed by sonication and filtered through a 0.2 μm membrane filter prior to use. Deionized water was produced using a water purification system by Millipore Co. (Bedford, MA, USA). Diatomaceous earth extraction cartridges (Extrelut-20) and refill material were provided by Merck Co. (Darmstadt, Germany). Bond Elut PRS (500 mg) and C_{18} (100 and 500 mg) cartridge and stopcocks were from Varian Co. (Harbor, CA, USA). Cartridge coupling pieces were from Supelco Co. (Bellefonte, PA, USA).

Sixteen HAAs standards, IQ, MeIQ, 2-amino-3-methyl-imidazo [4,5-f]-quinoxaline(IQx), MeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline- (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx), 2-amino-3,4,7,8-tetramethylimidazo[4,7,8-f]quinoxaline (4,7,8-TriMeIQx), PhIP, Harman, Norharman, Glu-P-1, 2-amino-dipyrido[1,2-a: 3′,2′-d]imidazole (Glu-P-2), Trp-P-1, Trp-P-2, AaC and MeAaC were purchased from Toronto Research Chemicals (Downsview, ON, Canada). Stock standard solutions of 100 μg/mL in methanol were prepared and used for further dilutions. 4,7,8-TriMeIQx was used as internal standards (1 μg/mL methanolic solution).

The poultry, chicken breast and duck breast were obtained from a local supermarket in Nanjing. The poultry breasts were properly thawed before cooking, and skin and bones were removed.

2.2. Instrumentation

An Agilent Technologies model Series 1100 (Waldbronn, Germany) equipped with a model G1322A on-line vacuum degasser, a model G1311A quaternary pump system, a model G1367A autosampler and model G1315B photodiode-array detector and model G1321A fluorescence detector, both driven by the Chemstation. The column was a TSK-gel ODS-80TM (5 μm, 25 cm × 4.6 mm I.D.) from Tosoh Co.(Tokyo, Japan), equipped with a Supelguard LC-18-DB precolumn (Supelco, Bellefonte, PA, USA).

A Supelco Visiprep and a Visidry SPE vacuum manifold (Supelco, Bellefonte, PA, USA) were used for manipulations with solid-phase extraction cartridges. The homogenizer (ULTRA-TURRAX T25 Basic) was from IKA works (Staufen, Germany).

2.3. Cooking of poultry meat

Before processing, the poultry breasts were trimmed to obtain a uniform size and shape of each. After cleaning, poultry breasts (weighing about 169.76 ± 5.80 g) were prepared using several common household cooking conditions: pan-frying, deep-frying, charcoal grilling and roasting. For pan-frying, the poultry breasts were fried for 5 min per side without fat or oil in a Teflon-coated pan, which was preheated and the surface temperature was measured as 180 °C. For the deep-frying for six poultry breasts, fresh soybean oil (2 L) was used. The poultry breasts were fried for 10 min in a commercial stainless steel deep-fat fryer, when the temperature of oil reached 180 °C. For the charcoal grilling, approximately 1 kg of charcoal was placed in the bottom of an oven, and 100 mL of gasoline was poured onto charcoal to start fire for 5 min. When all flame had subsided, the charcoal was leveled by raking. The poultry breasts were then grilled over charcoal for 10 min per side, total cooking time was 20 min, the distance between samples and charcoal was about 8 cm. The surface temperature of the samples was measured about 200 °C. For roasting, the poultry breasts were placed in an oven for roasting for 20 min at 200 °C. No salt, oil and flour were applied to poultry breast before and after cooking. The temperature of cooking was monitored by the thin chromium-aluminium thermocouples. All experiments were repeated three times, for each treatment, one poultry breast was used. After all processing methods, the cooked poultry breasts were cooled at room temperature, then homogenized using commercial blender to obtain a uniform sample and analyzed to determine proximate composition. All of the cooked poultry samples were wrapped in plastic wrap and kept frozen until analyzed.

2.4. Chemical analyses

Samples were analyzed for moisture content, protein, total lipid, glucose, creatine, creatinine content, weight loss and pH.

Moisture was determined, following the ISO recommended method 1442 (ISO, 1996).

Protein was determined by following the ISO recommended method 937 (ISO, 1978).

Fat was determined by following the ISO recommended method 1443 (ISO, 1973).pH was determined by following the ISO recommended method 2917 (ISO, 1999) using a HI92240 model pH meter (HANNA instrument, Portugal).

The weight loss during cooking was determined by weighing.

Glucose content was measured by glucose assay kit (Sigma Kit GAHK-20). The content of creatine were analyzed by the a-naphthol-diacetyl method (Wong, 1971), and creatinine by pierate method (Lan, Kao, & Chen, 2004).

HAAs were analyzed after solid-phase extraction using HPLC with UV and fluorescence detectors, as described by Gross and Grüter (1992), with some minor modifications. In addition, dichloromethane/toluene (95:5) was used instead of dichloromethane. To improve chromatographic efficiency, additional purification was carried out on some samples (Solyakov, Skog, & Jägerstad, 1999). The identification of HAAs was carried out by comparing