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.

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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

retention times of unknown peaks in the UV and fluorescence chromatograms with those of reference solutions and internal standard compound. Peaks of identified HAAs were verified by comparing on-line UV spectra library with those of reference solutions. Recoveries were determined by spiking one sample from each cooking session with a standard HAAs mixture. All values reported were corrected for the calculated recovery. Content of each HAAs (ng/g) were calculated as previously reported (Liao, Xu, & Zhou, 2009).

2.5. Statistical analysis

In the present study, a completely randomized design was employed (tree replicates). Means were compared using Duncan's multiple-range test at the significance level of 0.05. All analyses were carried out using the SPSS11.5 software.

3. Results and discussion

The moisture, protein, fat, glucose, creatine, creatinine and pH values of raw and cooked chicken and duck breast are given in Table 1. The compositions of the raw and cooked poultry products were similar to the findings of other researchers (Cobos, Veiga, & Díaz, 2000; Liu, Xu, & Zhou, 2007; Solyakov & Skog, 2002).

While the raw chicken breast had a higher ($P < 0.05$) moisture content than cooked chicken breast, the chicken breast cooked by deep-frying had the lowest ($P < 0.05$) moisture content than those cooked by other methods. Similar trends were showed in the experiment on duck breast. For chicken breast, all cooking methods seemed to result in a higher protein proportion, and the chicken breast cooked by deep-frying had the highest protein content. The protein content in cooked duck breast was significantly increased compared to the raw duck breast, and duck breast cooked by deep-frying had the higher ($P < 0.05$) protein content than those cooked by other methods. As a result of meat cooking, the content of water decreases and the protein proportion increases in general. In this study, the weight losses of cooked poultry were significantly different among the cooking methods, the weight losses of poultry cooked by deep-frying were the highest whilst the lowest for poultry cooked by pan-frying ($P < 0.05$). Therefore, the protein proportion in cooked poultry was higher than that in raw poultry, and the poultry cooked by deep-frying had the highest protein content. However, the contents of fat, glucose, creatine and creatinine in raw poultry were higher than those in cooked poultry, with the exception of fat content in poultry cooked by deep-frying. This can be explained by the dripping of fat and the degradation of glucose, creatine and creatinine during the processing. Also, poultry cooked by deep-frying, where the

poultry is surrounded by hot fat, may increase the content of fat. Glucose, creatine and creatinine were the precursors in HAAs forming reactions. In the present study, the glucose content and the total contents of creatine and creatinine decreased in cooked meat. It was likely due to the reaction with Maillard browning products (Skog & Jägerstad, 1990), or the formation of the HAAs. The pH values of raw chicken and duck breast were 5.93 and 6.10, respectively. The changes in pH of both raw and cooked chicken and duck breast were not significant ($P > 0.05$) as depicted in Table 1.

The effects of cooking methods on the formation of HAAs in poultry meat was investigated, the levels of polar and apolar HAAs in cooked poultry are shown in Tables 2 and 3, respectively. Representative chromatograms from the HPLC analysis of one of the samples of duck breast pan-fried at 180 °C for 10 min are shown in Figs. 1 and 2. No HAAs was detected in the raw poultry meat. Table 2 shows the polar HAAs in cooked chicken and duck breast. Four polar HAAs, including IQ, MeIQx, 4,8-DiMeIQx and PhIP were detected in cooked chicken and duck breast (Table 2). Table 3 shows the apolar HAAs in cooked chicken and duck breast. Six apolar HAAs, including Norharman, Harman, Trp-P-2, Trp-P-1, AaC and MeAaC were detected in cooked chicken and duck breast (Table 3). IQx, MeIQ, 7, 8-DiMeIQx, Glu-P-1, Glu-P-2 were not detected in any cooked poultry samples. The quantification limits for IQ, MeIQx, 4, 8-DiMeIQx, PhIP, Trp-P-1, Norharman, Harman, Trp-P-2, AaC and MeAaC were 0.08 ng, 0.05 ng, 0.05 ng, 2 pg, 0.5 pg, 1 pg, 2 pg, 0.5 pg, 1 pg and 1 pg, respectively. The recovery rates of extraction varied depending on the meat matrix and cooking conditions. Recoveries for the 10 HAAs were as follows: IQ (94.6 ± 2.7%), MeIQx (82.1 ± 2.2%), 4,8-DiMeIQx (86.9 ± 3.5%), PhIP (60.8 ± 5.2%), Trp-P-1 (72.7 ± 4.8%), Norharman (74.0 ± 9.5%), Harman (81.9 ± 8.4%), Trp-P-2 (82.8 ± 9.8%), AaC (76.6 ± 8.8%) and MeAaC (78.0 ± 4.6%). These recoveries matched the range of published results (Lan & Chen 2002; Lan et al. 2004; Salmon, Knize, & Felton, 1997). Tai, Lee, and Chen (2001) reported average recoveries of 73%, 75%, 82% and 85% for AaC, MeAaC, Norharman and Harman, respectively, from fried fish fiber. Toribio, Galceran, and Puignou (2000) reported that the recovery rates for all HAAs are broadly ranged from 5% to 98% because of complex food matrix and several separation steps. The extraction efficiencies were used to correct for incomplete recoveries in samples.

Poultry meat is sometimes referred to as white meat, in contrast to red meat, for example, beef and pork. The main difference is the low fat content in poultry meat, but the amino acid pattern and the content of glucose and creatine also differ (Borgen, Solyakov, & Skog, 2001). PhIP seems to form more easily in chicken than in beef, pork or fish during cooking, while the content of, for example, MeIQx is generally lower in cooked chicken than in cooked beef and pork (Skog, Johansson, & Jägerstad, 1998).Poultry can be pre-

Table 1
Means for moisture, protein, fat, glucose, creatine, creatinine and pH values of raw and cooked chicken and duck breast[A].

Poultry	Condition	Moisture (%)	Protein (%)	Fat (%)	Glucose(umol/g dry matter)	Creatine + creatinine(mg/g dry matter)	pH	Weight loss (%)
Chicken breast	Raw	74.42 ± 0.70[a]	23.69 ± 0.75[d]	3.07 ± 0.96[b]	10.46 ± 2.33[a]	21.74 ± 1.22[a]	5.93 ± 0.06[b]	–
	Pan-frying	69.52 ± 0.81[b]	28.13 ± 0.80[c]	1.37 ± 0.04[c]	8.72 ± 2.49[ab]	14.15 ± 1.04[b]	5.91 ± 0.06[b]	22.28 ± 1.11[d]
	Deep-frying	53.77 ± 1.64[d]	43.69 ± 0.55[a]	4.62 ± 0.63[a]	4.57 ± 0.24[b]	16.10 ± 1.10[b]	6.10 ± 0.11[a]	45.42 ± 0.53[a]
	Charcoal grilling	65.20 ± 0.47[c]	32.77 ± 1.31[b]	1.54 ± 0.02[c]	6.50 ± 3.40[b]	11.25 ± 0.85[c]	6.00 ± 0.06[ab]	31.44 ± 1.16[b]
	Roasting	65.81 ± 0.19[c]	31.41 ± 1.20[b]	1.65 ± 0.07[c]	8.84 ± 2.91[ab]	15.99 ± 1.08[b]	5.84 ± 0.19[b]	28.74 ± 1.43[c]
Duck breast	Raw	76.50 ± 1.21[a]	21.14 ± 0.80[e]	3.11 ± 0.60[b]	23.26 ± 3.76[a]	20.29 ± 1.01[a]	6.10 ± 0.12[a]	–
	Pan-frying	69.44 ± 0.15[b]	27.63 ± 0.25[d]	1.23 ± 0.52[c]	5.82 ± 0.99[c]	14.39 ± 1.29[c]	6.14 ± 0.02[a]	21.90 ± 2.78[c]
	Deep-frying	52.66 ± 1.31[d]	43.79 ± 0.52[a]	5.23 ± 0.54[a]	9.35 ± 2.61[bc]	17.04 ± 0.99[b]	6.09 ± 0.07[a]	44.23 ± 1.23[a]
	Charcoal grilling	65.20 ± 0.42[c]	31.32 ± 0.17[c]	1.81 ± 0.18[c]	10.42 ± 2.66[bc]	11.51 ± 1.07[d]	6.14 ± 0.04[a]	30.74 ± 3.01[b]
	Roasting	65.32 ± 1.62[c]	33.97 ± 1.57[b]	1.81 ± 0.16[c]	12.71 ± 4.46[b]	17.01 ± 0.95[b]	6.13 ± 0.05[a]	29.85 ± 2.03[b]

[A] Values are shown as mean ± standard deviation of three replicates. Symbols bearing different letters in the same column are significantly different ($P < 0.05$).

Table 2
Polar heterocyclic aromatic amines (ng/g) in cooked chicken and duck breast[A].

Poultry	Cooking method	IQ	MeIQx	4,8-DiMeIQx	PhIP
Chicken breast	Pan-frying	1.76 ± 0.68[a]	1.83 ± 0.86[a]	1.05 ± 0.40[b]	18.33 ± 3.63[b]
	Deep-frying	ND	0.77 ± 0.19[a]	0.38 ± 0.15[b]	2.16 ± 0.60[c]
	Charcoal grilling	ND	1.16 ± 0.55[a]	3.55 ± 1.03[a]	31.06 ± 4.53[a]
	Roasting	ND	ND	ND	0.04 ± 0.01[c]
Duck breast	Pan-frying	5.20 ± 1.08[a]	3.44 ± 1.26[a]	2.02 ± 0.68[a]	21.88 ± 3.13[a]
	Deep-frying	ND	0.68 ± 0.14[b]	1.76 ± 0.54[a]	1.47 ± 0.35[c]
	Charcoal grilling	2.74 ± 0.69[a]	2.40 ± 0.69[a]	1.34 ± 0.38[a]	11.80 ± 1.66[b]
	Roasting	ND	ND	ND	ND

ND: Not detected.
[A] Values are shown as mean ± standard deviation of three replicates. Symbols bearing different letters in the same column are significantly different ($P < 0.05$).

Table 3
Apolar heterocyclic aromatic amines (ng/g) in cooked chicken and duck breast[A].

Poultry	Cooking method	Norharman	Harman	Trp-P-2	Trp-P-1	AaC	MeAaC
Chicken breast	Pan-frying	1.41 ± 0.20[c]	2.77 ± 0.40[c]	ND	ND	0.23 ± 0.06[b]	0.02 ± 0.01[b]
	Deep-frying	5.39 ± 1.04[b]	12.32 ± 1.83[b]	ND	ND	0.27 ± 0.11[b]	0.02 ± 0.00[b]
	Charcoal grilling	32.18 ± 3.76[a]	31.67 ± 3.23[a]	3.58 ± 0.62[a]	1.46 ± 0.06[a]	5.58 ± 1.02[a]	1.57 ± 0.54[a]
	Roasting	3.05 ± 0.40[bc]	0.69 ± 0.06[c]	0.02 ± 0.00[b]	0.02 ± 0.01[b]	0.05 ± 0.01[b]	0.05 ± 0.01[b]
Duck breast	Pan-frying	6.15 ± 1.26[a]	12.90 ± 2.09[a]	0.20 ± 0.00[a]	0.05 ± 0.00[a]	1.26 ± 0.21[a]	0.21 ± 0.04[a]
	Deep-frying	3.77 ± 0.78[b]	6.03 ± 1.48[b]	ND	ND	0.14 ± 0.03[c]	0.04 ± 0.01[c]
	Charcoal grilling	4.95 ± 1.25[ab]	7.81 ± 1.23[b]	0.13 ± 0.04[b]	0.04 ± 0.00[a]	0.62 ± 0.02[b]	0.13 ± 0.02[b]
	Roasting	6.12 ± 1.19[a]	0.56 ± 0.04[c]	0.02 ± 0.00[c]	0.01 ± 0.00[b]	0.06 ± 0.01[c]	0.05 ± 0.01[c]

ND: Not detected.
[A] Values are shown as mean ± standard deviation of three replicates. Symbols bearing different letters in the same column are significantly different ($P < 0.05$).
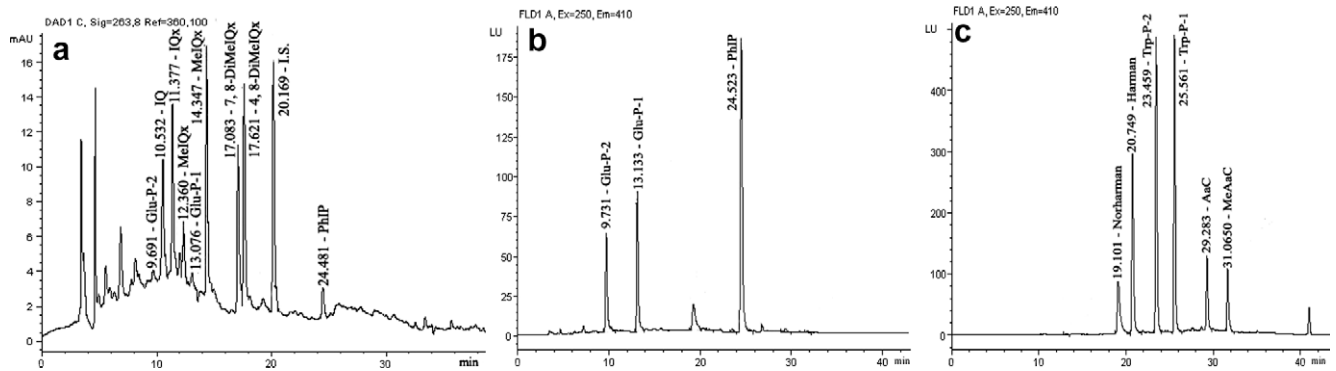

Fig. 1. HPLC chromatograms of heterocyclic aromatic amines (HAAs) extracts from pan-frying duck breast spiked with HAAs standards. (a) Polar extracts of duck breast fried at 180 °C for 10 min spiked with HAAs standards (UV detection). (b) Polar extracts of duck breast fried at 180 °C for 10 min spiked with HAAs standards (fluorescence detection). (c) Apolar extracts of duck breast fried at 180 °C for 10 min spiked with HAAs standards (fluorescence detection).
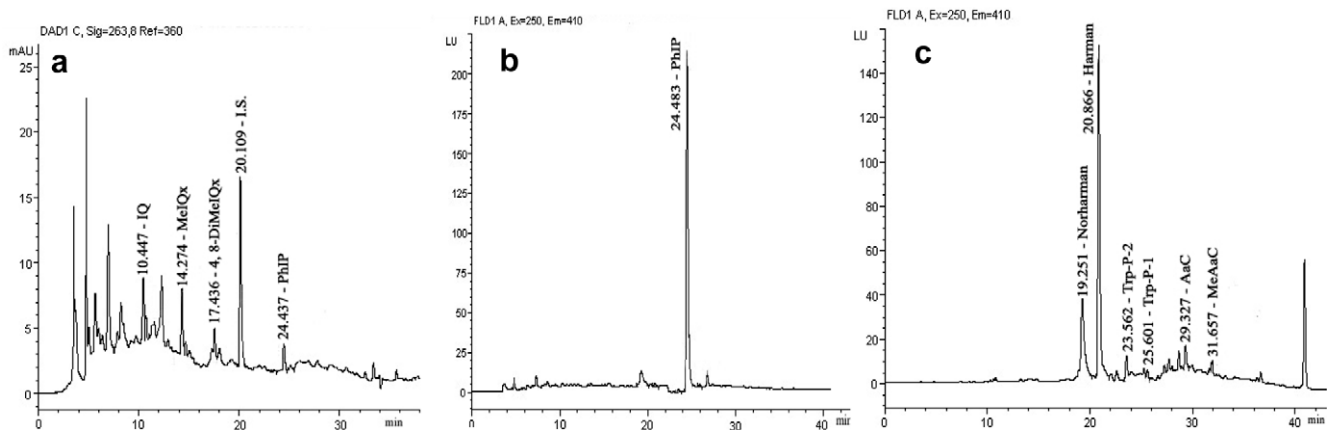

Fig. 2. HPLC chromatograms of HAAs extracts from pan-frying duck breast. (a) Polar extracts of duck breast fried at 180 °C for 10 min (UV detection). (b) Polar extracts of duck breast fried at 180 °C for 10 min (fluorescence detection). (c) Apolar extracts of duck breast fried at 180 °C for 10 min (fluorescence detection).

pared under many different cooking conditions and will thus contain variable levels of HAAs. To date, more than 20 reports on HAAs content in poultry, mainly chicken, have been published (Skog & Solyakov, 2002). To the best of our knowledge, this is the first report on analysis of HAAs in cooked duck meat. Many previous studies have shown that cooking conditions are of crucial importance to the formation of HAAs (Salmon et al., 2006; Solyakov & Skog, 2002).

Pan-frying generally produced MeIQx at levels below 2 ng/g, but there is one report of higher content of MeIQx, as high as 10.4 ng/g (Krul et al., 2000). PhIP is usually found at higher levels than MeIQx in cooked chicken, and there are several reports of PhIP content of around 20 ng/g (Krul et al., 2000; Norrish et al., 1999; Sinha et al., 1995; Solyakov & Skog, 2002). Sinha et al. (1995) reported that the highest content of PhIP (70 ng/g) in pan-fried samples was detected in skinless, boneless chicken breasts, followed by pan-fried turkey breast (64.9 ng/g) (Brockstedt & Pfau, 1998). In addition, a high concentration of AaC, 19 ng/g, was reported in the same pan-fried turkey sample (Brockstedt & Pfau, 1998). In the present study, our data are in the same range as those found in the literature. One gram of pan-fried chicken breast was estimated to contain 2 ng IQ, 2 ng MeIQx, 1.1 ng 4,8-DiMeIQx, 18.3 ng PhIP, 1.4 ng Norharman, 3 ng Harman, 0.2 ng AaC and 0.02 ng MeAaC. While pan-frying of duck breasts produced IQ, MeIQx, 4, 8-DiMeIQx, PhIP, Norharman, Harman, Trp-P-2, Trp-P-1, AaC and MeAaC at 5.2, 3.4, 2, 22, 6.2, 13, 0.2, 0.1, 1.3 and 0.2 ng/g, respectively. The total content of HAAs in duck breast cooked by pan-frying was 53.3 ng/g, which was about two times higher than that in pan-fried chicken breast (27.4 ng/g). For the similar cooking condition, this discrepancy probably results from the differences of precursors (glucose, creatine, creatinine and free amino acids) concentrations in raw poultry (Table 1).

Deep-frying mainly produced traces of MeIQx, 4, 8-DiMeIQx and PhIP, and Harman and Norharman in contents below 1 ng/g (Solyakov & Skog, 2002). In another study, up to 12 different HAAs were identified in chicken legs deep-fried at 100–200 °C for 5–15 min. The content of these HAAs were as high as 1 ng/g, and furthermore the content of both Harman and PhIP were 2–3 ng/g (Chiu, Yang, & Chen, 1998). In our study, Seven HAAs were detected in deep-fried chicken breast. Harman was present at the highest content (12.3 ng/g), followed by Norharman (5.4 ng/g), PhIP (2.2 ng/g), MeIQx (1 ng/g), 4, 8-DiMeIQx (0.4 ng/g), AaC (0.3 ng/g) and MeAaC (0.02 ng/g). The total content of HAAs in deep-fried chicken breast was 21.3 ng/g. The types and content of HAAs formed in deep-fried duck breast were similar to those formed in deep-fried chicken breast.

Charcoal grilling is a common method of poultry preparation. After charcoal grilling for 20 min, the chicken breast was well done, and nine HAAs were identified. Norharman was present at the highest content (32.2 ng/g), followed by Harman (32 ng/g), PhIP (31.1 ng/g), AaC (6 ng/g), Trp-P-2 (4 ng/g), 4,8-DiMeIQx (4 ng/g), MeAaC (2 ng/g), Trp-P-1 (2 ng/g), and MeIQx (1.2 ng/g). The total content of HAAs in charcoal grilled chicken breast was 112 ng/g. While charcoal grilling of duck breast produced ten HAAs, including PhIP (12 ng/g), Harman (8 ng/g), Norharman (5 ng/g), IQ (3 ng/g), MeIQx(2.4 ng/g), 4,8-DiMeIQx (1.3 ng/g), AaC (1 ng/g), MeAaC (0.1 ng/g), Trp-P-2 (0.1 ng/g) and Trp-P-1 (0.04 ng/g). The total content of HAAs in charcoal grilled duck breast was 32 ng/g, and less than that in charcoal grilled chicken breast. The level of HAAs agrees well with results from another study (Solyakov & Skog, 2002). In most reports, the content of MeIQx are below 3 ng/g and PhIP below 40 ng/g. Extremely high content of MeIQx, over 100 ng/g, were reported in chicken breasts prepared over a gas flame for 6 min (Holder, Preece, Conway, Pu, & Doerge, 1997), but these experiments were probably performed at very high temperatures. PhIP was detected at levels up to 480 ng/g in chicken

breasts barbecued at 177–260 °C (Sinha et al., 1995), 330 ng/g in chicken barbecued at 339–365 °C (Salmon et al., 1997), and 270 ng/g in grilled chicken breast (Knize, Salmon, Hopmans, & Felton, 1997). High content of AaC have been reported: 180.4 ng/g in chicken grilled at "high-heat" settings (Matsumoto, Yoshida, & Tomita, 1981), 170 ng/g in chicken grilled at temperatures exceeding 350 °C (Knize et al., 1996), and over 100 ng/g in chicken slices prepared over a naked gas flame (Totsuka et al., 1999). The latter study also showed remarkably high levels of Harman and Norharman (Totsuka et al., 1999), but no information was given on cooking temperature or time.

Roasting does not seem to result in the formation of high content of HAAs. The highest values reported are 3.2 ng/g for MeIQx and 5.3 ng/g for PhIP (Richling, Häring, Herderich, & Schreier, 1998). When chicken was prepared in a clay pot or a roasting bag in the oven, at 200 °C for 25 min, the only HAAs detected were Harman and Norharman (Solyakov & Skog, 2002). On the other hand, when chicken breast and chicken thigh were heated at 275 °C for 30 min in a lab furnace, the content of MeIQx did not exceed 0.5 ng/g, while the content of PhIP were 37.5 ng/g (breast) and 8.0 ng/g (thigh) (Pais, Salmon, Knize, & Felton, 1999). However, such a cooking method is probably not employed in normal food preparation. In this study, roasted chicken breast was found to contain seven apolar HAAs. Norharman was present at highest concentration (5 ng/g), followed by Harman (1 ng/g), and the contents of the other HAAs were below 0.1 ng/g. While duck breast cooked by roasting only produced six apolar HAAs, including Norharman (6.1 ng/g), Harman (1 ng/g), AaC (0.1 ng/g), MeAaC (0.1 ng/g), Trp-P-2 (0.02 ng/g), and Trp-P-1 (0.01 ng/g). The total content of HAAs in roasted chicken breast (4 ng/g) was less than that of roasted duck breast (6.8 ng/g).

4. Conclusions

In conclusion, the presented study shows that the content of HAAs in cooked poultry products varies with cooking methods and cooking conditions. Different types and content of analyzed HAAs were produced in chicken and duck breast cooked by pan-frying, deep-frying, charcoal grilling and roasting. Charcoal grilling and pan-frying are the common methods of poultry preparation that may produce higher content of HAAs than other methods. The data from this study could be used to assess the human intake of HAAs and also to identify conditions that minimise the formation and intake of HAAs. Further research is necessary to determine the content of HAAs in commercial poultry production.

Acknowledgements

References

Abdulkarim, B. G., & Smith, J. S. (1998). Heterocyclic amines in fresh and processed meat products. *Journal of Agricultural and Food Chemistry, 46*(11), 4680–4687.

Borgen, E., Solyakov, A., & Skog, K. (2001). Effects of precursor composition and water on the formation of heterocyclic amines in meat model systems. *Food Chemistry, 74*(1), 11–19.

Brockstedt, U., & Pfau, W. (1998). Formation of 2-amino-carbolines in pan-fried poultry and ^{32}P-postlabelling analysis of DNA adducts. *Zeitschrift fur Lebensmittel Untersuchung und – Forschung A, 207*(6), 472–476.

Chiu, C. P., Yang, D. Y., & Chen, B. H. (1998). Formation of heterocyclic amines in cooked chicken legs. *Journal of Food Protection, 61*(6), 712–719.

Cobos, Á., Veiga, A., & Díaz, O. (2000). Chemical and fatty acid composition of meat and liver of wild ducks (*Anas platyrhynchos*). *Food Chemistry, 68*(1), 77–79.

de Meester, C. (1995). Genotoxic potential of beta-carbolines: A review. *Mutation Research, 339*(3), 139–153.

Felton, J. S., Jägerstad, M., Knize, M. G., Skog, K., & Wakabayashi, K. (2000). Contents in food, beverages and tobacco. In M. Nagao & T. Sugimura (Eds.), *Food borne*

carcinogens: Heterocyclic amines (pp. 31–72). Chichester, West Sussex: John Wiley & Sons.

Felton, J. S., & Knize, M. G. (1990). Heterocyclic amine mutagens/carcinogens in foods. In C. S. Copper & P. L. Grover (Eds.), Handbook of experimental pharmacology (pp. 471–502). Berlin: Springer-Verlag.

Gross, G. A., & Grüter, A. (1992). Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. Journal of Chromatography, 592(1–2), 271–278.

Holder, C. L., Preece, S. W., Conway, S. C., Pu, Y. M., & Doerge, D. R. (1997). Quantification of heterocyclic amine carcinogens in cooked meats using isotope dilution liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 11(15), 1667–1672.

IARC (1993). IARC monographs on the evaluation of carcinogenic risks to humans. No. 56. Some naturally occurring substances: Food items and constituents. Heterocyclic aromatic amines and mycotoxins. International Agency for Research on Cancer, Lyon.

ISO 1443:1973 (E) (1973). Meat and meat products –Determination of total fat content. International standard, 1st ed.

ISO 937:1978 (E) (1978). Meat and meat products –Determination of nitrogen content (reference). International standard, 1st ed.

ISO 1442:1997 (E) (1996). Meat and meat products –Determination of moisture content. International standard, 2nd ed.

ISO 2917:1999 (E) (1999). Meat and meat products – Measurement of pH-reference method. International standard, 2nd ed.

Jägerstad, M., Laser Reutersward, A., Olsson, R., Grivas, S., Nyhammar, T., Olsson, K., et al. (1983). Creatin(in)e and Maillard reaction products as precursors of mutagenic compounds: Effects of various amino acids. Food Chemistry, 12(4), 255–264.

Knize, M. G., Dolbeare, F. A., Carroll, K. L., Moore, D. H., & Felton, J. S. (1994). Effects of cooking time and temperature on the heterocyclic amine content of fried beef patties. Food and Chemical Toxicology, 32(7), 595–603.

Knize, M. G., & Felton, J. S. (2005). Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors in meat. Nutrition Reviews, 63(5), 158–165.

Knize, M. G., Salmon, C. P., Hopmans, E. C., & Felton, J. S. (1997). Analysis of foods for heterocyclic aromatic amine carcinogens by solid-phase extraction and high-performance liquid chromatography. Journal of Chromatography A, 763(1–2), 179–185.

Knize, M. G., Sinha, R., Salmon, C. P., Mehta, S. S., Dewhirst, K. P., & Felton, J. S. (1996). Formation of heterocyclic amine mutagens/carcinogens during home and commercial cooking of muscle foods. Journal of Muscle Foods, 7(3), 271–279.

Krul, C., Luiten-Schuite, A., Baandagger, R., Verhagen, H., Mohn, G., Feron, V., et al. (2000). Application of a dynamic in vitro gastrointestinal tract model to study the availability of food mutagens, using heterocyclic aromatic amines as model compounds. Food and Chemical Toxicology, 38(9), 783–792.

Kuhn, W., Muller, T., Grosse, H., & Rommelspacher, H. (1996). Elevated levels of Harman and Norharman in cerebrospinal fluid of parkinsonian patients. Journal of Neural Transmission, 103(12), 1435–1440.

Lan, C. M., & Chen, B. H. (2002). Effect of soy sauce and sugar on the formation of heterocyclic amines in marinated foods. Food and Chemical Toxicology, 40(7), 989–1000.

Lan, C. M., Kao, T. H., & Chen, B. H. (2004). Effect of heating time and antioxidants on the formation of heterocyclic amines in marinated foods. Journal of Chromatography B, 802(1), 27–37.

Liao, G. Z., Xu, X. L., & Zhou, G. H. (2009). Effects of cooked temperatures and addition of antioxidants on formation of heterocyclic aromatic amines in pork floss. Journal of Food Processing and Preservation, 33(2), 159–175.

Liu, Y., Xu, X. L., & Zhou, G. H. (2007). Changes in taste compounds of duck during processing. Food Chemistry, 102(1), 22–26.

Matsumoto, T., Yoshida, D., & Tomita, H. (1981). Determination of mutagens, amino-alpha-carbolines in grilled foods and cigarette smoke condensate. Cancer Letters, 12(1–2), 105–110.

Nagao, M. (1999). A new approach to risk estimation of food-borne carcinogen-heterocyclic amines-based on molecular information. Mutation Research, 431(1), 3–12.

Nagao, M., & Sugimura, T. (2000). Food borne carcinogens, heterocyclic amines. West Sussex, UK: Wiley.

Nagao, M., Yahagi, T., & Sugimura, T. (1978). Differences in effects of Norharman with various classes of chemical mutagens and amounts of S-9. Biochemical and Biophysical Research Communications, 83(2), 373–378.

Norrish, A. E., Ferguson, L. R., Knize, M. G., Felton, J. S., Sharpe, S. J., & Jackson, R. T. (1999). Heterocyclic amine content of cooked meat and risk of prostate cancer. Journal of the National Cancer Institute, 91(23), 2038–2044.

Pais, P., Salmon, C. P., Knize, M. G., & Felton, J. S. (1999). Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. Journal of Agricultural and Food Chemistry, 47(3), 1098–1108.

Richling, E., Häring, D., Herderich, M., & Schreier, P. (1998). Determination of heterocyclic aromatic amines (HAA) in commercially available meat products and fish by high performance liquid chromatography electrospray tandem mass spectrometry (HPLC–ESI–MS–MS). Chromatographia, 48(3–4), 258–262.

Salmon, C. P., Knize, M. G., & Felton, J. S. (1997). Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. Food and Chemical Toxicology, 35(5), 433–441.

Salmon, C. P., Knize, M. G., Felton, J. S., Zhao, B., & Seow, A. (2006). Heterocyclic aromatic amines in domestically prepared chicken and fish form Singapore Chinese households. Food and Chemical Toxicology, 44(4), 484–492.

Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P., Knize, M. G., Swanson, C. A., et al. (1995). High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-b] pyridine (PhIP) occur in chicken but are dependent on the cooking method. Cancer Research, 55(20), 4516–4519.

Skog, K., & Jägerstad, M. I. (1990). Effect of monosaccharides and disaccharides on the formation of food mutagens in model systems. Mutation Research, 230(2), 263–272.

Skog, K., Johansson, M., & Jägerstad, M. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: A review on formation, occurrence and intake. Food and Chemical Toxicology, 36(9–10), 879–896.

Skog, K., & Solyakov, A. (2002). Heterocyclic amines in poultry products: A literature review. Food and Chemical Toxicology, 40(8), 1213–1221.

Solyakov, A., & Skog, K. (2002). Screening for heterocyclic amines in chicken cooked in various ways. Food and Chemical Toxicology, 40(8), 1205–1211.

Solyakov, A., Skog, K., & Jägerstad, M. (1999). Heterocyclic amines in process flavours, process flavour ingredients, bouillon concentrates and a pan residue. Food and Chemical Toxicology, 37(1), 1–11.

Sugimura, T. (1997). Overview of carcinogenic heterocyclic amines. Mutation Research, 376(1–2), 211–219.

Sugimura, T. (2000). Nutrition and dietary carcinogens. Carcinogenesis, 21(3), 387–395.

Sugimura, T., Nagao, M., Kawachi, T., Honda, M., Yahagi, T., Seino, Y., et al. (1977). Mutagens–carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. In H. H. Hiatt, J. D. Watson, & J. A. Winsten (Eds.), Origins of human cancer (pp. 1561–1577). New York: Cold Spring Harbor Laboratory.

Sugimura, T., Nagao, M., & Wakabayashi, K. (1982). Metabolic aspects of the comutagenic action of Norharman. Advances in Experimental Medicine and Biology, 136B, 1011–1025.

Tai, C. Y., Lee, K. H., & Chen, B. H. (2001). Effect of various additives on the formation of heterocyclic amines in fried fish fibre. Food Chemistry, 75(3), 309–316.

Toribio, F., Galceran, M. T., & Puignou, L. (2000). Separation of heterocyclic aromatic amines in food products. Journal of Chromatography B. Biomedical Sciences and Applications, 747(1–2), 171–202.

Totsuka, Y., Ushiyama, H., Ishihara, J., Sinha, R., Goto, S., Sugimura, T., et al. (1999). Quantification of the co-mutagenic beta-carbolines, Norharman and Harman, in cigarette smoke condensates and cooked foods. Cancer Letters, 143(2), 139–143.

Wong, T. (1971). Creatine determination by a-naphtnol-diacetyl reaction. Analytical Biochemistry, 40(2), 18–28.