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Occurrence of Heterocyclic Amines in Cooked Meat Products

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ABSTRACT

Heterocyclic amines (HCAs), potent mutagens and a risk factor for human cancers, are produced in meats cooked at high temperature. The aim of this study was to determine the HCA content in cooked meat products (beef, chicken, pork, fish) prepared by various cooking methods (pan frying, oven broiling, and oven baking at 170 to 230 °C) that are preferred by U.S. meat consumers. The primary HCAs in these samples were PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine) (1.49-10.89 ng/g), MeIQx (2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline) (not detected-4.0 ng/g), and DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-*f*]quinoxaline) (not detected-3.57 ng/g). Type and content of HCAs in cooked meat samples were highly dependent on cooking conditions. The total HCA content in well-done meat was 3.5 times higher than that of medium-rare meat. Fried pork (13.91 ng/g) had higher levels of total HCAs than fried beef (8.92 ng/g) and fried chicken (7.00 ng/g). Among the samples, fried bacon contained the highest total HCA content (17.59 ng/g).

Keywords: heterocyclic amines, cooking, beef, pork, chicken, fish

1. Introduction

Heterocyclic amines (HCAs) are mutagenic and carcinogenic compounds that are present at parts per billion levels in cooked muscle foods, mainly meat and fish, via the Maillard reaction with create(ni)ne, amino acids, and sugars as the precursors (Janoszka, Blaszczyk, Damasiewicz-Bodzek, & Sajewicz, 2009; Pais, Salmon, Knize, & Felton 1999; Sugimura 2002). More than 25 HCAs have been isolated from different cooked muscle foods; however, the most common HCAs found in foods are the thermic HCAs, which include 2-amino-3-methyl-imidazo [4,5-*f*]quinoline (IQ), 2-amino-3-methylimidazo [4,5-*f*]quinoxaline (IQx), 2-amino-3,4-dimethylimidazo [4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx), DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-*f*]quinoxaline), and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) (Knize, Dolbeare, Carroll, Moore, & Felton, 1994). These HCAs are listed in the U.S. Department of Health and Human Services's 11th Report of Carcinogens (2005) as compounds *reasonably anticipated to be a human carcinogen*. The International Agency for Research on Cancer (1993) categorized MeIQ, MeIQx, and PhIP as *reasonably anticipated to be a human carcinogen* and IQ as a *probable human carcinogen*. The epidemiological studies over the past 10 years have shown that high intake of well-done meat and high exposure to meat carcinogens, particularly HCAs, may increase the risk of stomach, colon, and breast cancers in humans (Kampman, Slattery, Bigler, Leppert, & Samowitz, 1999).

The concentration and type of HCAs formed in thermally treated meat and fish depend on many factors including cooking method, cooking time and temperature, the concentration of precursors, and presence of water and fat in the raw product (Janoszka et al., 2009). The content of HCAs formed increases with increasing temperature and time (Knize et al., 1994). High

cooking loss is related to the formation of large contents of HCAs (Knize et al., 1994; Skog, Steineck, Augustsson, & Jägerstad, 1995), and the content of cooking loss during cooking depends on several factors including the muscle tension and direction of muscle fibers (Pais et al., 1999). Many cooking methods, including frying, roasting, smoking, broiling, and baking have been reported to induce HCA formation, and the type HCAs formed can be different for various cooking methods (Chen & Chiu, 1998). For example, IQ, MeIQx, PhIP were detected in broiled beef, whereas MeIQx and DiMeIQx were detected in fried ground beef (Starvic, 1994).

The studies on HCA levels in cooked meat products have yielded inconsistent results, and there are gaps in the available HCA data. It is difficult to directly compare results between studies because of the differences in food items, cooking procedures (cooking methods, cooking levels, fat or oil usage, frequency of turning), and food preparation. In some previous studies, samples were cooked at high temperature or for a long time; these cooking conditions exceed those needed to produce acceptable cooked meat products (Murkovic, Friedrich, & Pfannhauser, 1997; Pais et al., 1999). Reports from some previous studies did not include the information on internal temperature of the cooked samples (Janoszka et al., 2009; Jo, Sim, Lee, Ryeom, & Myung, 2008; Murkovic et al., 1997; Oz, Kaban, & Kaya, 2007). Internal temperature is usually used to evaluate the safety of cooked meat products. Collecting this type of data would allow researchers to better monitor HCA levels in meat products cooked under normal household conditions and develop more accurate estimates of human HCA exposure. The main objective of the study was to determine HCA contents of the major categories of cooked meat products prepared with various cooking methods that are preferred by U.S. meat consumers. These data can be combined with food consumption survey data to estimate exposure to HCAs due to meat

consumption.

2. Materials and Methods

2.1. Chemicals

The HCA standards IQ (2-amino-3-methyl-imidazo [4,5-*f*]quinoline), IQx (2-amino-3-methyl-imidazo [4,5-*f*]quinoxaline), MeIQ (2-amino-3,4-dimethyl-imidazo [4,5-*f*]quinoline), MeIQx (2-amino-3,8-dimthylimidazo [4,5-*f*]quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-*f*]quinoxaline, TriMeIQx (2-amino-3,4,7,8-tetramethyl-imidazo [4,5-*f*]quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine were obtained from Toronto Research Chemicals (Toronto, Canada). Ammonium acetate and triethylamine were purchased from Aldrich Chemicals (Milwaukee, WI, USA). Phosphoric acid was obtained from Sigma Chemicals (St. Louis, MO, USA). Deionized water was processed by a Sybron/Branstead PCS unit (Barnstead/Thermolyne, Dubuque, IA, USA). The solid-phase extraction Extrelut NT 20 columns and diatomaceous earth refill material were purchased from VWR International (Bristol, CT, USA). Bond Elut propyl-sulfonic acid (PRS) cartridges, C-18 cartridges, and coupling adaptors were purchased from Varian Sample Preparation (Harbor City, CA, USA). Trichloroacetic acid, diacetyl, 1-napthol, and NaOH were obtained from Sigma Aldrich (St. Louis, MO, USA). Solvents and chemicals such as acetonitrile (HPLC grade), methanol (HPLC grade), and sodium hydroxide (ACS-grade) were purchased from Fisher Scientific (Fairlawn, NJ, USA).

84 2.2. Fresh meat samples

85 The following fresh meat samples were purchased from local grocery stores: consisting
86 of beef (top loin, round tip, and ground beef), pork (top loin, ground pork, and bacon), chicken
87 (breast without skin, breast with skin, thigh without skin, and thigh with skin), and fish (catfish,
88 salmon, and tilapia).

89

90 2.3. Chemical analyses

91 The pH of uncooked samples was measured according to the method of Jang et al. (2008)
92 with slight modifications. Five grams of fine ground sample was added to 45 mL of distilled
93 water and blended for 30 s at medium speed in a Waring blender (Waring Laboratory,
94 Torrington, CT, USA). The pH of each sample was measured with an Accumet AP115 portable
95 pH meter (Fisher, Pittsburgh, PA, USA).

96 Fat and moisture for each sample were determined by rapid microwave drying and
97 nuclear magnetic resonance using the CEM Smart Trac system (CEM Corporation, Matthews,
98 NC, USA). Crude protein was determined with a LECO FP-2000 protein analyzer (Leco Corp, St
99 Joseph, MI, USA).

100 Creatine content was determined according to the method described by Polak, Došler,
101 Žlender, and Gašperlin (2009) with slight modifications. A 0.25-g finely ground sample was
102 homogenized for 5 min at 9500 rpm (IKA, Ultra-Turrax T18, Wilmington, NC, USA) in 100 mL
103 trichoroacetic acid (30 g/L in distilled water), and then the samples were filtered through
104 Whatman #4 filter paper. Twenty milliliters of the filtrate was defatted with 10 mL diethylether,
105 and then samples were shaken vigorously and allowed to stand for 10 min to separate the phases.

After the phases were separated, 4 mL of defatted extract (bottom layer) was mixed with 2 mL of diacetyl (0.2 g/L in distilled water) and 2 mL of 1-napthol (25 g/L in 20 g/L of sodium hydroxide solution). The mixture heated for 5 min at 40 °C. Each sample's absorbance was measured at 520 nm against a reagent blank. The creatine content was expressed as milligrams per gram of meat sample. The chemical analyses of raw meat samples are summarized in Table 1.

2.5. Cooking procedure and cooking loss

Fresh meat products were removed from refrigerator and allowed to approach room temperature before they were cooked (Infrared thermometer model ST20XE, Raytek, Santa Cruz, CA, USA). Spear-point thermocouple temperature probes were inserted horizontally to the midpoint of samples, and temperature was monitored with a data logger (USB-TC model, Measurement Computing, Norton, MA, USA). Each meat sample was cooked as described in Table 2. All meats were cooked one sample at a time, except for fried bacon, which was cooked three slices at a time. No salt, spice, food additive, or oil was used in the cooking procedures. Cooked sample were allowed to cool at room temperature for approximately 30 min, and then cooking loss was determined using the following equation:

$$\% \text{ cooking loss} = [(\text{before cook weight} - \text{after cook weight})/\text{before cook weight}] \times 100$$

The cooking information for each type of meat consists of the data for uncooked meat (weight of raw meat, thickness of raw meat), and cooking data (desired cooking internal temperature, cooking temperature, cooking time, and cooking loss); this information is summarized in Table 3. After samples were cooled at room temperature, they were refrigerated

overnight. For the samples of chicken with skin, the skin was removed from the muscle. Approximately 2 mm of the surface was removed from chilled meats with a commercial-grade meat slicer (Cebela's commercial grade slicer, 1/3 hp, Sidney, NE, USA). The meat surface was ground and homogenized with a food processor (KitchenAid, model KFP 750), and refrigerated at 4 °C, and extraction of HCAs in meat samples was performed on the next day.

2.6. Extraction and analysis of HCAs

The HCAs were extracted from meat samples and purified using the method described by Gross and Grüter (1992) except that ethyl acetate was used as the extraction solvent (Puangsombat & Smith, 2010; Santos et al., 2004; Smith, Ameri, & Gadgil, 2008). Each sample (3 g) was homogenized with 12 mL of 1 M NaOH in a commercial Waring blender (Fisher, Pittsburgh, PA, USA). The homogenate was then mixed with 24 g of Extrelut refill material (Merck, Darmstadt, Germany) and poured into an empty Extrelut 20 column. For determination of recovery, selected homogenate samples were spiked with 50 ng of each of the HCA standards. The HCAs were eluted from the Extrelut columns with 60 mL ethyl acetate into a PRS cartridge conditioned with 7 mL of ethyl acetate. The PRS cartridge was then rinsed with 6 mL of 0.1 M HCl, 15 mL of methanol/0.1 M HCl (45:55 v/v), and 2 mL of distilled water to wash out the nonpolar HCAs and other impurities. The HCAs were eluted from the PRS cartridge with 20 mL of 0.5 M ammonium acetate pH 8.5 into 100-mg C-18 cartridges preconditioned with 5 mL of methanol followed by 5 mL of distilled water. The HCAs were then eluted from the C-18 cartridge with 1 mL of methanol/ammonium hydroxide (9:1, v/v) into the vial. The HCA extract was concentrated until dry under a stream of nitrogen and dissolved in 25 µL of methanol before

it was injected into the HPLC. The HCAs were analyzed on an HP1090A Series II HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled with a photodiode array UV-visible detector (HP 1040) and an HP 1046A programmable fluorescence detector. The HCA separation was performed on a reversed-phase TSK gel ODS-80 TM column (25 cm × 4.6 mm, 5 µm, 80 Å, Tosohass, Montgomeryville, PA, USA) with a mobile phase of 0.01 M triethylamine pH 3.6 (A) and acetonitrile (B). The HCA separation was achieved using a linear gradient that started with 95%A and 5% B and changed to 75% A and 25% B in 30 min at a flow rate of 1 mL/min and a column temperature of 40 ºC. After 30 min, the mobile phase returned to its original ratio (95% A, 5 % B) for 10 min to allow the column to equilibrate before the next injection. The UV detector was set at 252 nm for IQ, IQx, MeIQ, MeIQx, and DiMeIQx, and the fluorescence detector was programmed accordingly to the excitation/emission wavelengths of 229 and 437 nm for PhIP. Data were analyzed with an HP 9000 series 300 ChemStation. The identities of HCA peaks were confirmed by comparing the retention times and the UV absorbance spectrum of each peak with library spectra acquired from standard solutions.

2.6. Quantitation, recovery, and spectral matching

The HCA concentrations were quantitated by the internal standard method to compensate for variations in injection volume and also for small changes in detector sensitivity that might occur (Lindsay, 1992). A known content of TriMeIQx (used as internal standard) was added to samples before they were injected into the HPLC. The limit of detection for the HCAs was 0.5 ng/mL for IQ, IQx, MeIQ, MeIQx, and PhIP. The HCA identities were verified in the cooked meat extracts by online UV spectral matching to a spectral library made from pure standards.

Match factors typically were observed at 95% or greater (Tsen, Ameri, & Smith, 2006). Average recoveries for the HCAs were 72% for IQx, 61% for IQ, 63% for MeIQ, 68% for MeIQx, 60% for DiMeIQx, and 65% for PhIP. The HCA identities were verified in the cooked meat extracts by online UV spectral matching to a spectral library made from pure standards. Match factors typically were observed at 95% or greater (Puangsombat & Smith, 2010). Average recoveries for the HCAs were 72% for IQx, 61% for IQ, 63% for MeIQ, 68% for MeIQx, 60% for DiMeIQx, and 65% for PhIP. The recoveries of MeIQx and PhIP are in agreement with previous reports from this laboratory (Puangsombat & Smith, 2010; Smith et al., 2008; Tsen et al., 2006) and from Cheng et al. (2007).

2.7. Statistical analyses

The experimental design was a randomized complete block with repeated measurements, and each experiment was replicated four times. Duplicate measurements taken on the same experimental unit were averaged for statistical analysis. All statistical significance tests were analyzed using SAS version 9.1. Data were examined by analysis of variance (ANOVA) followed by Tukey's multiple comparison test (Tukey, 1993), and means were considered significant at $p < 0.05$.

3. Results and discussion

The choice of meat samples in our study was based on a previous internet-based survey of U.S. consumers' preference for method of cooking and degree of doneness of meat and fish. The survey was conducted by Exponent, Inc. developed to assess customer's HCA intake

(unpublished data). Meat samples selected for the present study included beef (fried beef and broiled beef cooked to medium-rare and well-done, baked beef, and fried beef patty), pork (fried pork, baked pork, fried pork patty, and fried bacon), chicken (fried-chicken breast and fried-chicken thigh with skin and without skin), and fish (fried and baked catfish, salmon, and tilapia).

3.1. Chemical analyses

Table 2 summarizes the results of chemical analyses in the selected fresh meat products. The pH of beef samples (5.47 to 5.89) was lower than that of pork samples (6.01 to 6.71) and chicken samples (6.19 to 6.70); fish samples had the highest pH (6.94 to 7.91). The moisture level of fresh meat products ranged between 69 to 82%, except in the high fat parts (bacon, breast skin, and thigh skin), which contained low moisture levels (approximately 37%). The fat levels of raw meat samples ranged from 1.07 to 53.67%; tilapia contained the lowest content of fat and skin of chicken breast contained the highest contents of fat. The protein levels of raw meat samples ranged from 9.04 to 23.37%; chicken thigh skin contained the lowest content of protein, and skin of chicken breast contained the highest content of protein. Creatine in the uncooked meat samples ranged from 1.02 to 2.95 mg/g. There was not much difference in creatine level among these samples.

3.2. Identification and quantification of HCAs

Because consumption of undercooked meat and fish has been linked epidemiologically to foodborne outbreaks, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS, 1998) has established guidelines for both consumers and the food service industry

for safe handling and preparation of cooked meat and fish. USDA-FSIS recommends a minimum instantaneous internal cooking temperature of 63 °C (145 °F) for beef steak and fish, 71 °C (160 °F) for pork and ground beef, and 74 °C (165 °F) for chicken. According to the survey by Exponent Inc. (unpublished data), people mostly consume meat and fish that are cooked until their internal temperatures reach the minimum temperature recommended by USDA-FSIS, except for steak and bacon. Two doneness levels of steak, medium-rare and well-done, are most often consumed. In our study, all samples except beef steak and bacon were cooked until their internal temperature reached the temperatures recommended by USDA-FSIS. Fried and broiled beef steak samples were cooked until their internal temperature reached 57 °C (135 °F) for medium-rare and 71 °C (160 °F) for well-done. Fried bacon was cooked at 172 °C for 3 min on each side (three slices at a time) as recommended to minimize carcinogenic nitrosamine level (Ikins et al., 1986). We investigated the presence of five HCAs (IQ, IQx, MeIQ, MeIQx, and PhIP) that can be found in cooked meat products and have been commonly studied and reported in many papers. Level of HCAs in each sample was analyzed the outer layer (2 mm) of meat samples to increase analysis sensitivity because HCAs are mainly present on the outer surface (Busquets, Bordas, Toribio, Puignou, & Galceran, 2004). The content of HCAs on the surface was then used to calculate the content of HCAs in the whole cooked meat samples (Busquets et al., 2004). The values were corrected for incomplete recovery. To our knowledge, this is the first report of HCA contents in food samples commonly consumed in the U.S. and prepared by domestic cooking procedures to internal temperatures recommended by USDA to eliminate foodborne illness.

HCAs were extracted and quantified by HPLC. The UV and FLD chromatograms of

HCA standards, sample, and spiked sample are shown in Figure 1. The quantitative analyses of HCAs in cooked meat products are summarized in Tables 4 to 8. The total content of HCAs ranged from 1.72 ng/g (medium-rare broiled-beef) to 17.59 ng/g (fried-bacon). In all meat samples, PhIP was found at the highest level (1.49 to 10.89 ng/g), followed by MeIQx (not detected to 4.00 ng/g), DiMeIQx (not detected to 3.57 ng/g), and IQx (not detected to 3.11 ng/g); neither IQ nor MeIQ was found in any sample. The highest level of PhIP was found in fried tilapia (10.89 ng/g), followed by fried pork (9.20 ng/g). IQx was not found except in fried bacon (3.11 ng/g) and baked fish (0.38 to 0.85 ng/g).

We investigated the occurrence of HCAs for three types of fried meat (beef, pork, chicken) and the results of the HCA quantitative determinations are summarized in Table 4. All of these samples contained MeIQx, DiMeIQx, and PhIP. Although the target internal temperatures of the meat samples fried at 204 °C were slightly different, total HCAs in fried pork (13.91 ng/g, PhIP accounting for 9.20 ng/g) were significantly higher than those in fried beef (8.92 ng/g, PhIP accounting for 6.60 ng/g) and fried chicken (7.06 ng/g, PhIP accounting for 6.06 ng/g). There were no significant differences in total HCAs between fried beef and fried pork ($p > 0.05$). This is in agreement with results of Skog, Augustsson, Steineck, Stenberg, and Jägerstad (1997) who reported higher contents of total HCAs in fried pork (21.3 ng/g) than fried chicken breast (10.7 ng/g) when cooked at 225 °C. In contrast, our result is not in agreement with data from Pais et al. (1999), who reported that total HCAs were higher for chicken (38.2 ng/g) than for pork (8.6 ng/g) and beef (2.83 ng/g) when cooked at 275 °C for 30 min. Also, Iwasaki et al. (2010) reported a lower content of total HCAs in fried chicken (1.01 ng/g), fried pork (0.5 ng/g), and fried beef (0.1 ng/g) when cooked to well-done (internal temperature 75 °C

for chicken, 88 °C for pork, and 78 °C for beef). The inconsistent results could be due to different cooking methods, different weight/thickness of meat samples, and different ways of preparing meat before cooking, as well as the efficiencies of heat transfer. The high level of HCAs in the fried pork in present study is an important finding because of the three meats studied, the consumption of pork is growing the fastest (1.6 % annually) (FAPRI 2010 U.S. and world agricultural outlook, 2010).

Table 5 shows the HCA levels of cooked chicken samples. MeIQx, DiMeIQx, and PhIP were detected in all chicken samples and the values were in agreement with the HCA found in fried chicken reported by Solyakov and Skog (2002) and Liao, Wang, Xu, and Zhou (2010). All chicken samples had more PhIP than MeIQx and DiMeIQx. For the chicken samples without skin, total HCA levels in the chicken breasts (7.06 ng/g) were higher than those in the chicken thighs (5.58 ng/g); this may be because the weight of raw chicken breast (250 to 280 g) was higher than that of raw chicken thigh (140 to 180 g), therefore, a longer cooking time was needed for chicken breast to reach the same internal temperature of 74 °C, leading to higher cooking loss (Table 2) and increasing the level of HCAs. This result is in agreement with data by Pais et al. (1999), who reported that chicken breast had more total HCAs (38.2 ng/g) than chicken thigh (8.07 ng/g). For the chicken samples with skin (both breasts and thighs), the meat and skin were analyzed separately. MeIQx, DiMeIQx, and PhIP levels in the skin were much higher than the levels detected in the meat ($p < 0.05$). Total HCAs were 7.06 ng/g for skin and 2.89 ng/g for meat in chicken breast and 4.87 ng/g for skin and 2.07 ng/g for meat in chicken thigh. The cooking loss of chicken breast with skin (24.39%) was lower than that of chicken breast without skin (27.88%); the cooking loss of chicken thigh with skin (22.74%) was lower than that of

chicken thigh without skin (24.96%) (Table 2). This suggests that the skin present at the surface acts as an insulating layer for the meat and can help retain moisture during frying, thus decreasing HCA formation. This is in agreement with results of Chiu,Yang, and Chen (1998) and Solyakov and Skog (2002). The high level of HCAs in the skin can be explained by the direct exposure to the cooking surface. In addition, the higher fat content in skin might affect HCA formation. Lipids are known to conduct heat more efficiently into the product, which favors the formation of HCAs (Johansson & Jägerstad, 1994). It is possible that lipids, and perhaps lipid oxidation, form products that may enhance the formation of certain Maillard reation products and lead to an increased content of HCA formation (Barnes, Maher, & Weisburger, 1983; Johansson & Jägerstad, 1994). Removing the skin portion before consumption could significantly reduce total HCA levels from 7.06 to 2.89 ng/g in chicken breast ($p < 0.05$), and from 5.58 to 2.06 ng/g in chicken thigh ($p < 0.05$). Although chicken skin contains a high level of HCAs, the weight of skin portion is much less than meat portion. The total HCA levels in chicken cooked with skin (3.13 ng/g in breast and 2.33 ng/g in thigh) were still significantly lower than the levels in chicken cooked without skin (7.06 ng/g in breast and 5.58 ng/g in thigh) ($p <0.05$). These results agree with studies by Gašperlin, Lukan, Žlender, & Polak (2009), who reported a lower content of total HCAs in chicken with skin (3.49 ng/g) than in chicken without skin (4.75 ng/g) grilled at a temperature of 220 °C to an internal temperature of 82 °C, and Chiu et al. (1998), who reported a lower content of total HCAs in chicken with skin (6.67 ng/g) than in chicken without skin (12.71 ng/g) fried at 200 °C for 15 min. Taken together, these results indicated that presence of skin reduces HCA formation. However, it is still best to remove skin before consuming chicken to minimize HCA intake.

Table 6 shows the HCA levels of fried beef and broiled beef cooked to medium-rare (internal temperature 57 °C) and well-done (internal temperature 71 °C). MeIQx, DiMeIQx, and PhIP were detected in fried and broiled beef. There was a dramatic increase in total HCAs (approximately 3.5-fold) for both fried beef and broiled beef with the increase in cooking time (degree of doneness) from medium-rare to well-done (from 2.73 ng/g to 8.92 ng/g for fried beef and from 1.72 ng/g to 6.04 ng/g for broiled beef) ($p < 0.05$). We observed approximate increases of 2-fold for MeIQx level, 8-fold for DiMeIQx, and 6-fold for PhIP when comparing medium-rare with well-done fried beef. This result is in agreement with studies by Skog et al. (1997) and Janoszka et al. (2009). When cooking time increases, more proteins are denatured, pressing more water, which contains water-soluble HCA precursors, out of the protein network to the meat surface. Thus more of these precursors are transferred to the surface for HCA formation (Persson, Oroszvári, Tornberg, Sjöholm, & Skog, 2008; Skog et al., 1997). Different cooking methods affected total HCA formation. Total HCAs in fried beef (2.73 ng/g) were slightly higher than those in broiled beef (1.72 ng/g) for medium-rare samples, but the difference was not significant ($p > 0.05$). Total HCAs of fried beef (8.92 ng/g) were significantly higher than those of broiled beef (6.04 ng/g) for well-done samples ($p < 0.05$). Cooking time may have more influence on HCA formation than cooking temperature because the cooking temperature used for broiling (232 °C) was higher than that used for frying (204 °C); however, the cooking time used for broiling was less than that used for frying. Also, in oven broiling, the heat is transferred to the meat by air, this produces fewer HCAs than frying, in which the meat is in direct contact with a heated pan (Skog et al., 1997). This result clearly indicates that controlling cooking temperature is a way to minimize HCA formation.

The HCA quantitative determination in fried beef and pork patties, baked beef and pork, and fried bacon is summarized in Table 7. The level of total HCAs did not differ much between fried beef patty and fried pork patty, and between baked beef and baked pork. Baked beef had a lower content of HCAs than fried and broiled beef (Table 6) because baking is done at a lower temperature and because a higher weight of raw beef was used for baking. The level of HCAs in fried bacon was the highest of all meat samples in present study. The total content of HCAs in fried bacon was 17.59 ng/g (6.91 ng/g PhIP, 4.00 ng/g MeIQx, 3.57 ng/g DiMeIQx, and 3.11 ng/g IQx). The content of HCAs in fried bacon in the present study was much higher than that of fully cooked bacon, ready-to-eat meat product included in our previous studies. Fully cooked bacon (heated in a microwave for 30 s according to package direction) had only 0.91 ng/g HCAs (0.14 ng/g PhIP, 0.14 ng/g MeIQx, 0.60 ng/g IQ, and 0.04 ng/g IQx). We believed that the low content of HCAs in fully cooked bacon is due to the precooking process. Industrial fully cooked bacon is cooked at low temperature (162 °C) in the presence of steam induced high humidity either by using a continuous microwave oven or a continuous linear circulating oven. Cooking loss of fried bacon in the present study was high (71.94%). This may explain the high level of HCAs, especially PhIP, in fried bacon compared with fully cooked bacon. PhIP formation increases dramatically in the cooking conditions that generate high cooking loss (Messner & Murkovic, 2004). The content of cooking loss and total HCAs of fried bacon in the present study agree with results of a study by Johansson and Jägerstad (1994), who reported 50.3 to 71.4% cooking loss and 16.7 ng/g total HCAs of bacon fried at 150 °C for 5 min per side.

Table 8 summarizes the results of HCA quantitative determination in fried and baked fish (catfish, salmon, and tilapia). There was no significant difference in content of HCAs among the

three fish species ($p > 0.05$). Concentrations of MeIQx, DiMeIQx, and PhIP in fried fish (catfish, salmon, and tilapia) were similar to those reported earlier for fried mackerel (Gu et al., 2002) and fried salmon (Iwasaki et al., 2010). For all three fish species, total HCAs in fried fish (13.09 to 16.29 ng/g) were significantly higher than those in baked fish (7.85 to 8.70 ng/g) ($p < 0.05$); however, the small contents of IQx (0.38 to 0.52 ng/g) were detected only in baked fish samples.

The total content of HCAs can be used to order these cooked meat products from low to high. Low levels of total HCAs (less than 5 ng/g) were found in baked beef (2.34 ng/g), fried chicken thigh with skin (2.33 ng/g), medium-rare fried beef (2.73 ng/g), fried chicken breast with skin (3.13 ng/g), baked pork (3.29 ng/g), and fried pork patty (4.12 ng/g). Intermediate levels of total HCAs (5 to 10 ng/g) were found in fried beef patty (5.46 ng/g), fried chicken thigh (5.58 ng/g), well-done broiled beef (6.04 ng/g), fried chicken breast without skin (7.06 ng/g), baked fish (8.32 ng/g), and well-done fried beef (8.92 ng/g). High levels of total HCAs (higher than 10 ng/g) were found in fried pork (13.91 ng/g), fried fish (14.91 ng/g), and fried bacon (17.91 ng/g). The high levels of HCAs in some cooked meat products in the present study raises several interesting issues related to HCA intake and cancer aetiology. Data from the National Health and Nutrition Examination Survey 2003-2006 (unpublished data), which estimated meat consumption of U.S. populations, indicated that chicken breast without skin was the most frequently consumed meat item in the U.S. (9.57 g/day), followed by beef steak (8.52 g/day), pork chops (2.89 g/day), and bacon (1.39 g/day). Thus, according to our study, the high levels of HCAs found in fried bacon and fried pork and the intermediate levels of HCAs found in fried chicken breast without skin and well-done fried beef steak indicate that people consuming these products frequently have a high exposure to HCAs that could lead to the possibility of an

increased risk of cancers.

4. Conclusions

The HCA content in cooked meat depends on type of meat, cooking methods, and cooking time and temperature. The primary HCAs in these samples were PhIP, MeIQx, and DiMeIQx. Our results indicated that type and content of HCAs in cooked meat samples were highly dependent on cooking conditions. The total HCA contents in cooked meat were 3.5 times lower if cooked to medium-rare rather than well-done degree of doneness. Fried pork showed higher total HCAs than fried beef and chicken. The skin of fried chicken contained a significant HCA contents, therefore removing the skin before consuming can reduce HCA exposure. Total HCAs were briefly ranked in a decreasing order as follows: low HCA contents (< 5 ng/g) found in baked beef, fried chicken without skin, medium-rare steak, and fried pork patty; intermediate HCA contents (5-10 ng/g) found in fried beef patty, fried chicken with skin, baked fish, and well-done steak; and high HCA contents (> 10 ng/g) found in fried pork, fried fish, and fried bacon. Our data can help food safety professionals recommend cooking methods to be used at home or in the food industry to reduce HCA formation in cooked meat products, will provide important information for use in estimating HCA exposure, and will facilitate investigation of the role of HCAs in the etiology of cancer of population in the United States.

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508 Table 1: Chemical analyses (pH, moisture, fat, protein, and creatine) of raw meat samples prior to cooking

Sample		**pH**	**moisture (%)**	**fat (%)**	**protein (%)**	**creatine (mg/g)**
Beef	Top loin	5.62 ± 0.05	69.32 ± 0.83	7.25 ± 0.01	21.29 ± 0.18	2.93 ± 0.06
	Round tip	5.47 ± 0.04	71.21 ± 1.22	4.61 ± 2.27	22.50 ± 0.64	2.95 ± 0.23
	Ground beef	5.89 ± 0.04	69.79 ± 0.79	9.22 ± 1.46	19.66 ± 0.54	2.53 ± 0.09
Pork	Top loin	6.01 ± 0.36	75.07 ± 0.45	7.73 ± 0.32	20.90 ± 1.01	1.88 ± 0.69
	Ground pork	6.23 ± 0.08	60.12 ± 1.15	21.42 ± 0.59	15.48 ± 0.10	1.79 ± 0.14
	Bacon	6.71 ± 0.05	37.74 ± 2.14	47.99 ± 0.16	11.83 ± 1.15	1.23 ± 0.33
Chicken	Breast meat	6.19 ± 0.16	74.63 ± 0.62	4.87 ± 0.71	23.37 ± 0.25	2.21 ± 0.17
	Breast skin	6.35 ± 0.07	37.06 ± 3.56	53.67 ± 4.36	11.04 ± 1.56	1.02 ± 0.16
	Thigh meat	6.70 ± 0.10	74.44 ± 1.47	4.58 ± 0.64	19.85 ± 0.54	2.51 ± 0.07
	Thigh skin	6.64 ± 0.04	37.36 ± 2.47	52.98 ± 3.80	9.04 ± 1.29	1.18 ± 0.22
Fish	Catfish	6.94 ± 0.11	77.98 ± 0.39	4.99 ± 0.13	15.49 ± 0.13	2.81 ± 0.15
	Salmon	6.80 ± 0.05	78.66 ± 2.91	1.12 ± 1.11	18.21 ± 2.71	2.66 ± 0.23
	Tilapia	7.91 ± 0.14	82.03 ± 2.64	1.07 ± 1.02	15.72 ± 1.01	1.80 ± 0.12

509 Each value is represented as mean ± standard deviation (n = 3).

510 Table 2 : Cooking description

Cooking Method	Food item	Description
Frying	Beef, pork, chicken, fish	Meat was fried in a Teflon-coated frying pan without adding oil at a surface temperature of 204 °C. Meat was fried, turned once, and removed from the pan when the desired temperature was reached.
Broiling	Beef	Oven (convection, top and bottom heat) was preheated to 232 °C (monitored with oven thermometer). The meat was placed on a broiler pan to keep the broiled beef out of the drippings. The meat was removed when a final internal temperature was achieved.
Baking	Beef, Pork, Fish	Oven (convection, top and bottom heat) was preheated to 177 °C (monitored with oven thermometer). The meat was placed on a baking pan. The meat was removed when a final internal temperature was achieved.

*Convection oven with top and bottom heat (top heat 1.0 W and bottom heat 1.2 W)

511 Table 3: Cooking conditions and cooking loss in cooked meat samples

Meat type	Types/cuts of meat	Type of cooking	Raw meat (g)	Thickness, raw meat (cm)	Desired internal temperature (°C)	Cooking temperature (°C)	Cooking time (min per side)	Cooking loss (%)
Beef	Top loin	Frying (medium rare)	350-400	3.8	57	204	6	17.50 ± 1.84
	Top loin	Frying (well done)	350-400	3.8	71	204	12	31.86 ± 1.66
	Top loin	Broiling (medium rare)	350-400	3.8	57	232	5	23.57 ± 1.66
	Top Loin	Broiling (well done)	350-400	3.8	71	232	10	33.81 ± 2.19
	Round tip	Baking (well done)	650-680	9.0	71	177	80 (total)	30.75 ± 3.75
	Ground beef	Frying	140-160	2.3	71	204	6	35.30 ± 2.35
Pork	Top loin	Frying	230-250	2.3	71	204	8	26.12 ± 1.70
	Top loin	Baking	650-680	9.0	71	177	70 (total)	26.48 ± 2.24

	Ground pork	Frying	130-135	2.3	71	204	6	22.20 ± 1.93
	Bacon	Frying	18-25	0.3	-	172	3	71.94 ± 1.26
Chicken	Breast without skin	Frying	250-280	2.5	74	204	10	27.88 ± 1.26
	Breast with skin	Frying	280-310	2.5	74	204	10	24.39 ± 4.71
	Thigh without skin (bone-in)	Frying	140-180	2.5	74	204	7	24.96 ± 3.09
	Thigh with skin (bone-in)	Frying	150-200	2.5	74	204	7	26.74 ± 2.99
Fish	Catfish	Frying	170-190	1.8	63	204	6	27.28 ± 2.46
	Salmon	Frying	180-200	1.8	63	204	6	21.60 ± 2.39
	Tilapia	Frying	140-160	1.5	63	204	6	23.65 ± 1.84
	Catfish	Baking	170-190	1.8	63	177	15 (total)	20.68 ± 2.45
	Salmon	Baking	180-200	1.8	63	177	14 (total)	18.41 ± 2.24
	Tilapia	Baking	140-160	1.5	63	177	12 (total)	18.55 ± 3.73

512 Table 4: Heterocyclic amine content (MeIQx, DiMeIQx, PhIP, and total) of fried meat samples

Cooked items	Internal temperature (°C)	Heterocyclic amines (ng/g)			
		MeIQx	DiMeIQx	PhIP	Total
Fried beef (well done)	77	3.33 ± 0.38	0.33 ± 0.38	5.27 ± 0.81	8.92 ± 1.08
Fried pork	71	2.39 ± 0.50	2.33 ± 0.52	9.20 ± 1.20	13.91 ± 1.81
Fried chicken (breast without skin)	74	0.46 ± 0.34	0.54 ± 0.19	6.06 ± 0.10	7.06 ± 0.56

513 Each value is represented as mean ± standard deviation (n = 4). Means with different superscript letters within the same
514 column are significantly different at $p < 0.05$.

Table 5: Heterocyclic amine content (MeIQx, DiMeIQx, PhIP, and total) of fried chicken samples

Cooked items			Heterocyclic Amines (ng/g)			
			MeIQx	**DiMeIQx**	**PhIP**	**Total**
Breast	without skin	meat	0.46 ± 0.34 [b]	0.54 ± 0.19 [a]	6.06 ± 0.10 [a]	7.06 ± 0.56 [a]
	with skin	meat	0.23 ± 0.15 [b]	0.05 ± 0.01 [c]	2.61 ± 0.63 [c]	2.89 ± 0.72 [b]
		skin	1.61 ± 0.72 [a]	0.93 ± 0.50 [a]	4.52 ± 0.37 [b]	7.07 ± 1.43 [a]
		meat and skin	0.31 ± 0.15 [b]	0.10 ± 0.02 [b]	2.72 ± 0.60 [c]	3.13 ± 0.67 [b]
Thigh	without skin	meat	0.09 ± 0.05 [b]	0.06 ± 0.04 [b]	5.43 ± 0.43 [a]	5.58 ± 0.38 [a]
	with skin	meat	nd	nd	2.06 ± 0.04 [c]	2.07 ± 0.05 [b]
		skin	0.47 ± 0.18 [a]	0.24 ± 0.14 [a]	4.16 ± 0.42 [b]	4.87 ± 0.65 [a]
		meat and skin	0.05 ± 0.03 [b]	0.02 ± 0.02 [b]	2.25 ± 0.10 [c]	2.33 ± 0.14 [b]

Each value is represented as mean ± standard deviation (n = 4). Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 6: Heterocyclic amine content (MeIQx, DiMeIQx, PhIP, and total) of fried beef and broiled beef

Cooked items		Heterocyclic amines (ng/g)			
		MeIQx	**DiMeIQx**	**PhIP**	**Total**
Fried beef	Medium rare	1.75 ± 1.43 [a]	0.04 ± 0.07 [a]	0.94 ± 0.70 [b]	2.73 ± 2.01 [c]
	Well done	3.33 ± 0.38 [a]	0.33 ± 0.38 [a]	5.27 ± 0.81 [a]	8.92 ± 1.08 [a]
Broiled beef	Medium rare	0.08 ± 0.07 [b]	0.06 ± 0.04 [a]	1.58 ± 0.36 [b]	1.72 ± 0.43 [c]
	Well done	0.12 ± 0.07 [b]	0.11 ± 0.02 [a]	5.63 ± 0.95 [a]	6.04 ± 0.97 [b]

Each value is represented as mean ± standard deviation (n = 4). Means with different superscript letters within the same column are significantly different at $p < 0.05$.

Table 7: Heterocyclic amine content (IQx, MeIQx, DiMeIQx, PhIP, and total) of fried beef and pork patties, baked beef and pork, and fried bacon

Cooked items	Heterocyclic amines (ng/g)				
	IQx	**MeIQx**	**DiMeIQx**	**PhIP**	**Total**
Fried beef patty	nd	3.11 ± 0.69	ND	2.35 ± 0.30	5.46 ± 0.78
Fried pork patty	nd	1.09 ± 0.16	1.24 ± 0.75	1.80 ± 0.10	4.12 ± 0.72
Baked beef	nd	0.33 ± 0.05	0.53 ± 0.12	1.49 ± 0.10	2.34 ± 0.11
Baked pork	nd	0.23 ± 0.06	0.86 ± 0.24	2.20 ± 0.12	3.29 ± 0.36
Fried bacon	3.11± 1.38	4.00 ± 1.46	3.57 ± 1.12	6.91 ± 2.06	17.59 ± 5.18

Each value is represented as mean ± standard deviation (n = 4).
nd = not detected

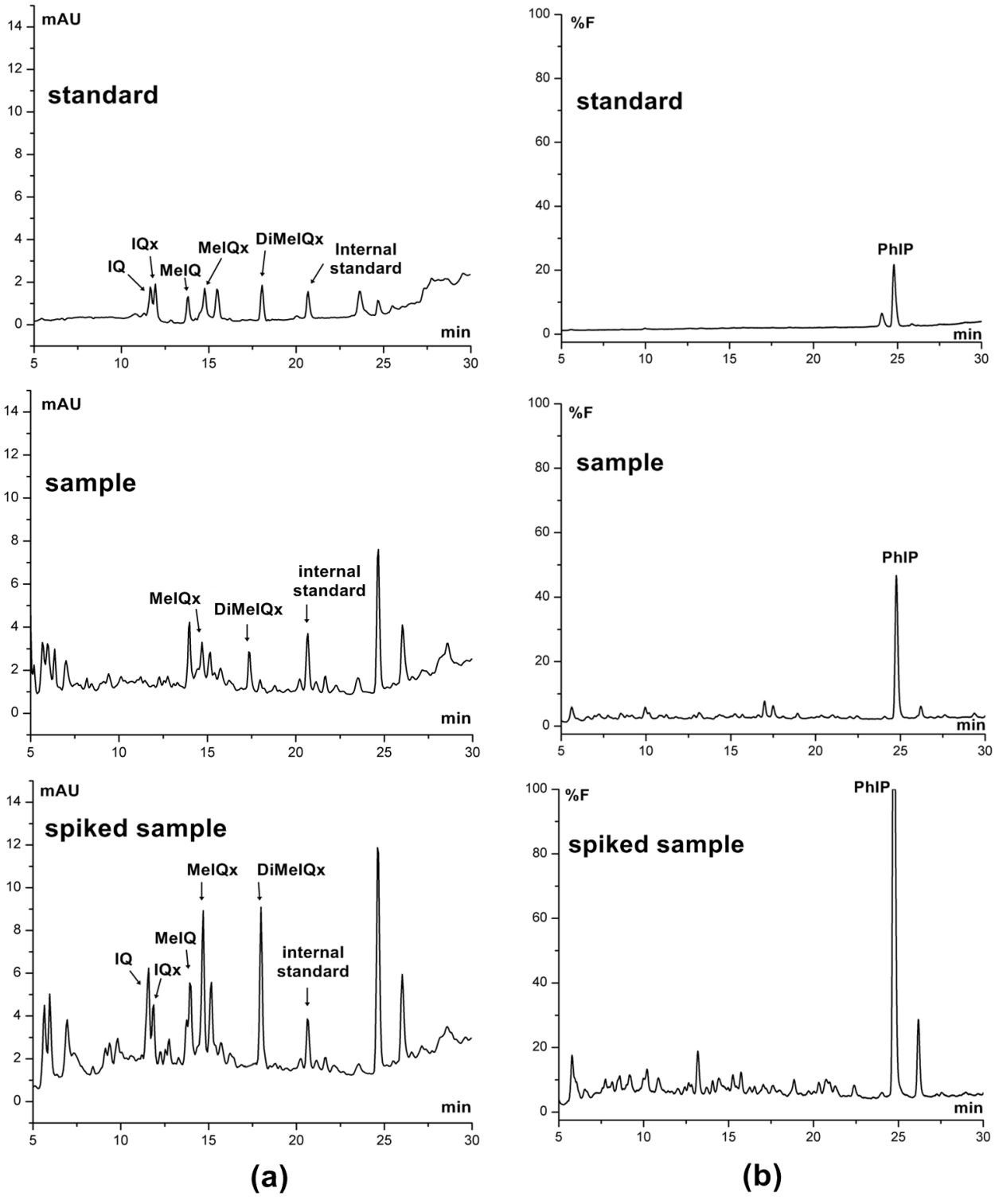
Table 8: Heterocyclic amine content (IQx, MeIQx, DiMeIQx, PhIP, and total) of fried and baked fish

Cooked items		Heterocyclic amines (ng/g)				
		IQx	**MeIQx**	**DiMeIQx**	**PhIP**	**Total**
Fried	Catfish	nd	2.31 ± 0.10 [b]	2.72 ± 0.08 [a]	10.31 ± 0.83 [a]	15.35 ± 0.78 [a]
	Salmon	nd	2.05 ± 0.50 [b]	1.93 ± 0.12 [ab]	9.11 ± 1.25 [a]	13.09 ± 0.90 [a]
	Tilapia	nd	3.11 ± 0.42 [a]	2.29 ± 0.35 [a]	10.89 ± 1.35 [a]	16.29 ± 1.98 [a]
Baked	Catfish	0.85 ± 0.45 [a]	2.95 ± 0.70 [ab]	0.51 ± 0.03 [c]	4.40 ± 0.64 [b]	8.70 ± 1.61 [b]
	Salmon	0.38 ± 0.19 [a]	2.03 ± 0.85 [b]	1.66 ± 0.77 [b]	4.34 ± 0.48 [b]	8.41 ± 1.09 [b]
	Tilapia	0.52 ± 0.21 [a]	1.27 ± 0.16 [c]	0.29 ± 0.23 [c]	5.67 ± 0.44 [b]	7.85 ± 0.65 [b]

Each value is represented as mean ± standard deviation (n = 4). Means with different superscript letters within the same column are significantly different at $p < 0.05$.
nd = not detected

Figure Legend

Figure 1. UV chromatogram (a) and FLD chromatogram (b) of HCA standards (250 ppb each), sample (fried pork), and spiked sample at concentration 25 ppb of each HCA.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(a) (b)

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