Final Report to American Meat Institute Foundation

Project Title	Assessment of Human Exposure to Heterocyclic Amines (HCAs) from Cooked Meat Products
Principal Investigator	J. Scott Smith, Terry A. Houser, Melvin C. Hunt
Research Institution	Kansas State University
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EXECUTIVE SUMMARY

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Heterocyclic amines (HCAs) are cancer-causing compounds found in meat products cooked at temperatures higher than 300 °F. Several studies have shown that high intake of well-done meat and exposure to HCAs may increase risk of human cancers such as colorectal, breast, pancreatic and prostate. In this study the HCA levels in ready-to-eat (RTE) meat products and meat products prepared by cooking methods common to the U.S. were investigated. HCA levels in RTE meat products, including hot dogs, deli meat products, pepperoni, and fully-cooked bacon, are generally low, but some items (e.g. rotisserie chicken) may contain elevated amounts of HCAs. Cooked meat products (pork, beef, chicken, fish) prepared by pan frying, oven broiling, and oven baking had HCA levels 10-50 fold higher than the RTE meat products. The effect of enhancement and marinating on HCA formation in products was investigated. Product enhanced with a solution containing water, salt, and phosphate showed greatly improved water-holding capacity and decreased HCA formation (up to 58%). Greater reductions in HCA levels (up to 79%) were found in marinated fresh meat; especially when the enhancement solution contained ingredients possessing high antioxidant activity. The results from this study can be used to recommend cooking methods for use at home or in the food industry, or used as guidelines for the meat industry on how to modify a formulation process to minimize HCA formation in cooked meat products. Also these data will provide important information for use in estimating HCA exposure and will facilitate investigation of the role of HCAs in the etiology of cancer in the United States.

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Technical Abstract

Heterocyclic amines (HCAs) are produced in meats cooked at high temperature, which are potent mutagens and a risk factor for human cancers. Occurrence of HCAs in ready-to-eat (RTE) meat products and cooked meat products were evaluated. The type of meat products and cooking methods were chosen based on U.S. meat consumer preferences. The primary HCAs detected were 2-amino-3-methyl-imidazo [4,5-*f*]quinoline (IQ), 2-amino-3-methylimidazo [4,5-*f*]quinoxaline (IQx), 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP). Overall, the HCA levels in RTE meat products are generally low, but some items may contain elevated amounts of HCAs. RTE meat products were ranked in the following order of increasing total HCA content: pepperoni (0.05 ng/g) < hot dogs and deli meat products (0.5 ng/g) < fully cooked bacon (1.1 ng/) < rotisserie chicken meat (1.9 ng/g) < rotisserie chicken skin (16.3 ng/g).

The HCA content in cooked meat depends on the type of meat, cooking methods, and cooking temperature. Total amount 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).

Although, it is impossible to prevent HCA formation completely, a reduction of the HCA levels in cooked meat and fish can be achieved by several methods. The addition of salt and phosphate greatly improved the water-holding capacity and decreased HCA formation (up to 58%) in enhanced fresh meat products. However, enhancement with water alone did not reduce HCA formation because the meat did not retain the injected water. A greater reduction of HCAs (up to 79%) was found in marinated fresh meat where the enhancement solution contained ingredients rich in antioxidant compounds.

Taken together, the results from this study can be used to recommend cooking methods for use at home or in the food industry, or used as guidelines for the meat industry on how to modify a formulation process to minimize HCA formation. These data provide information for use in estimating HCA exposure and will facilitate investigation of the role of HCAs in the etiology of cancer in the United States.

Keywords: heterocyclic amines, ready-to-eat meat products, cooked meat products, enhancement, marination

Introduction

Researchers have reported that the diet is strongly associated with a broad range of human diseases, including cancers (Sugimura 2002). The public considers cancer a potentially life-threatening disease that affects people of all ages (Lynch and others 1995), and cancers are the second leading cause of death worldwide after cardiovascular diseases (Oliveira and others 2007). Heterocyclic amines (HCAs) are mutagenic and carcinogenic compounds present at parts per billion levels in cooked muscle foods. The three main precursors of HCA formation are creatine/creatinine, sugars, and amino acids originally found in muscle foods. 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), and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) (Knize and others 1994). Four of these HCAs (IQ, MeIQ, MeIQx, and PhIP) 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*. Epidemiological studies have shown that dietary intake of HCAs through consumption of cooked meat products increased the risk of stomach, colon, and breast cancers in humans (Kampman and others 1999).

The major HCAs formed in cooked meat and fish are PhIP, MeIQx and DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-*f*]quinoxaline) (Pais and others 1999, Janoszka and others 2009). 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 and others 2009). The levels of HCAs increase with increasing temperature and time (Knize and others 1994). High cooking loss is related to the formation of high amounts of HCAs (Knize and others 1994, Skog and others 1995) and the amount of cooking loss during cooking depends on several factors including the muscle tension and direction of muscle fibers (Pais and others 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 and Chiu 1998). For example, IQ, MeIQx, and 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 yield 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, and food preparation. In some previous studies, samples were cooked at high temperature or for a long time; under conditions exceeding those needed to produce an acceptable cooked products (Murkovic and others 1997, Pais and others 1999). Some previous studies lacked information on internal temperature of the cooked samples (Murkovic and others 1997, Oz and others 2007, Jo and others 2008, Janoszka and others 2009). 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 develops more accurate estimates of human HCA exposure.

Ready-to-eat (RTE) products are defined in CFR Title 9 Part 430 (2005) as, "A meat or poultry that is in a form that is edible without additional preparation to achieve food safety and may receive additional preparation for palatability or aesthetic, epicuream, gastronomic, or culinary purposes. RTE product is not required to bear a safe-handling instruction (as required for non-RTE products by 9 CFR 317.2(I) and 381.125(b)) or other labeling that directs that the product must be cooked or otherwise treated for safety, and can include frozen and poultry products." Demand for RTE meat products has increased over the years and are widely consumed in modern society because of their convenience and variety. Few studies have reported on the HCA content in foods from restaurants, fast-food outlets, and RTE meat products.

Case-ready fresh meat products are defined as products that come in a packaged state from the supplier and are not repackaged at the store (Belcher 2006). The prevalence of case ready products has grown at a tremendous rate, increasing from 50% in 2002 to 64% of total fresh meat packages in 2007 (Baczwaski and Mandigo 2003, Belcher 2006), and the number is expected to rise above 70% by 2010 (Young 2009). Two technologies that are commonly used in case-ready meat products are enhancement and marination. Enhancement is the process of injecting a solution of water, salt, and sodium phosphates that typically adds 7 to 15% to the beginning weight of fresh meat to improve the eating quality (juiciness, tenderness, and flavor) of the final product (Baczwaski and Mandigo 2003, Sheard and Tali 2004, Knock and others 2006). Marination expands the solution by using ingredients with additional flavor and texture profiles. A marinade typically contains the same ingredients as enhancement solutions, plus flavor components such as caramel coloring and spices (Baczwaski and Mandigo 2003). In the Sealed Air Corporation study, the National Cattlemen's Beef Association and the National Pork Board assessed case-ready meat products across the country. Within enhanced meat products, pork had the greatest number of products (35%), followed by chicken (19%), beef (13%), and turkey (6%); within marinated meat products, pork also had the greatest number of products (42%), followed by beef (30%), chicken (16%), and turkey (12%) ("Today's Retail Meat Case," 2007). Non-meat ingredients used in enhanced and marinated meat products play various roles. Water is used mainly to dissolve other non-meat ingredients and increase yield; it also contributes to juiciness and tenderness (Miller 1998). Salt and phosphate are used in combination to provide a synergistic action on increasing water-holding capacity and improving texture and flavor (Sheard and Tali 2004). Although some research has been conducted regarding methods to minimize HCA formation in cooked meat products; however, details of the effects of increasing water-holding capacity of fresh meats by means of enhancement and marination on HCA formation are still lacking.

Objectives

The main objective of the study was to evaluate various processing procedures and ingredients that may influence the levels of HCA formation in the major muscle food categories. The major types of products investigated were separated into three categories: fresh meat, RTE meat products, and enhanced/marinated products. Products evaluated in each category included the following:

Fresh meat

Meat samples were selected based on an Internet-based survey of U.S. consumers' preference for method of cooking and degree of doneness of meat and fish conducted by Exponent, Inc. (2009). Meat samples selected 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).

RTE meat products

Eight RTE meat products selected in this study included hot dogs, deli meat products, fully-cooked bacon, pepperoni, and rotisserie chicken.

Enhanced/marinated products

Three type of commercial products (non-enhanced/marinated products, enhanced products, and marinated products) in each meat species (beef, chicken, and pork) and manufactured pork loin samples from the Kansas State University meat laboratory were used in this study.

Materials and Methods

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, triethylamine, phosphoric acid, trichloroacetic acid, diacetyl, 1-napthol, Folin-Ciocalteau's reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), sodium carbonate, gallic acid , and sodium hydroxide were obtained from Sigma-Aldrich (St. Louis, MO). 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 the coupling adaptors were purchased from Varian Sample Preparation (Harbor City, CA, USA).Solvents and chemicals such as acetonitrile (high-performance liquid chromatography [HPLC] grade), methanol (HPLC grade), and sodium hydroxide (ACS-grade) were purchased from Fisher Scientific (Fairlawn, NJ, USA). Rosemary extract (Fortium® R-WS 10 liquid) was supplied by Kemin Industries, Inc. (Des Moines, IA, USA).

Meat samples

Fresh meat

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

RTE meat products

Eight types of RTE meat products were purchased from a local grocery store: hot dog (beef and beef-pork-turkey), deli meat (roast beef, ham and turkey), fully cooked bacon, pepperoni as on frozen pizza, and rotisserie chicken.

Enhanced/marinated products

Three types of commercial products (non-enhanced/marinated products, enhanced products, and marinated products) in each meat species (beef, chicken, and pork) were purchased from local grocery stores. Selected beef products consisted of non-enhanced/marinated beef (control), 12% enhanced beef, and peppercorn-marinated beef. Selected pork products consisted of non-enhanced/marinated pork (control), 12% and 30% enhanced pork, peppercorn-marinated pork, and apple bourbon-marinated pork. Selected chicken products consisted of non-enhanced/marinated chicken breast without skin (control), 15% enhanced chicken breast without skin, and BBQ-marinated chicken breast without skin.

Enhanced pork samples were prepared at the Kansas State University meat laboratory using a multi-needle brine injector. Two individual loins were selected, and each was divided into four sections. Each section was randomly assigned to one of four treatments: (1) no injection, (2) injection with 12% water, (3) injection with 12% enhancement brine (0.4% sodium chloride and 0.35% sodium tripolyphosphate), or (4) injection with 12% enhancement brine (0.075% rosemary extract - Fortium® R-WS 10 liquid). After pumping, loins were vacuum packed and held for 72 h at 4 °C to allow the injected solution to equilibrate throughout the loins.

Chemical analyses

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

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

Creatine content was determined according to the method described by Polak and others (2009). A 0.25 g finely ground sample was homogenized for 5 min at 9500 rpm (IKA, Ultra-Turrax T18, Wilmington, NC, USA) in 100 mL trichloroacetic acid (30 g/L in distilled water), and then the samples were filtered through Whatman #4 filter paper. Twenty milliliters of the filtrate was defatted with 10 mL diethylether, and then the 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) and the mixture was 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 total phenolic content of the enhanced/marinated products was determined using Folin-Ciocalteau's reagent according to the method described by Jang and others (2008). A 5.00 g sample of finely ground meat in 80% ethanol (100 mL) was homogenized for 2 min at 9500 rpm (IKA, Ultra-Turrax T18) (Wilmington, NC, USA). The samples were filtered through Whatman #1 filter paper. Two hundred milliliters of filtrate was mixed with 2 mL of deionized water in a test tube, 200 μL of Folin-Ciocalteu reagent was added, and the tubes were allowed to stand for 6 min at room temperature. Then 1 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The mixture was stored in the dark for 2 h at room temperature. Each sample's absorbance was measured at 765 nm with a UV/VIS spectrophotometer. A standard curve was evaluated from 0 to 100 μg of gallic acid per milliliter. The total phenolic content was expressed as milligram gallic acid equivalents per one gram of meat sample.

Diphenyl-2-picryl-hydrazyl (DPPH) antioxidant/ scavenging activity was evaluated for the enhanced/marinated products using the method of Jang and others (2008). A 5-g sample of finely ground meat in 80% ethanol (100 mL) was homogenized for 2 min at 9500 rpm (IKA, Ultra-Turrax T18) (Wilmington, NC, USA). The samples were filtered through Whatman #1 filter paper. Each 500 μL of filtrate was mixed with 2.5 mL of 0.1 mM freshly prepared DPPH methanolic solution and stored in the dark for 30 min at room temperature before absorbance

was measured at 517 nm. Ethanol (95%) was used as a blank. The control solution consisted of 0.1 mL of 95% ethanol and 2.9 mL of DPPH solution. The radical scavenging activity was expressed as the inhibition percentage and calculated using the following equation:

Antioxidant activity or DPPH scavenging activity (%)

$$= [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Sample preparation and cooking procedure

Fresh meat

Fresh meat products were removed from the refrigerator and allowed to approach room temperature before they were cooked. For frying, meat (beef, pork, chicken and fish) was fried in a Teflon-coated pan without adding oil at a surface temperature 400 °F; turned once, and removed from the pan when the desired temperature was reached. For broiling, meat (beef) was cooked in an oven preheated to 450°F; meat was placed on a broiler pan and removed when the desired temperature was reached. For baking, meat (beef, pork, and fish) were cooked in an oven preheated to 350°F; meat was placed on a baking pan and removed when the desired temperature was reached. Cooked sample was allowed to cool at room temperature for approximately 30 min, and then cooking loss was determined.

RTE meat products

Two hot dogs of each kind were heated in a microwave (1000 W) on high power according to package directions (35 s wrapped in a paper towel). The fully-cooked bacon was heated in a microwave (1000 W) on high for 30 and 60 s as per package directions. Pepperoni taken from the top of the frozen pizza was analyzed as unheated pepperoni. Oven-cooked pepperoni was taken from pizzas that had been cooked for approximately 23 min in an oven at 204 °C (400 °F). Microwave-cooked pepperoni was taken from pizzas cooked in a microwave (1000 W) on high for approximately 4 min per package directions. For the rotisserie chicken, skin and meat were separated before analysis. Deli roast beef, deli ham, and deli turkey were used as obtained.

Enhanced/marinated products

The following commercial products were used as obtained: 12% enhanced beef, 12% enhanced pork, 15% enhanced pork, and BBQ-marinated chicken. Non-enhanced products served as the controls. Peppercorn-marinated beef, 30% enhanced pork, peppercorn-marinated pork, and apple bourbon-marinated pork were manually sliced to a thickness of 2 cm and then stored at 4 °C. For the enhanced pork that was prepared in our meat laboratory, after the

equilibration, loins were sliced to a thickness of 2 cm with a meat slicer and then stored at 4 °C before cooking. The samples used for chemical analyses were further chopped and ground with a food processor and refrigerated at 4 °C before analysis. Each sample was cooked in a Teflon-coated frying pan at a surface temperature of 204 °C (400 °F) to an internal core temperature of 77 °C (170 °F). The cooked sample was allowed to cool at room temperature for approximately 30 min, and then cooking loss was determined.

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. 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 acetae. 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 containing the internal standard before injection into the HPLC. The HCAs were analyzed on an HP1090A Series II HPLC (Agilent Technologies) 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 reequilibrate 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 according to excitation/emission wavelengths of 229 and 437 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.

Quantitation, recovery, and spectral matching

The HCA concentrations were quantitated by the internal standard method (Lindsay 1992). A known amount of TriMeIQx (used as internal standard) was added to samples before they were injected into the HPLC. 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.

Statistical analyses

The experimental design was a randomized complete block with repeated measurements and each experiment was replicated three 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 (SAS Institute Inc., Cary, NC, USA, 2002). Data were examined by analysis of variance (ANOVA) followed by Tukey's multiple comparison test, means were considered significant at $p< 0.05$.

Results

Fresh products

Table 1 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 amount of fat and chicken breast contained the highest amounts of fat. The protein levels of raw meat samples ranged from 9.04 to 23.37%; chicken thigh skin contained the lowest amount of protein, and skin of chicken breast contained the highest amount 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.

HCA levels in cooked pork products are summarized in Table 2 and their pictures are shown in Figure 1. Fried pork contained significant amount of HCAs (13.91 ng/g, PhIP accounting for 9.20 ng/g) and was higher than those in fried beef (Table 3) and fried chicken (Table 4) when they were cooked at the same temperature. 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). The level of total HCAs did not differ much between fried pork patty and fried beef patty,

and between baked beef and baked pork. The level of HCAs in fried bacon was the highest of all meat samples. The total amount 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).

Table 3 summarizes the HCA levels in cooked beef products and their pictures are shown in Figure 2. 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). 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 and others 1997). This result clearly indicates that controlling cooking temperature is a way to minimize HCA formation.

Table 4 summarizes the HCA levels in cooked chicken products, and their pictures are shown in Figure 3. HCA levels in the chicken breasts were higher than in the chicken thighs. Interestingly, the weight loss of chicken breasts/thighs without skin was slightly higher than the loss in chicken breasts/thighs with skin. This suggests that the skin present at the surface can help retain moisture during frying, thus decreasing the HCAs. This is in agreement with results observed by Chiu and others (1998). 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 muscle. The high level of HCAs in the skin can be explained by the direct exposure to the cooking surface, whereas the skin acts as an insulating layer for the meat. Removing the skin portion before consumption could reduce the total HCA levels from 3.13 ng/g to 2.89 ng/g in chicken breasts and from 5.58 ng/g to 2.07 ng/g in chicken thighs. Therefore, it is obvious that the intake of HCAs from fried chicken can be decreased by not consuming the skin.

Table 5 summarizes the results of HCA quantitative determination in fried and baked fish (catfish, salmon, and tilapia), and their pictures are shown in Figure 4. There was no difference in amount of HCAs among the three fish species. For all three fish species, total HCAs in fried fish (13.09 to 16.29 ng/g) were higher than those in baked fish (7.33 to 8.03 ng/g); however, the small amounts of IQx (< 0.5 ng/g) were detected only in baked fish samples (data not shown).

The total amount 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 (above 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 etiology. 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 United States (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.

Table 1. Chemical analyses of pH, moisture, fat, protein, and creatine in uncooked meat samples

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

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

Table 2. HCAs in ppb (ng/g) in cooked pork products (ND = not detected)

Sample	Description	IQx	MeIQX	DiMeIQX	PhIP	Total
Pork chop	Top loin, 230-250 g fried, well-done internal temp 160 °F cooking time 16 min cooking loss 26.1 %	ND	2.39 ± 0.50	2.33 ± 0.52	9.20 ± 1.20	13.91 ± 1.81
Roast pork	Top loin, 650-680 g baked, well-done internal temp 160 °F cooking time 70 min cooking loss 26.5 %	ND	0.23 ± 0.06	0.86 ± 0.24	2.20 ± 0.12	3.29 ± 0.36
Pork patty	Ground pork, 130-135 g fried, well-done internal temp 160 °F cooking time 12 min cooking loss 22.2 %	ND	1.09 ± 0.16	1.24 ± 0.75	1.80 ± 0.10	4.12 ± 0.72
Bacon	Bacon, 18-25 g fried at 342 °F (3 slices at a time) cooking time 3 min cooking loss 71.9 %	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).

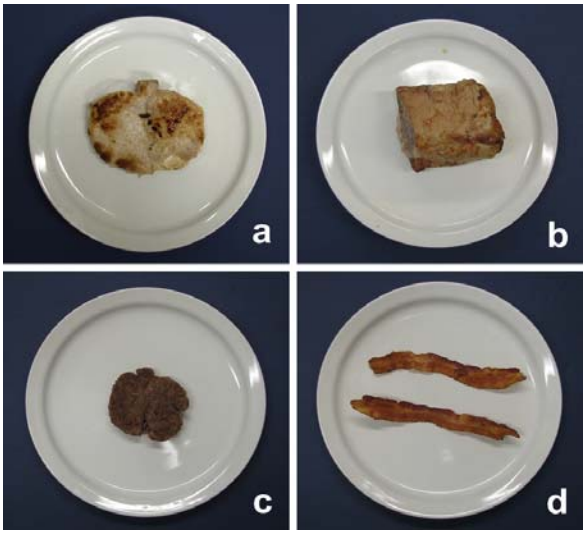

Figure 1.Cooked pork products: fried pork (a), baked pork (b), pork patty (c), and fried bacon (d)

Table 3. HCAs in ppb (ng/g) in cooked beef products (ND = not detected)

Sample	Description	MeIQX	DiMeIQX	PhIP	Total
Steak	Top loin, 350-400 g fried, medium-rare internal temp 135 °F cooking time 12 min cooking loss 17.5 %	1.75 ± 1.43	0.04 ± 0.07	0.94 ± 0.70	2.73 ± 2.01
Steak	Top loin, 350-400 g fried, well-done internal temp 160 °F cooking time 24 min cooking loss 31.9 %	3.33 ± 0.38	0.33 ± 0.38	5.27 ± 0.81	8.92 ± 1.08
Steak	Top loin, 350-400 g broiled, medium-rare internal temp 135 °F cooking time 10 min cooking loss 23.6 %	0.08 ± 0.06	0.06 ± 0.04	1.58 ± 0.36	1.72 ± 0.43
Steak	Top loin, 350-400 g broiled, well-done internal temp 165 °F cooking time 20 min cooking loss 33.8 %	0.12 ± 0.07	0.11 ± 0.02	5.63 ± 0.95	6.04 ± 0.97
Roast beef	Round tip, 650-680 g baked, well done internal temp 160 °F cooking time 80 min cooking loss 30.8 %	0.33 ± 0.05	0.53 ± 0.12	1.49 ± 0.10	2.34 ± 0.11
patty	Ground beef, 140-160 g fried, well done internal temp 160 °F cooking time 12 min cook loss 35.3 %	3.11 ± 0.69	ND	2.35 ± 0.30	5.46 ± 0.78

Each value is represented as mean ± standard deviation (n = 4).

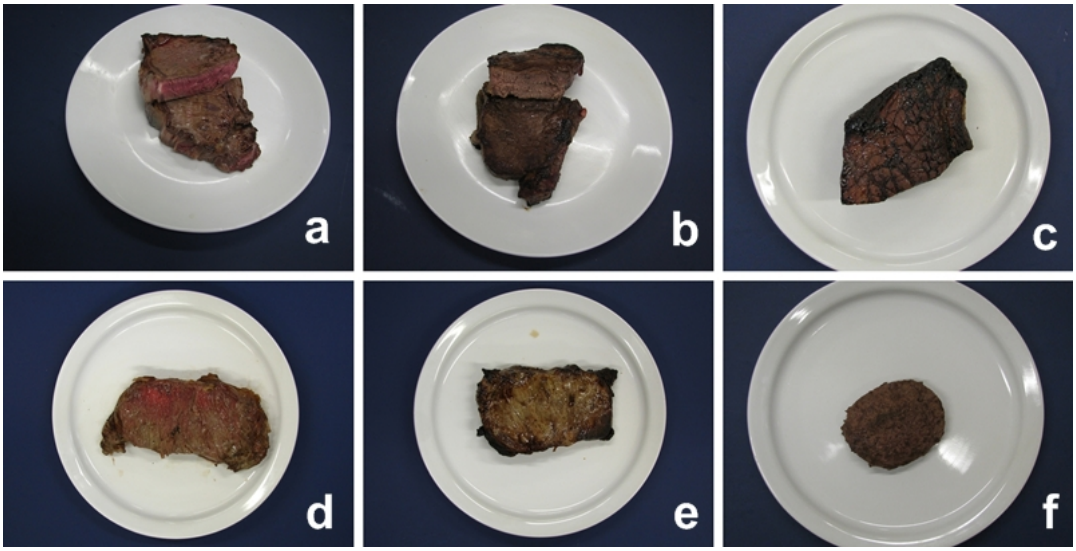

Figure 2. Cooked beef products: medium-rare fried beef (a), well-done fried beef (b), baked beef (c), medium-rare broiled beef (d), and well-done broiled beef (e), and beef patty (f).

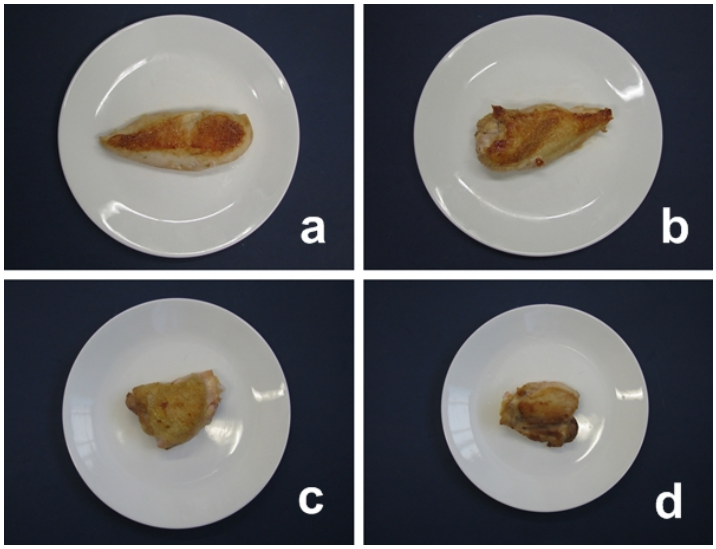

Figure 3. Cooked chicken products: Fried breast without skin (a), fried breast with skin (b), fried thigh without skin (c), and fried thigh with skin (d).

Table 4. HCAs in ppb (ng/g) in cooked chicken products (ND= not detected)

Sample	Description	MeIQX	DiMeIQX	PhIP	Total
Chicken breast skinless and boneless	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 28-35%	0.46 ± 0.34	0.54 ± 0.19	6.06 ± 0.10	7.06 ± 0.56
Chicken breast with skin and boneless (skin removed)	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 22-29%	0.23 ± 0.15	0.05 ± 0.01	2.61 ± 0.63	2.89 ± 0.72
Chicken breast with skin and boneless (skin included)	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 22-29%	0.31 ± 0.15	0.10 ± 0.02	2.72 ± 0.60	3.13 ± 0.67
skin of chicken breast	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 22-29%	1.61 ± 0.72	0.93 ± 0.50	4.52 ± 0.37	7.07 ± 4.13
Chicken thigh skinless and boneless	fried, just done internal temp 165 °F cooking time 20-25 min cooking loss 26-33%	0.09 ± 0.05	0.06 ± 0.04	5.43 ± 0.43	5.58 ± 0.38
Chicken thigh with skin and boneless (skin removed)	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 22-28%	ND	ND	2.06 ± 0.04	2.07 ± 0.05
Chicken thigh with skin and boneless (skin included)	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 22-28%	0.05 ± 0.03	0.02 ± 0.02	2.25 ± 0.10	2.33 ± 0.14
skin of chicken thigh	fried, just done internal temp 165 °F cooking time 25-30 min cooking loss 22-28%	0.47 ± 0.18	0.24 ± 0.14	4.16 ± 0.42	4.87 ± 0.65

Each value is represented as mean ± standard deviation (n = 4).

Table 5. HCAs in ppb (ng/g) in cooked fish products (ND= not detected)

Sample	Description	MeIQX	DiMeIQX	PhIP	Total
Catfish	raw weight 170-190 g fried at 400 °F internal temp 145 °F cooking time 12 min cooking loss 27.3 %	2.31 ± 0.10	2.72 ± 0.08	10.31 ± 0.83	15.35 ± 0.78
Salmon	raw weight 180-200 g fried at 400 °F internal temp 145 °F cooking time 12 min cooking loss 21.6 %	02.05 ± 0.50	1.93 ± 0.12	9.11 ± 1.25	13.09 ± 0.90
Tilapia	raw weight 140-160 g fried at 400 °F internal temp 145 °F cooking time 12 min cooking loss 23.7 %	3.11 ± 0.42	2.29 ± 0.75	10.89 ± 1.35	16.29 ± 1.98
Catfish	raw weight 170-190 g baked at 350 °F internal temp 145 °F cooking time 15 min cooking loss 20.7 %	2.35 ± 0.70	0.51 ± 0.03	4.40 ± 0.64	7.85 ± 1.61
Salmon	raw weight 180-200 g baked at 350 °F internal temp 145 °F cooking time 14 min cooking loss 18.4 %	2.03 ± 0.85	1.66 ± 0.77	4.34 ± 0.48	8.03 ± 1.09
Tilapia	raw weight 140-160 g baked at 400 °F internal temp 145 °F cooking time 12 min cooking loss 18.6 %	1.27 ± 0.16	0.27 ± 0.16	5.67 ± 0.44	7.33 ± 0.65

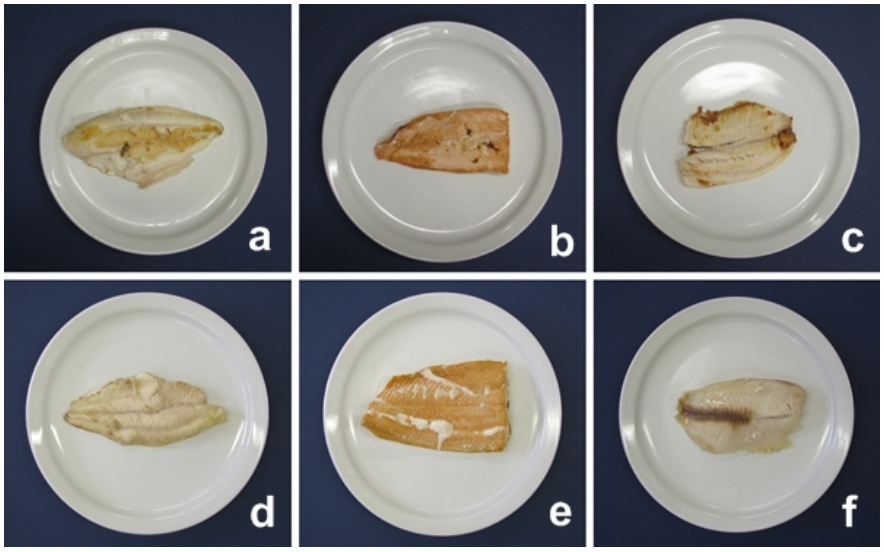

Figure 4. Cooked fish products: fried catfish (a), fried salmon (b), fried tilapia (c), baked catfish (d), baked salmon (e), and baked tilapia (f).

RTE meat products

The pH and composition of each RTE sample are shown in Table 6. The pH of most RTE samples ranged from 5.0 to 6.5; pepperoni, which is a fermented meat product had a low pH level (pH 4.78). Both types of hot dogs had a low amount of creatine (less than 1 mg/g). Creatine in the deli meat ranged from 1.9 to 2.3 mg/g, and pepperoni contained 1.37 mg/g creatine. Bacon had the highest amount of creatine at 3 ng/g. Deli meat products had high moisture levels (69 to 76%) followed by hot dogs (47 to 50%). Pepperoni and bacon had low moisture levels (24.4% for pepperoni and 15.3% for bacon). Deli meat contained low levels of fat (less than 10%), and hot dogs contained approximately 30% fat. Bacon and pepperoni had high fat content (37.9% for bacon and 44.5% for pepperoni). Protein content was lowest for hot dogs (10%), intermediate for deli meat products (20%), and highest for bacon (42.8%).

Figure 5 shows the pictures of RTE meat products in this study. The result of HCA quantitative determinations in the eight selected RTE products are summarized in Table 7. Total contents of the five measured HCAs (IQ, IQx, MeIQx, DiMeIQx, and PhIP) of RTE products ranged from 0.05 to 13.07 ng/g. Hot dogs, deli meat products, and pepperoni generally had relatively low levels of total HCAs. Bacon and rotisserie chicken, especially the skin, had high HCA levels. All three pepperoni sample types had very low levels of total HCAs, and there were no statistically significant differences in HCAs among types. PhIP was the only HCA found in the pepperoni samples. Bacon heated for 30 or 60 s had higher levels of IQ, MeIQx, and PhIP than unheated bacon. The increased amount of HCAs after microwave heating may be due to the loss of water during heating, which could lead to more concentrated HCAs or formation of more

HCAs. Furthermore, there was considerable variation in HCA levels of the rotisserie chicken skin. Of the four replications of rotisserie chicken, one of the skin samples had visible black charred areas and contained higher amount of HCA levels than the other three replications.

If the total amount of HCAs is considered, the RTE meat products can be arranged in order from high to low. The chicken skin was the product with the highest levels of HCA (13.82 ng/g), probably due to more extreme heating conditions than for the other products. The deli turkey, deli ham, beef hot dog, combo hot dog, deli roast beef, bacon and chicken breast had levels of HCAs in the range of 0.4-2.0 ng/g. Most of these products contain salt, sodium phosphate and/or modified food starch which confer a better water-holding capacity, thus reducing the transport of precursors towards the surface during cooking. The pepperoni contained spices, oleoresin of paprika, BHA and BHT, which can act as antioxidants. This may explain the low levels of HCAs in pepperoni (0.02-0.05 ng/g) relative to the other products. Our results indicated that the level of HCAs in RTE products are generally low, but that some items may contain elevated amounts. Taken together, our results show that cooking conditions and ingredients influence the levels of HCA in the ready to eat products.

Table 6. pH and composition of RTE meat products.

Sample	**pH**	**creatine (mg/g)**	**moisture (%)**	**fat (%)**	**protein (%)**
hot dog beef	6.17 ± 0.03	0.75 ± 0.08	47.40 ± 0.44	30.78 ± 0.17	10.53 ± 0.17
hot dog beef-pork-turkey	6.39 ± 0.11	0.57 ± 0.06	49.86 ± 0.71	28.54 ± 0.61	10.61 ± 0.15
deli roast beef	5.47 ± 0.04	2.23 ± 0.13	69.41 ± 0.65	5.67 ± 1.09	21.33 ± 0.68
deli ham	6.40 ± 0.04	2.02 ± 0.28	71.63 ± 1.69	4.24 ± 0.61	19.20 ± 1.41
deli turkey	6.32 ± 0.02	1.95 ± 0.12	75.18 ± 0.48	1.74 ± 0.21	18.28 ± 1.92
pepperoni (unheated)	4.78 ± 0.20	1.37 ± 0.10	24.40 ± 0.42	44.52 ± 1.17	21.15 ± 1.05
bacon (unheated)	6.44 ± 0.73	3.00 ± 0.61	15.31 ± 0.82	37.86 ± 1.37	42.79 ± 1.69

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

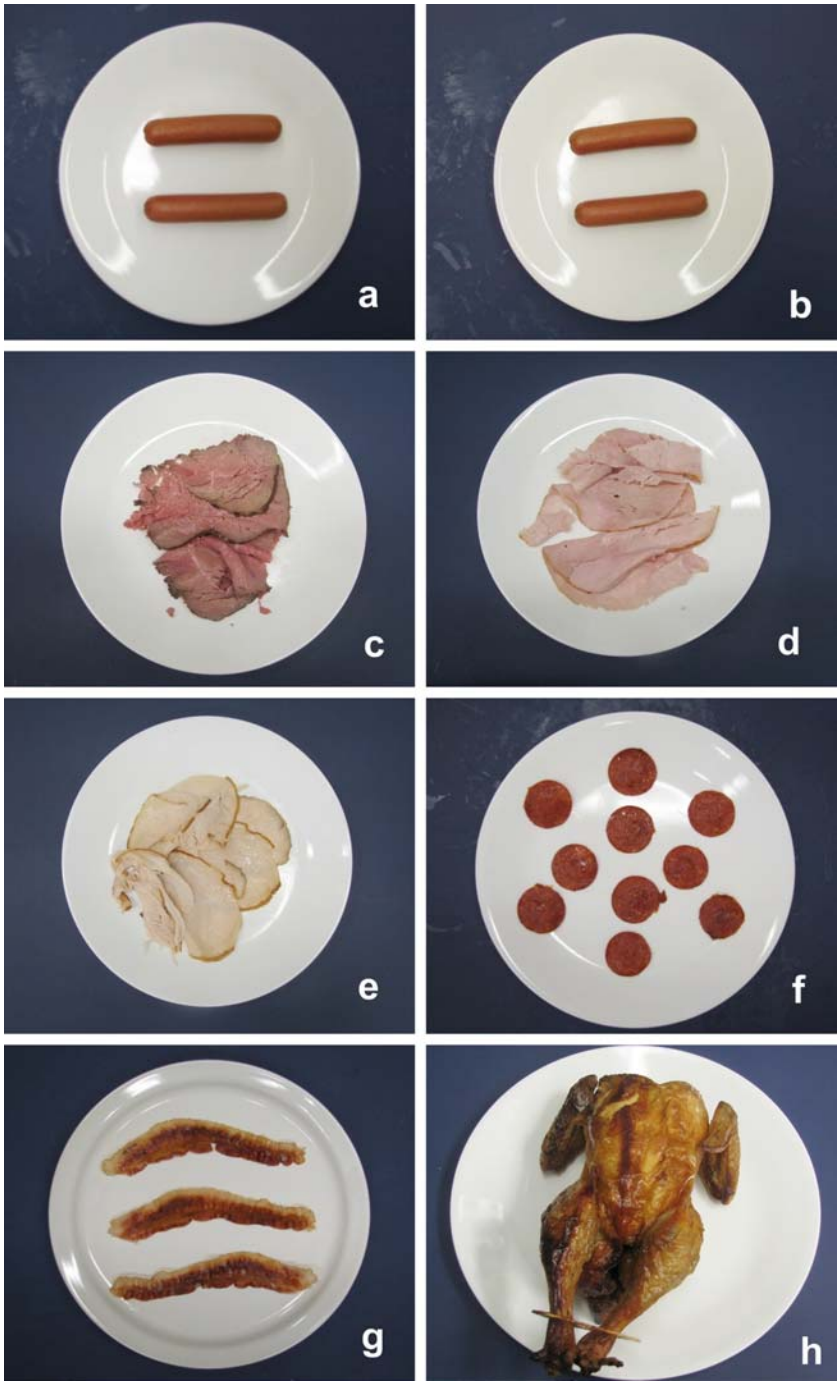

Figure 5. RTE meat products: hot dog beef heated in microwave for 35 second (a), hot dog beef-pork-turkey heated in microwave for 35 second (b), deli roast beef (c), deli ham (d), deli turkey (e), pepperoni heated in an oven for 23 min at 400 °C (f), and bacon heated in microwave for 30 s (g), and rotisserie chicken (h).

Table 7. Heterocyclic amine contents (IQ, IQx, MeIQx, DiMeIQx, PhIP, and total of RTE meat products (ND = not detected)

Sample	**IQ**	**IQx**	**MeIQX**	**DiMeIQX**	**PhIP**	**Total**
Hotdog beef	0.31 ± 0.09	ND	0.07 ± 0.02	ND	0.06 ± 0.01	0.44 ± 0.08
Hot dog beef-pork-turkey	0.28 ± 0.10	ND	0.07 ± 0.03	ND	0.07 ± 0.03	0.42 ± 0.10
Deli roast beef	0.20 ± 0.09	ND	0.08 ± 0.03	ND	0.15 ± 0.15	0.44 ± 0.19
Deli ham	0.29 ± 0.13	ND	0.03 ± 0.01	ND	0.14 ± 0.08	0.53 ± 0.06
Deli turkey	0.22 ± 0.10	ND	0.13 ± 0.07	ND	0.09 ± 0.01	0.46 ± 0.11
Unheated pepperoni	ND	ND	ND	ND	0.03 ± 0.02	0.03 ± 0.02
Oven-cooke d pepperoni	ND	ND	ND	ND	0.05 ± 0.01	0.05 ± 0.01
Microwaved-cooked pepperoni	ND	ND	ND	ND	0.01 ± 0.01	0.01 ± 0.01
Unheated bacon	0.33 ± 0.07	0.00 ± 0.00	0.09 ± 0.06	ND	0.10 ± 0.02	0.53 ± 0.11
Bacon, heated for 30 s	0.60 ± 0.05	0.04 ± 0.03	0.14 ± 0.02	ND	0.14 ± 0.03	0.91 ± 0.06
Bacon, heated for 60 s	0.52 ± 0.03	0.03 ± 0.02	0.36 ± 0.15	ND	0.18 ± 0.00	1.10 ± 0.14
Rotisserie chicken meat	0.75 ± 1.44	0.24 ± 0.13	0.17 ± 0.07	0.02 ± 0.03	0.39 ± 0.62	1.56 ± 2.02
Rotisserie chicken skin	0.32 ± 0.53	0.39 ± 0.07	3.62 ± 4.24	0.86 ± 0.98	7.89 ± 12.92	13.07 ± 18.63

Each value is represented as mean ± standard deviation (n = 4).

Enhanced/marinated products

Commercial enhanced/marinated products

Table 8 summarizes the results of chemical analyses and HCA quantitative determinations in the non-enhanced, enhanced, and marinated commercial meat products (pork, beef, and chicken). For pork products, there was not much difference in MeIQx and DiMeIQx levels, PhIP levels ranged broadly from 1.96 to 17.58 ng/g. Total HCA levels were higher for control pork than for 12% and 30% enhanced pork products. Both marinated pork products had lower total HCA levels than control and both enhanced pork products; total HCAs were lowest for apple bourbon-marinated pork. Antioxidant compounds in some ingredients (e.g., tomato powder, onion powder, garlic powder, turmeric, and mustard seed) of apple bourbon marinated pork are believed to play a role in inhibiting HCAs (Shishu and Kaur 2008, Janoszka 2010). For beef products, total HCAs were higher for control than for 12% enhanced beef and peppercorn-marinated beef, and there was not much difference between 12% enhanced and peppercorn-marinated beef products. In all three products, PhIP was found at the highest levels, followed by MeIQx and DiMeIQx. There was not much difference in MeIQx and PhIP among these three beef products; DiMeIQx was higher in control than in 12% enhanced beef and was not detected in peppercorn-marinated beef. For chicken products, there was not much difference in MeIQx, DiMeIQx, and PhIP levels among the three chicken products.

Taken together, the results for these commercial products indicate that enhancing meat products with water, salt, and phosphate can help meat retain water resulting in a lower amount of HCA formation (as seen in enhanced beef and pork). Meat enhanced with only water without the addition of salt and phosphate (as seen in enhanced chicken) could not hold water very well; this increased the cooking loss and led to an increase in the amount of HCAs. However, this conclusion is based on the meat species represented in commercial products; meat type and other ingredients may also influence HCA formation. Therefore, to gain a better understanding of the effect of the enhancement process on HCA formation, we decided to prepare experimental enhanced pork loin products in our meat laboratory.

Table 8. Heterocyclic amine contents (IQ, IQx, MeIQx, DiMeIQx, PhIP, and total of RTE meat products.

Product		pH	cooking loss (%)	total phenolic (mg GAE/g)	Antioxid-ant activity (%)	Heteocyclic amines (ng/g)			
						MeIQx	DiMeIQx	PhIP	Total
non-enhanced pork	mean	6.07	33.54	1.50	10.44	3.20	1.07	13.31	17.58
	SD	0.09	0.18	0.02	0.82	1.74	0.27	0.94	2.94
12% enhanced pork	mean	6.11	34.92	1.45	11.21	1.71	0.75	9.60	12.06
	SD	0.14	2.96	0.02	0.76	0.80	0.38	1.19	0.46
30% enhanced pork	mean	6.21	40.47	1.52	6.23	2.89	1.80	9.85	14.54
	SD	0.22	0.81	0.03	0.38	0.46	0.34	0.83	1.34
Peppercorn-marinated pork	mean	5.84	42.78	1.30	13.67	0.88	0.60	5.83	7.32
	SD	0.18	0.11	0.02	0.49	0.39	0.03	0.27	0.57
Non-enhanced beef	mean	5.39	40.75	1.45	58.78	0.90	0.61	0.46	1.96
	SD	0.04	0.57	0.04	1.53	0.20	0.14	0.15	0.07
12% enhanced beef	mean	5.70	36.37	1.35	7.68	2.59	0.67	6.03	9.30
	SD	0.07	1.17	0.01	0.40	1.15	0.11	0.72	0.37

Peppercorn-marinated beef (30% enhancement)	mean	6.15	42.98	1.22	13.33	3.13	0.00	6.16	9.29
	SD	0.28	2.15	0.01	0.19	0.33	0.00	0.28	0.07
Non-enhanced chicken	mean	5.77	27.08	1.35	12.88	1.20	0.64	5.35	7.20
	SD	0.01	1.35	0.02	0.84	0.25	0.11	0.76	0.86
15% enhanced chicken	mean	5.92	33.74	1.36	12.95	2.65	1.13	5.01	8.80
	SD	0.01	3.27	0.06	1.42	1.01	0.35	0.54	0.71
BBQ-marinated chicken	mean	6.00	28.47	1.48	17.06	1.87	0.96	4.14	6.97
	SD	0.04	2.64	0.02	1.85	0.22	0.47	0.51	0.20

Experimental enhanced products

The pH, creatine levels, total phenolic, and antioxidant activity in manufactured pork loins are summarized in Table 9. The pH was significantly ($p < 0.05$) higher for salt/phosphate-injected loin (pH 6.07) than for water-injected loin (pH 5.67), and the control (pH 5.70). Creatine content of loins injected with water (4.6 mg/g) and salt/phosphate (4.3 mg/g) was slightly lower than that of the control (5.0 mg/g); perhaps the injected solution caused little dilution of the creatine level originally present in the loins. Table 10 summarizes the results of HCA quantitative determinations in control, water- injected loin, salt/phosphate-injected loin, and rosemary extract-injected loin. The amounts of PhIP, MeIQx, and DiMeIQx in water-injected loin were slightly higher than those in the control, but the differences were not statistically significant ($p > 0.05$). In contrast, the amounts of PhIP, MeIQx, and DiMeIQx in salt/phosphate-injected loin were significantly lower than those in the control and water-injected loin ($p < 0.05$). Injection of water with a combination of water and rosemary extract had lower HCA concentration than injection of water alone ($p < 0.05$). This is due to the presence of polyphenol compounds in rosemary extract that have been reported to have strong antioxidant effects, e.g. rosmarinic acid, carnosol and carnosic acid (Tsen and others 2006).

Although amounts of total phenolic were not different among treatments, the antioxidant activity of rosemary extract-injected loin was significantly higher than that in control, water-injected loin, and salt/phosphate-injected loin ($p < 0.05$) (Table 9). Injection of water with salt

and phosphate showed more reduction of HCA formation than injection of water with rosemary extract ($p < 0.05$). This may be due to rosemary-extract injected loin was not able to hold injected water inside the product very well, thus some of water-soluble antioxidant compounds present in rosemary extract may loss along with water during storage and cooking. Injection of salt/phosphate reduced the level of PhIP by 42.5% (reduce from 13.12 to 7.54 ng/g), MeIQx by 79.0% (reduce from 7.59 to 1.57 ng/g), and DiMeIQx by 75.6% (reduce from 1.64 to 0.40 ng/g) compared with the control. This is in agreement with a study by Persson and others (2003) who reported that addition of 1.5% sodium chloride and 0.3% sodium tripolyphosphate to beefburgers decreased the formation of PhIP (up to 38%), MeIQx (up to 38%), and DiMeIQx (up to 12.5%) when beefburgers were fried at 180 °C and 220 °C. In addition, Persson and others (2003) stated that addition of salt and phosphate may inhibit the conversion of creatine to creatinine during cooking, resulting in reduced HCA formation. Because creatine is less water soluble than creatinine, inhibiting the conversion of creatine to creatinine means that fewer HCA precursors are transported to the meat surface during cooking. Therefore, addition of salt and phosphate has a great impact on improving water-holding capacity and reducing HCA formation in pork loins. It may be that injected salt and phosphate can hold the water in the sample, thus decreasing the transport of water and water-soluble precursors (creatine/creatinine, glucose, and amino acids) to the surface.

Table 9. The pH, creatine levels, total phenolic, and DPPH radical scavenging activity in manufactured pork loins

Treatment	**pH**	**creatine**	**Total phenolic (mg GAE/g sample)**	**Antioxidant activity (%)**
control (no injection)	5.70 ± 0.12 [b]	5.00 ± 0.29 [a]	1.23 ± 0.05 [a]	9.12 ± 1.88 [b]
water injection	5.67 ± 0.20 [b]	4.60 ± 0.34 [b]	1.13 ± 0.08 [ab]	7.70 ± 0.91 [b]
salt/phosphate injection	6.07 ± 0.17 [a]	4.28 ± 0.25 [b]	1.10 ± 0.07 [b]	8.38 ± 1.21 [b]
rosemary extract injection	5.71 ± 0.13 [b]	4.30 ± 0.43 [b]	1.11 ± 0.06 [b]	12.12 ± 1.83 [a]

Table 10. Cooking loss and heterocyclic amines (MeIQx, DiMeIQx, PhIP, and total) in manufactured pork loins

Treatment	Cooking loss (%)	Heterocyclic amines (ng/g)			
		MeIQx	DiMeIQx	PhIP	Total
Control (no injection)	25.23 ± 1.02 b	7.59 ± 0.77 [a]	1.64 ± 0.31 [a]	13.12 ± 0.68 [a]	22.34 ± 1.33 [ab]
water injection	34.69 ± 4.24 [a]	8.21 ± 1.61 [a]	2.71 ± 1.62 [a]	15.05 ± 1.35 [a]	25.98 ± 4.09 [a]
salt/phosphate injection	26.39 ± 2.63 [b]	1.57 ± 0.44 [c]	0.40 ± 0.06 [b]	7.54 ± 2.67 [b]	9.50 ± 2.48 [c]
rosemary injection	33.42 ± 3.11 [a]	5.33 ± 1.24 [b]	0.88 ± 0.32 [b]	12.15 ± 1.98 [a]	18.35 ± 3.08 [b]

Conclusions

Heterocyclic amines (HCAs), potent mutagenic and carcinogenic compounds, are formed during the cooking of meat and fish. The major HCAs found in cooked meat and fish are PhIP, MeIQx, and DiMeIQx. Most RTE meat products contain very low HCA levels, except for rottisserie chicken that contain elevated amounts of HCAs. We concluded that consumption of RTE meat products contributes very little to HCA intake. In cooked meat products (beef, chicken and fish) prepared by various cooking methods (pan frying, oven broiling, and oven baking). High levels of total HCAs were found in fried pork, fried fish, and fried bacon. The formation of HCAs in cooked samples is highly dependent on the method and level of cooking, and the content of HCAs in cooked meat and fish will be low if an appropriate cooking procedure is selected.

Because of the increasing evidence of the risk of cancers, it is necessary to reduce the exposure to HCAs. Although, it is impossible to prevent the HCA formation completely, a reduction of the HCA levels in cooked meat and fish can be achieved by several methods. The enhancement and marination were found to reduce the amount of HCAs formed in cooked meat products. Addition of salt and phosphate greatly improved water-holding capacity and decreased HCA formation in enhanced fresh meat products. An even greater reduction of HCAs was found in marinated fresh meat; the enhancement solution for this meat contained ingredients that exhibited food antioxidant properties.

These data 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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