Heterocyclic Amines in Fresh and Processed Meat Products

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Heterocyclic amines (HCAs), potent mutagens/carcinogens, are pyrolysis products formed during the cooking of meat and fish. Processed meats (bratwurst, fresh pork sausage, Italian sausage, and light smoked sausage) were evaluated for heterocyclic amine content. Eye round steak and ground beef with two fat levels (5 and 15%) also were evaluated. Meat samples fried at 150, 190, and 230 °C or grilled at 200 and 240 °C were analyzed by HPLC using ultraviolet and fluorescence detection. Both the interior and external surfaces of the patties were evaluated. The crust of the Italian sausage showed the highest level of heterocyclic amine 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (3.44 ng/g), whereas that of smoked sausage showed the highest levels of 2-amino-methyl-6-phenylimidazo[4,5-*b*]pyridine (5.83 ng/g), 1-methyl-9*H*-pyrido[3,4-*b*]indole (10.6 ng/g), and 9H-pyrido[3,4-*b*]-indole (2.51 ng/g). The 5% fat beef patties showed higher HCA content than the 15% fat beef patties.

Keywords: *Fresh; processed; meat products; heterocyclic amines; MeIQx; PhIP*

INTRODUCTION

Heterocyclic amines (HCAs) are the main mutagenic and probably carcinogenic substances produced in cooked muscle meat, specifically beef, pork, mutton, chicken, and fish. These compounds are mutagenic in the Ames/*Salmonella* assay (Sugimura, 1985). Several long-term animal studies on mice, rats, and nonhuman primates have suggested a correlation between HCA consumption and cancer (Layton et al., 1995). Furthermore, epidemiological studies have suggested a relationship between high consumption of meat and fish and different types of human cancer such as pancreatic, colorectal, and urothelial cancer (Gerhardsson de Verdier, 1995).

Physical variables such as temperature, time, and method of cooking significantly affect the mutagenic activity of cooked meat. Cooking temperature is the most important factor (Sugimura and Sato, 1983). Studies show a marked decrease in mutagenic activity of meat when it is fried at lower temperatures (Skog et al., 1995). Although all cooked meat has some mutagenic activity, meat cooked at ≤150 °C to rare or medium-rare doneness showed fewer mutagens at lower content than meat cooked to well-done (≥150 °C) (Hatch et al., 1988). Moreover, the surface of well-done charcoal-broiled steak contained much higher levels of HCAs than that of boiled beef.

Several studies have postulated that the products of the Maillard reaction, such as pyridines or pyrazines formed by Strecker degradation, undergo an aldol-type condensation (Jägerstad et al., 1990). The resulting vinylpyridines or vinylpyrazines go through a conjugation addition with creatinine. The reaction is completed by ring closure, water elimination, and desaturation to give imidazoquinolines, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]-quinoline (MeIQ); imidazoquinoxalines, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx); and the imidazopyridine 2-amino-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).

The other suggested route for the formation of these mutagens is condensation of an aldehyde with creatinine, followed by combination with pyrazines to form imidazoquinoline or imidazoquinoxaline compounds (Jones and Weisburger, 1988). Cyclization and water elimination from a creatine molecule provide the essential 2-amino-3-methylimidazole part of the HCA molecule. This reaction occurs readily if the temperature of the meat rises above 100 °C.

Several studies have suggested that these mutagens form in different types of meats. Layton et al. (1995) listed the contents of HCAs in a variety of cooked muscle foods, such as ground beef, beef extract, beef steak, chicken, lamb, pork, and fish, and also in nonmuscle foods, such as mushrooms and Worcestershire sauce. Stavric et al. (1997) surveyed for the presence of HCAs in processed, ready-to-eat meat products such as turkey breast, salami, and chicken loaf. They concluded that no serious health risk will be encountered from the consumption of such meat products due to undetectable levels of HCAs. Sinha et al. (1998) analyzed the HCA content of different pork products cooked by frying, oven-broiling, boiling, or grilling/barbecuing to different degrees of doneness. Their results indicated that well- and very well-done oven-broiled bacon showed the highest levels of PhIP, whereas very well-done pan-fried pork chops contained the highest level of MeIQx. No research has evaluated the levels of HCAs in a wide variety of processed meat products, including cured/fermented meat products. Therefore, this study was undertaken to determine the level of HCAs in bologna, summer sausage, ham, bratwurst, fresh pork sausage, smoked sausage, Italian sausage, and eye round steak and to investigate the effect of fat content (5 and 15%) in ground beef on the levels of HCA formation.

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MATERIALS AND METHODS

Meat Samples. Bologna, summer sausage, ham, bratwurst (20% fat), fresh pork sausage (17% fat), Italian sausage (15% fat), ground beef (5% and 15% fat), and eye round steak were obtained from the Kansas State University Meat Laboratory. Light smoked sausage (14% fat) was purchased from a local supermarket. Ingredients of the bratwurst were pork, water, salt, corn syrup, dextrose, spices, flavoring, and monosodium glutamate. The light smoked sausage was made of skinless turkey, pork, water, beef, corn syrup, salt, natural spices, dextrose, monosodium glutamate, ascorbic acid, flavorings, and sodium nitrite. The fresh pork sausage contained salt and spices, whereas the Italian sausage contained pork, salt, caraway seeds, garlic powder, and other spices.

Chemicals. All chemicals and solvents were of HPLC grade. Deionized water was used throughout the experimental procedures. Standards for polar HCAs (IQ, MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP) and nonpolar HCAs (Trp-p-1, Trp-p-2) and β-carbolines, harman and norharman, were obtained from Toronto Research Chemicals (Toronto, Canada). Extrelut extraction cartridges (20 mL) and refill material were obtained from VWR Scientific Products (Chicago, IL). Bond Elut propylsulfonic silica (PRS) (500 mg) cartridges, and C-18 (100 and 500 mg) cartridges were obtained from Varian Sample Preparation Products (Harbor City, CA). Cartridge coupling adaptors were obtained from Supelco (Bellefonte, PA).

Sample Preparation and Cooking Conditions. The various processed meat samples (precooked and raw) were allowed to reach room temperature (20 °C). The cured/fermented meat samples, bologna, ham, and summer sausage, were sampled and analyzed for HCA content. Raw meat samples were formed into 100 g patties, 1.5 cm thick and 10 cm in diameter, which were fried in a thermostat-controlled Teflon-coated electric griddle (Presto, model 073202) at 150 °C for 6 min, at 190 °C for 10 min, or at 230 °C for 14 min. The smoked sausages were fried by rolling them in the griddle at the same temperatures and times of cooking as the raw meat patties. These samples were also analyzed for HCA content after being charcoal grilled at two temperatures and times, 240 °C for 7 min or 200 °C for 12 min. The temperature of the grill was monitored with a 20-gauge hypodermic probe-type thermocouple connected to a Doric temperature recorder (Trendicator 410A, San Diego, CA). This device was also used to measure the internal temperature of the meat patties by inserting the probe into the geometric center of the patty at a 45° angle. For each treatment, three meat samples were cooked (fried or barbecued) at the same time. All of the cooked meat samples were wrapped in plastic wrap and kept frozen until analyzed.

Solid-Phase Extraction of Samples. Before analyses, each sample was divided equally in random fashion without bias to heavily browned areas. The brown surfaces (1−2 mm thickness) of identically treated samples were removed with a scalpel. Next, the scrapes of the crusts were pooled and used for analyses after grinding with a Micro-Mill. The remaining halves of the identically treated samples were pooled and used for whole sample analysis. With the precooked sausages, samples from the product's skin were taken after the casing was removed. Sample cleanup and HCA extraction were based on the method described by Gross and Grüter (1992). At the point of analyses, 3.00 g samples from cooked and precooked meat products were homogenized in 20.0 mL of 1.0 M sodium hydroxide using a commercial Waring blender for 3 min at medium speed. The homogenates were then mixed thoroughly with Extrelut refill material and loaded to empty Extrelut columns.

Bond Elut PRS cartridges were fitted with coupling adapters and conditioned with 5 mL of dichloromethane (DCM), and another 2 mL of DCM was pipetted into each cartridge. A Supelco Visiprep SPE vacuum manifold was used for manipulation of eluent flow rate with the sample solid-phase extraction cartridges. To elute HCAs from the Extrelut columns to PRS cartridges, 60 mL of dichloromethane was used. The PRS cartridges were dried for 4 min under maximum vacuum (15 in. of Hg) and then rinsed successively at 1.5 mL/min with 6 mL of 0.1 M hydrochloric acid, 15 mL of methanol/0.1 M hydrochloric acid (45:55 v/v), and 2 mL of water. These three washes, containing nonpolar amines, were collected into empty Extrelut columns for further extraction. The polar amines were still bonded on the PRS cartridges.

Bond Elut C-18 cartridges (100 mg) were used to concentrate the polar amines. These cartridges first were conditioned with 1 mL of methanol, followed by 10 mL of water, and then they were coupled to the PRS cartridges that contained the polar amines. The HCAs were eluted from the PRS cartridge to the C-18 cartridge with 20 mL of 0.5 M ammonium acetate (adjusted to pH 8.0 with concentrated ammonium hydroxide), and the PRS cartridges were discarded. The C-18 cartridges were rinsed with 2 mL of water and dried by applying strong positive nitrogen pressure. The adsorbed polar amines were eluted into microvials using 0.8 mL of methanol-concentrated ammonium hydroxide solution (9:1 v/v).

The eluates containing the nonpolar amines were neutralized by adding 0.5 mL of concentrated ammonium hydroxide and diluted with water to <20% methanol. These mixtures were passed through previously conditioned Bond-Elut C-18 cartridges (500 mg) at a rate of 4−5 mL/min. The C-18 cartridges were conditioned with 2 mL of methanol and then 10 mL of water. The cartridges were then dried by applying strong positive nitrogen pressure. Finally, the adsorbed nonpolar amines were eluted into Teflon-lined screw-capped amber vials using 1.4 mL of methanol-concentrated ammonium hydroxide solution (9:1 v/v).

Polar and nonpolar amine extracts were concentrated under nitrogen and dissolved in 25 μL of methanol. For HPLC analysis, 8.0 μL aliquots were injected into a 20 μL loop by the partial filling technique.

The extraction of meat samples was repeated three times with each treatment, and each extract was analyzed by two injections onto the HPLC. Means, standard deviations, and coefficients of variance (CVs) were determined for each treatment.

High-Performance Liquid Chromatography (HPLC) Analysis. The instrumentation used for the separation and analysis of the HCAs was a Hewlett-Packard 1090 A, series II HPLC (Palo Alto, CA) fitted with both a photodiode array UV−visible detector (HP 1040) and a programmable fluorescence detector (HP 1046 A). Data were collected and analyzed with an HP 9000 series 300 ChemStation. Separation was achieved with a silica-based reversed-phase TSK-GEL ODS-80TM column (25 cm × 4.6 mm, 5 μm, 80 Å), protected by an ODS-80TM guard column (TosoHaas, Montgomeryville, PA).

Three solvents were used for the HPLC mobile phase: solvent A, 0.01 M triethylamine (pH 3.2); solvent B, 0.01 M triethylamine (pH 3.6); and solvent C, acetonitrile. The pH for solvents A and B was adjusted with 1.0 M phosphoric acid. The gradient profile was linear, and the program was 0−10.00 min, 5−15% C in A; 10−10.01 min, exchange of A with B; 10.01−20.00 min, 15−25% C in B; 20.01−26.00 min, 25−43% C in B. A 25 min postrun time was used for column rinse and equilibration. Retention time and on-line UV spectral library matching were used for peak identification in the samples. The HPLC analyses were done at 40 °C at a flow rate of 1 mL/min.

Standard Curves. Samples of 10.00 mg of pure HCAs were transferred to 10 mL volumetric flasks and made to volume with methanol to give solutions that were 1000 μg/mL. These solutions were used for serial dilutions for each HCA compound. Dilutions of 250, 100, 50, and 25 ng/mL were made with methanol. Coefficients of determination (r^2) for HCA standard curves were 0.985 for IQ, 0.982 for MeIQ, 0.996 for MeIQx, 0.999 for 4,8-DiMeIQx, 0.999 for PhIP, 0.990 for norharman, 0.990 for harman, and 0.992 for Trp-p-2.

RESULTS AND DISCUSSION

HCAs in Uncooked Sausages. Tables 1 and 2 summarize the amounts of MeIQx, PhIP, harman,

Table 1. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Produced on the Crusts of Processed Meat Samples Fried at Three Different Temperatures for Three Different Times

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
fresh pork sausage	3 + 3	150	ND[c]	ND	ND	ND	ND
	5 + 5	190	ND	0.04 ± 0.01	2.84 ± 0.19	0.62 ± 0.20	0.16 ± 0.01
	7.5 + 7.5	230	0.92 ± 0.33	0.08 ± 0.01	3.21 ± 0.33	1.90 ± 0.05	0.68 ± 0.01
bratwurst	3 + 3	150	ND	ND	0.54 ± 0.01	0.16 ± 0.03	0.05 ± 0.01
	5 + 5	190	0.79 ± 0.03	0.44 ± 0.10	0.30 ± 0.20	0.17 ± 0.02	0.14 ± 0.01
	7.5 + 7.5	230	1.20 ± 0.09	0.71 ± 0.06	3.14 ± 0.55	0.83 ± 0.14	0.81 ± 0.19
Italian sausage	3 + 3	150	ND	ND	1.95 ± 0.80	0.44 ± 0.17	0.05 ± 0.01
	5 + 5	190	0.81 ± 0.02	3.01 ± 0.63	4.87 ± 0.16	1.37 ± 0.06	0.16 ± 0.06
	7.5 + 7.5	230	3.44 ± 0.16	3.00 ± 0.25	7.66 ± 0.61	2.14 ± 0.13	0.27 ± 0.02
smoked sausage	6[d]	150	ND	0.67 ± 0.02	1.10 ± 0.12	0.22 ± 0.04	0.13 ± 0.07
	10	190	0.28 ± 0.04	1.15 ± 0.04	1.90 ± 0.23	0.43 ± 0.08	0.17 ± 0.02
	15	230	1.52 ± 0.05	5.83 ± 0.76	10.6 ± 0.63	2.51 ± 0.28	1.23 ± 0.22

[a] Values are the averages of three replicates. [b] Each side of the meat patty was fried for 3, 5, or 7.5 min. [c] Not detected. [d] Sausage rolled in the frying pan for 6, 10, or 15 min.

Table 2. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Produced on the Crusts of Processed Meat Samples Barbecued at Two Different Temperatures for Two Different Times[a]

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD_	Trp-p-2 (ng/g ± SD)
fresh pork sausage	6 + 6	200	ND[c]	0.09 ± 0.01	2.19 ± 0.53	1.27 ± 0.49	0.22 ± 0.00
	3.5 + 3.5	240	1.97 ± 0.97	1.89 ± 0.93	2.44 ± 0.93	2.25 ± 0.99	1.92 ± 0.89
bratwurst	6 + 6	200	1.23 ± 0.26	0.37 ± 0.20	2.28 ± 0.21	1.53 ± 0.20	0.95 ± 0.25
	3.5 + 3.5	240	0.89 ± 0.13	0.57 ± 0.27	1.94 ± 0.37	0.56 ± 0.19	0.56 ± 0.11
Italian sausage	6 + 6	200	1.22 ± 0.04	2.35 ± 0.13	9.18 ± 0.21	2.53 ± 0.15	0.29 ± 0.06
	3.5 + 3.5	240	0.89 ± 0.11	1.96 ± 0.15	7.30 ± 0.20	1.67 ± 0.22	0.28 ± 0.08

[a] Values are the averages of three replicates. [b] Each side of the meat patty was grilled for 6 or 3.5 min. [c] Not detected.

Table 3. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Produced on the Crust of Ground Beef Patties and Eye Round Steak Fried at Three Different Temperatures for Three Different Times[a]

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
15% ground beef	3 + 3	150	ND[c]	0.43 ± 0.10	0.96 ± 0.18	0.31 ± 0.08	ND
	5 + 5	190	0.84 ± 0.00	0.57 ± 0.14	2.00 ± 0.12	0.83 ± 0.30	1.70 ± 0.28
	7.5 + 7.5	230	1.00 ± 0.11	0.25 ± 0.04	5.65 ± 0.01	1.70 ± 0.70	1.50 ± 0.01
5% ground beef	3 + 3	150	ND	0.56 ± 0.06	1.28 ± 0.43	0.23 ± 0.09	0.71 ± 0.40
	5 + 5	190	2.01 ± 0.45	2.60 ± 0.88	5.00 ± 1.60	0.42 ± 0.06	0.46 ± 0.16
	7.5 + 7.5	230	2.34 ± 0.35	3.13 ± 0.52	5.06 ± 0.90	1.36 ± 0.25	0.78 ± 0.25
eye round steak	15 + 15	150	ND	0.26 ± 0.01	1.35 ± 0.35	0.92 ± 0.28	0.16 ± 0.03
	10 + 10	190	ND	0.15 ± 0.05	1.16 ± 0.12	0.75 ± 0.06	0.13 ± 0.01
	7.5 + 7.5	230	1.16 ± 0.37	1.70 ± 0.12	3.88 ± 0.18	1.00 ± 0.30	0.63 ± 0.02

[a] Values are the averages of three replicates. [b] Each side of the meat patty was fried for 3, 5, or 7.5 min. [c] Not detected.

norharman, and Trp-p-2 found on the surfaces of processed meat samples that were fried or charcoal grilled for various times and at various temperatures. The internal parts of the meat products showed lower HCA contents than the surfaces. The levels of these HCA were low or nondetectable in sausage samples fried at 150 °C but increased at higher cooking temperatures such as 190 or 230 °C. A sharp increase in the formation of most HCAs occurred when the temperature increased from 190 to 230 °C. Among sausages, Italian sausage fried at 230 °C showed the highest level of MeIQx (3.44 ng/g), and smoked sausage fried at 230 °C showed the highest levels of PhIP (5.83 ng/g), Trp-p2 (1.23 ng/g), norharman (10.6 ng/g), and harman (2.51 ng/g). Low levels of MeIQ (0.14 ng/g) were detected in smoked sausage fried at 230 °C for 7.5 min per side. Other than MeIQx, the smoked sausage represents the meat product with the highest levels of HCAs compared to the other processed meat products fried under identical conditions.

Studies have shown that a higher fat content in ground meat resulted in shorter time needed to reach a fixed meat surface temperature (Holtz et al., 1985). That is because fat is an effective heat-transfer agent. In our study, the internal parts of the meat products showed lower HCA content than the surfaces (Tables 5 and 6). The effect of high fat content in bratwurst was more pronounced inside the meat sample than on the outer surfaces. Comparison of the level of HCAs in bratwurst (20% fat) to that of Italian sausage (15% fat) shows this effect clearly. For example, the crust of the Italian sausage contained 2.8 times more PhIP than the bratwurst crust. However, a comparison of both meat samples for their internal content of PhIP showed that Italian sausage had 4 times more than bratwurst. This difference is probably due to the high fat content in bratwurst that enhances heat transfer. The effect of fat is more pronounced on the surface where more fat melting occurs because of the higher exposure to heat. Yet in this study the exact role of the fat content on the

Table 4. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Produced on the Crust of Ground Beef Patties and Eye Round Steak Barbecued at Two Different Temperatures for Two Different Times[a]

meat sample	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
15% ground beef	6 + 6	200	0.27 ± 0.09	2.35 ± 1.16	1.87 ± 0.78	0.61 ± 0.20	ND[c]
	3.5 + 3.5	240	0.80 ± 0.21	0.10 ± 0.03	2.17 ± 1.22	0.88 ± 0.01	ND
5% ground beef	6 + 6	200	1.51 ± 0.04	0.71 ± 0.01	2.64 ± 0.32	0.99 ± 0.14	0.15 ± 0.02
	3.5 + 3.5	240	1.70 ± 0.44	3.30 ± 0.35	1.30 ± 0.24	0.52 ± 0.08	0.14 ± 0.07
eye round steak	15 + 15	200	2.40 ± 0.11	4.20 ± 0.20	1.70 ± 0.06	0.60 ± 0.06	0.07 ± 0.02
	7 + 7	240	4.00 ± 0.14	2.20 ± 0.37	30.0 ± 2.7	28.6 ± 2.10	1.59 ± 0.08

[a] Values are the average of three replications. [b] Each side of the meat sample was barbecued for 6, 3.5, 15, or 7 min. [c] Not detected.

Table 5. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Produced in the Entire Sample of Processed Meat Samples Fried at Three Different Temperatures for Three Different Times[a]

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
fresh pork sausage	3 + 3	150	ND[c]	ND	ND	ND	ND
	5 + 5	190	ND	0.18 ± 0.02	0.74 ± 0.09	0.22 ± 0.01	0.015 ± 0.001
	7.5 + 7.5	230	0.63 ± 0.080	0.38 ± 0.06	1.12 ± 0.04	0.39 ± 0.06	0.043 ± 0.007
bratwurst	3 + 3	150	ND	ND	ND	ND	ND
	5 + 5	190	0.30 ± 0.050	0.42 ± 0.03	0.72 ± 0.22	0.17 ± 0.01	0.080 ± 0.003
	7.5 + 7.5	230	0.72 ± 0.080	0.28 ± 0.01	1.42 ± 0.15	0.39 ± 0.12	0.200 ± 0.025
Italian sausage	3 + 3	150	ND	ND	ND	ND	ND
	5 + 5	190	0.33 ± 0.070	0.79 ± 0.16	2.47 ± 0.27	0.67 ± 0.08	0.060 ± 0.003
	7.5 + 7.5	230	0.57 ± 0.023	1.13 ± 0.15	3.08 ± 0.25	0.84 ± 0.08	0.060 ± 0.006
smoked sausage[d]	6	150	ND	ND	ND	ND	ND
	10	190	ND	0.21 ± 0.01	0.93 ± 0.03	0.22 ± 0.12	0.043 ± 0.006
	15	230	0.13 ± 0.050	0.22 ± 0.02	1.21 ± 0.20	0.22 ± 0.02	0.220 ± 0.020

[a] Values are the averages of three replicates. [b] Each side of the meat patty was fried for 5 or 7.5 min. [c] Not detected. [d] Smoked pork sausage rolled in the frying pan for 10 or 15 min.

Table 6. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Produced in the Entire Sample of Fresh Pork Sausage, Bratwurst, Italian Sausage, and Smoked Pork Sausage Barbecued at Two Different Temperatures for Two Different Times[a]

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
fresh pork sausage	6 + 6	200	0.47 ± 0.17	0.30 ± 0.05	1.52 ± 0.22	0.39 ± 0.02	0.03 ± 0.008
	3.5 + 3.5	240	0.43 ± 0.03	0.31 ± 0.01	1.19 ± 0.14	0.36 ± 0.11	0.03 ± 0.003
bratwurst	6 + 6	200	0.43 ± 0.06	0.09 ± 0.04	0.79 ± 0.41	0.15 ± 0.07	0.13 ± 0.020
	3.5 + 3.5	240	0.35 ± 0.04	0.27 ± 0.09	0.57 ± 0.09	0.10 ± 0.02	0.10 ± 0.012
Italian sausage	6 + 6	200	0.80 ± 0.11	1.27 ± 0.15	4.20 ± 0.38	1.16 ± 0.11	0.13 ± 0.017
	3.5 + 3.5	240	0.40 ± 0.08	0.69 ± 0.09	1.98 ± 0.33	0.50 ± 0.06	0.07 ± 0.009

[a] Values are average of three replications. [b] Each side of the meat sample was barbecued for 6 or 3.5 min

formation of HCAs is not very clear. In addition, we are using different meat products with different additives that might enhance or prevent the development of mutagens.

HCAs in Ground Beef and Steak. The levels of heterocyclic amines were generally higher in the 5% fat ground beef than in 15% fat ground beef (Tables 3 and 4). The 5% fat ground beef showed 2.5 times higher MeIQx and 12.5 times higher PhIP content than did the 15% fat ground beef samples when both were fried at 230 °C. Our results are in agreement with the study by Knize et al. (1985), who also found that lower levels of mutagens formed in fried meat products with higher fat content. Eye round steak, on the other hand, showed the highest levels of norharman (30.0 ng/g), harman (28.6 ng/g), and Trp-p-2 (1.59 ng/g) when grilled at 240 °C for 14 min. Although norharman is not mutagenic, it will cause DNA adduct formation in the presence of aromatic amines such as alanine or toluidine isomers (Mori et al., 1996). The crust of steak grilled at 200 °C for 12 min contained 1.25 ng/g IQ and 0.54 ng/g MeIQ, whereas that of steak grilled at 240 °C for 7 min contained 4.11 ng/g IQ and 0.38 ng/g MeIQ. The imidazoquinoxaline (4,8-DiMeIQx) was detected at a level of 0.31 ng/g in ground beef (15% fat) fried at 230 °C. High levels of fat in meat products may result in lower levels of HCAs formed. This apparently results from dilution of the mutagenic precursors in meat by the fat (Skog, 1993). As in the processed meat products, the internal portions of the 15% ground beef, 5% ground beef, and steak contained lower HCAs when compared to the external surfaces (Tables 7 and 8). Again, these differences may be related to the fat serving as a more efficient heat-transferring agent on the surface. There were 2.3 times higher MeIQx on the surface of a 5% fat ground beef patty fried at 230 °C than on the surface of a 15% fat ground beef patty under the same conditions. On the other hand, the whole patty of the 5% fat ground beef showed 3.0 times higher MeIQx than the inside of 15% fat ground beef patty.

HCAs in Precooked Meats. Samples of the precooked meats (ham, summer sausage, and bologna) showed no HCA contents. Low levels or lack of thermally induced mutagens are expected in precooked

Table 7. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Formed in the Entire Sample of 15% Fat Ground Beef, 5% Fat Ground Beef, and Eye Round Steak Fried at Two Different Temperatures for Two Different Times[a]

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
15% ground beef	5 + 5	190	0.14 ± 0.02	ND[c]	1.13 ± 0.25	0.17 ± 0.004	ND
	7.5 + 7.5	230	0.27 ± 0.07	ND	1.04 ± 0.14	0.30 ± 0.015	0.38 ± 0.14
5% ground beef	5 + 5	190	1.11 ± 0.14	0.85 ± 0.18	2.60 ± 0.30	0.15 ± 0.024	0.36 ± 0.06
	7.5 + 7.5	230	0.78 ± 0.09	1.09 ± 0.30	1.60 ± 0.31	0.34 ± 0.060	0.29 ± 0.04
eye round steak	5 + 5	190	ND	0.06 ± 0.01	0.50 ± 0.05	0.24 ± 0.045	0.03 ± 0.01
	7.5 + 7.5	230	ND	0.40 ± 0.13	1.41 ± 0.20	0.83 ± 0.110	0.22 ± 0.05

[a] Values are average of three replications. [b] Each side of the meat patty was fried for 5 or 7.5 min. [c] Not detected.

Table 8. Levels of MeIQx, PhIP, Norharman, Harman, and Trp-p-2 Formed in the Entire Sample of 15% Fat Ground Beef, 5% Fat Ground Beef, and Eye Round Steak Barbecued at Two Different Temperatures for Two Different Times[a]

meat product	cooking time[b] (min)	cooking temp (°C)	MeIQx (ng/g ± SD)	PhIP (ng/g ± SD)	norharman (ng/g ± SD)	harman (ng/g ± SD)	Trp-p-2 (ng/g ± SD)
15% ground beef	6 + 6	200	0.14 ± 0.001	0.64 ± 0.03	1.40 ± 0.73	0.13 ± 0.04	ND
	3.5 + 3.5	240	ND[c]	0.31 ± 0.09	0.49 ± 0.07	0.09 ± 0.01	ND
5% ground beef	6 + 6	200	0.63 ± 0.04	0.47 ± 0.20	1.07 ± 0.62	0.53 ± 0.02	0.26 ± 0.047
	3.5 + 3.5	240	0.44 ± 0.05	1.40 ± 0.41	0.32 ± 0.09	0.23 ± 0.19	0.11 ± 0.067
eye round steak	6 + 6	200	1.30 ± 0.05	1.40 ± 0.33	6.40 ± 0.33	2.31 ± 0.37	0.12 ± 0.020
	3.5 + 3.5	240	1.61 ± 0.08	0.43 ± 0.15	7.18 ± 0.22	2.36 ± 0.10	0.14 ± 0.010

[a] Values are average of three replications. [b] Each side of the meat patty was fried for 6 or 3.5 min. [c] Not detected.

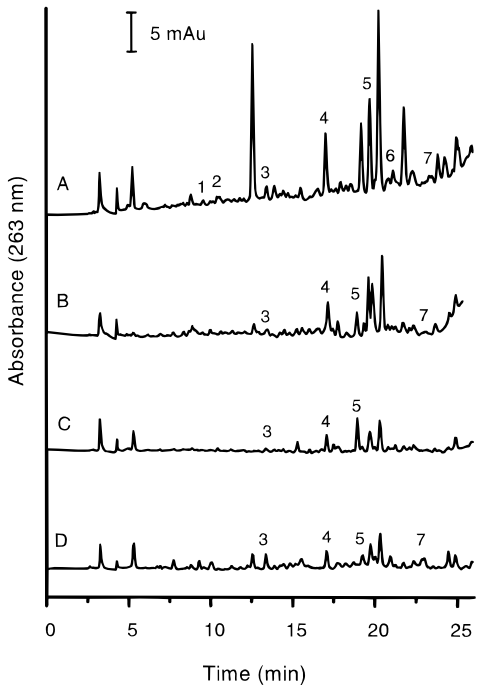

Figure 1. HPLC chromatograms of meat product extracts observed with UV detection: (A) crust of eye round steak; (B) crust of smoked sausage; (C) crust of bratwurst; (D) crust of Italian sausage. Ternary mobile phase system of A = 0.01 M triethylamine phosphate (TEAP), pH 3.2; B = 0.01 M TEAP, pH 3.6; C = acetonitrile used to separate HCAs on a TSK-GEL ODS-80TM column. All samples were fried at 230 °C for 15 min, except for the steak, which was grilled at 240 °C for 7 min. Peaks: (1) IQ; (2) MeIQ; (3) MeIQx; (4) norharman; (5) harman; (6) Trp-p-2; (7) PhIP.

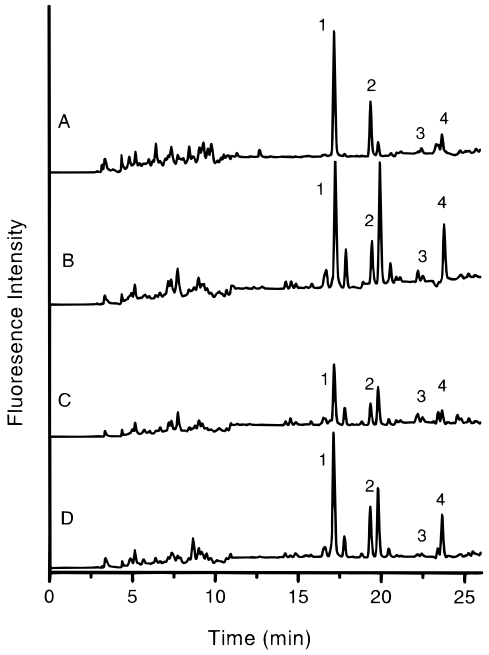

Figure 2. HPLC chromatograms of meat product extracts obtained by fluorescence detection: (A) crust of eye round steak; (B) crust of smoked sausage; (C) crust of bratwurst; (D) crust of Italian sausage. All samples were fried at 230 °C for 15 min, except for the steak, which was grilled at 240 °C for 7 min. Peaks: (1) norharman; (2) harman; (3) Trp-p2; (4) PhIP. For chromatographic conditions, refer to Figure 1.

sausages, because they are cooked at lower temperatures during processing. The highest temperature used in the traditional smoking/cooking operation for sausage products is 85 °C (Rust, 1987).

Conformation and Quantification of HCAs. Figures 1 and 2 show the UV and fluorescence chromatograms of different extracts obtained from the meat products. The peaks that corresponded to UV-absorbing heterocyclic amines were confirmed by comparing them to those of reference spectra. Figure 3 illustrates the match of shapes between the spectra recorded from the meat samples and the on-line library spectra created using pure standards. To quantitate the levels of PhIP, norharman, harman, Trp-p-1, and Trp-p-2, a programmable fluorescence detector set at the maximum excitation/emission wavelength for each compound was used.

The average recoveries of the HCAs from the extraction of spiked meat products were 66.8% for IQ, 48.2% for MeIQ, 72.5% for MeIQx, 42.0% DiMeIQx, 45.7% for PhIP, 30.5% for Trp-p-2, 77.0% for norharman, and

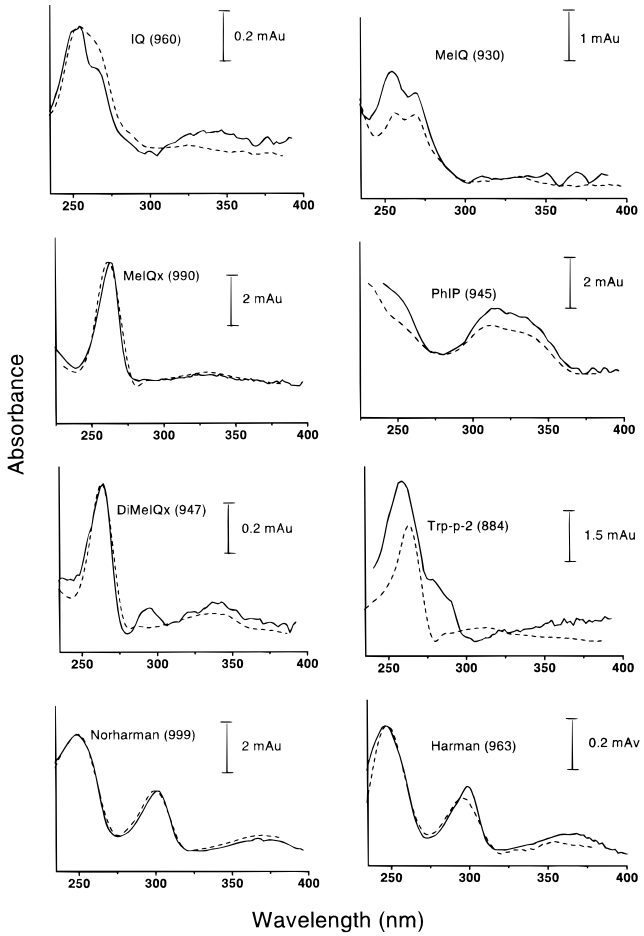

Figure 3. On-line UV spectra (solid line) of IQ, MeIQ, MeIQx, PhIP, norharman, and harman in different meat samples compared with library spectra (dashed lines) obtained from the standards. Numbers in parentheses are matching factors, where 1000 is a perfect spectral match. For chromatographic conditions, refer to Figure 1.

70.0% for harman. No corrections were made for the recovery of HCAs.

The limits of detection, which are dependent on the meat matrix used for analysis, were 0.06 ng/g for MeIQx, 0.08 ng/g for PhIP, 0.54 ng/g for norharman, 0.16 ng/g for harman, and 0.015 ng/g for Trp-p-2 at a signal-to-noise ratio of 5:1 using fried meat samples. Because of coextract interference, IQ, MeIQ, and Trp-p-1 could not be detected in all meat samples. Their detection limits were 0.81 ng/g for IQ, 0.28 ng/g for MeIQ, and 0.03 ng/g for Trp-p-1.

In this experiment, degree of doneness and the internal temperature were very important. The meat samples were cooked to different degrees of doneness, that is, rare, medium rare, medium, well done, and very well done. Table 9 shows the internal temperatures of the meat products and their degrees of doneness. The cooking times for the meat patties were sufficient to bring the products to an internal temperature that would kill any pathogenic microorganisms.

The contents of HCAs in the crusts of four processed meat products are illustrated in Figure 4. Differences in the fat content of meat products have a significant effect on the level of HCAs formed. Bratwurst showed the lowest amount when fried at 230 °C because of its high fat content compared to other meat products analyzed. Also, bratwurst contained water and carbohydrates, which may have acted to reduce the levels of HCAs formed. Fresh pork sausage and Italian sausage were both made from pork and different kinds of spices. As Figure 4 shows, the level of HCAs formed on the crust of fresh pork sausage is low compared to that on Italian sausage because of its higher fat content, when both were fried under identical conditions. Also, the presence of a reducing sugar, dextrose, in fresh pork sausage may have decreased the formation of HCAs. In this case, the reduction may have been due to a blocked reaction between creatinine and Maillard reaction intermediates such as 5-(hydroxymethyl)-2-furfural. Among these four processed meats, the greatest exposure to HCAs would be from eating smoked sausage fried at 230 °C for 15 min.

Exposure to HCAs from consuming four processed meat products as whole patties is shown in Figure 5. Because of the casing on the smoked sausage, which may have acted as a barrier to moisture and heat transfer, the total level of the HCAs was very low compared to other products. For the other processed meat products, the total level of HCAs was negatively correlated to the level of fat content when fried at 190 °C for 10 min. However, as the temperature of cooking increased to 230 °C, the trend changed. The level of HCAs in the bratwurst (20% fat) became higher than that of fresh pork sausage (17% fat). This may be due

Table 9. Surface Temperature, Internal Temperature, and Degree of Doneness of Fresh Pork Sausage, Bratwurst, Italian Sausage, and Smoked Sausage Fried at Different Temperatures for Different Times

meat product	cooking time[a] (min)	surface temp (°C)	internal temp (°C)	degree of doneness[b]
fresh pork sausage	3 + 3	150	55	very rare
	5 + 5	190	67	medium rare
	7.5 + 7.5	230	77	well done
bratwurst	3 + 3	150	60	rare
	5 + 5	190	72	medium
	7.5 + 7.5	230	79	well done
Italian sausage	3 + 3	150	58	very rare
	5 + 5	190	67	medium rare
	7.5 + 7.5	230	75	well done
smoked sausage	6[c]	150	60	rare
	10	190	70	medium
	15	230	75	well done

[a] Each side of the meat patty was fried for 3, 5, and 7.5 min [b] The degrees of doneness depending on the internal temperature defined according to the guidelines of Starrak and Kenneth (1982). [c] Total cooking time.

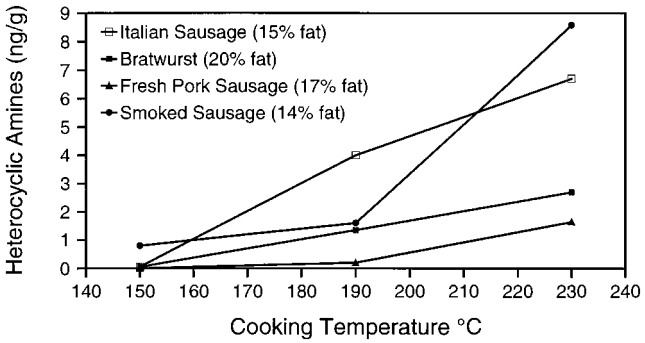

Figure 4. Total amounts of HCAs (ng/g) formed in the crusts of fresh pork sausage, bratwurst, Italian sausage, and smoked sausage fried at 150 °C (6 min), 190 °C (10 min), and 230 °C (15 min).

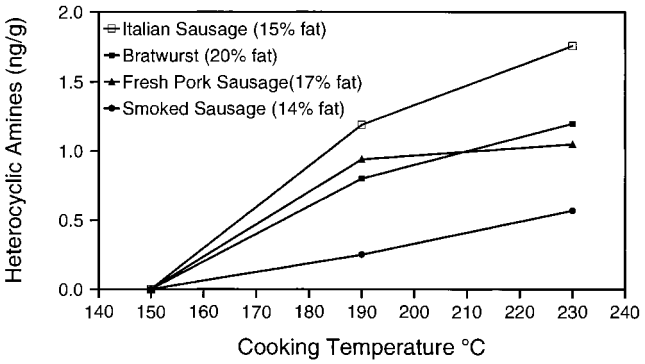

Figure 5. Total amounts of HCAs (ng/g) formed in the whole sample of fresh pork sausage, bratwurst, Italian sausage, and smoked sausage fried at 150 °C (6 min), 190 °C (10 min), and 230 °C (15 min).

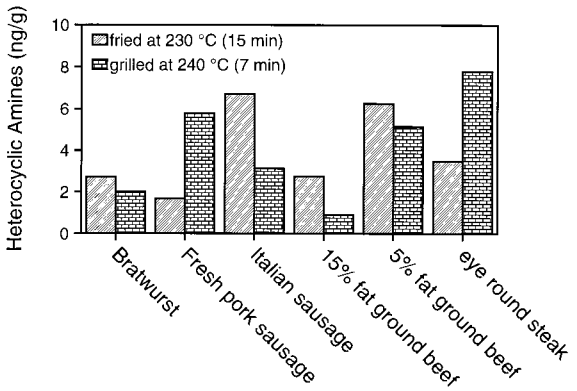

Figure 6. Level of total HCAs (ng/g) formed in fried versus grilled meat products cooked to a well-done temperature. Hatched bars represent meat products fried at 230 °C for 15 min, and blocked bars represent meat products grilled at 240 °C for 7 min.

to the effect of the fat as a heat transfer agent. The high level of total HCAs in the whole patty of Italian sausage (15% fat), Figure 5, is probably due to the high content of HCAs in the crust of this meat sample compared to the other processed meat products.

Effect of Methods of Cooking on the Level of HCAs. Comparing the results in Table 1 (cooking by frying) and Table 3 (cooking by grilling) shows that there was a difference in the level of HCAs formed. It was clear that the time and temperature of cooking had a profound effect on the level of HCA formation. Many studies have shown that barbecuing meat products mainly caused formation of polycyclic aromatic hydrocarbons, especially benzo[α]pyrene (Hattermer-Frey et al., 1991). As shown in Figure 6, grilling of meat products at 240 °C for 7 min versus frying them at 230 °C for 15 min did not cause a big reduction in the level of total HCA produced. In fact, the total levels of HCAs in fresh pork sausage were increased by 70% by grilling as contrasted to frying.

Ways to reduce exposure to HCAs have been explored (Knize et al., 1995). Cooking at temperatures below 200 °C for a short time has been shown to reduce the amounts of HCAs formed in meat products. Also, microwave pretreatment of meat patties before frying decreased the level of the mutagens to 10% of the level formed in fried meat patties with no microwave treatment. Adding glucose or lactose to the meat patties resulted in a significant reduction of HCA formation. All of these attempts are practical ways to lower human exposure to such genotoxic compounds.

Conclusions. The precooked meat samples (ham, summer sausage, and bologna) did not contain detectable levels of HCAs. That was due to the low temperature of cooking (85 °C) during processing/cooking of those products, which did not promote HCA formation. However, HCAs at different levels were found in raw nonprocessed and processed meat products after they were fried or grilled. When the temperature of cooking increased from 190 to 230 °C, levels of the mutagens increased 2−5 times. Levels were 2−4 times higher on the crusts of the meat samples compared to the interior portions. Smoked sausage showed the highest levels of HCAs on the external surfaces compared to the other meat products. When the internal portions were included in the analysis, Italian sausage was found to contain the highest levels of HCAs.

The results showed that frying meat products versus charcoal grilling at approximately the same temperature caused HCA levels to increase. This increase was correlated positively with the decrease in the fat content of the meat samples. Generally, as the cooking temperature exceeded 200 °C, the total level of HCAs increased drastically. This was seen especially in smoked sausage, 5% ground beef, 15% fat ground beef, and eye round steak.

ABBREVIATIONS USED

HCA, heterocyclic amine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ, 2-amino-3,4-imethylimidazo-[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-methyl-6-phenylimidazo[4,5-*b*]pyridine; Trp-p-2, 3-amino-1-methyl -5*H*-pyrido[4,3-*b*]indole; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole.

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