Formation of Mutagenic/Carcinogenic Heterocyclic Amines in Dry-Heated Model Systems, Meats, and Meat Drippings

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Mixtures of amino acids, creatine, and glucose simulating the composition of six different kinds of meats (beef, chicken breast, chicken thigh, turkey breast, pork, and fish) were dry-heated to simulate the formation of heterocyclic amines in meats. The presence of 16 heterocyclic amines was investigated in the model systems and in the six meats and their corresponding meat drippings to determine the importance of meat composition to heterocyclic amine formation. Nine mutagenic amines (IQ, MeIQ, 8-MeIQx, 4,8-DiMeIQx, PhIP, IQx, IFP, DMIP, and TMIP) were found to be present at concentrations >0.1 ng/g in some of the model systems and in some of the meats or pan residues. Heterocyclic amine concentrations clearly are affected by precursor composition in this model system, and the same nine heterocyclic amines formed in the meat and in the model system show that this is a well-controlled surrogate for the reaction conditions that occur in meats during cooking.

Keywords: *Heterocyclic amines; cooked meats; meat drippings; model systems; formation*

INTRODUCTION

During the cooking process a number of genotoxic compounds are formed from endogenous constituents of meat, and among these are various heterocyclic amines (Sugimura and Sato, 1983; Felton et al., 1986). Three of the most abundant amines, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (8-MeIQx), and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), are present in beef at levels of 1−50 ng/g for PhIP and 1−2 ng/g for 8-MeIQx and 4,8-DiMeIQx (Felton and Knize, 1990). These heterocyclic amines were first discovered because of their potent mutagenic activity on the Ames/*Salmonella* test, but PhIP and MeIQx are carcinogens in rodent bioassays, inducing tumors in a variety of tissues (Ohgaki et al., 1987; Kato et al., 1988). Colon and mammary gland tumors have been seen in all rat cancer bioassays in which PhIP has been tested (Ito et al., 1991; Ghoshal et al., 1994; El-Bayoumy et al., 1995). Recently, PhIP has also been reported to induce carcinomas in the prostate of the male rat (Shirai et al., 1997).

Heterocyclic amines are formed from free amino acids, creatine, and glucose present in foods [see review by Skog (1993)]. Factors reported to affect the formation of heterocyclic amines in foods include pH, precursor concentrations, type of amino acids, and processing time and temperature. Heterocyclic amines are formed at normal cooking temperatures, and in general, a higher temperature or longer cooking time increases the concentration of heterocyclic amines produced (Gross and Grüter, 1992; Knize et al., 1994; Skog et al., 1995). In a recent study, Arvidsson et al. (1997) examined the kinetics of formation of heterocyclic amines in a water-based model system and determined their temperature dependence. Modeling experiments are useful tools for studying the influence of physical and chemical parameters and precursors on heterocyclic amine formation and may lead to methods for preventing or limiting the formation of these mutagenic and carcinogenic compounds in foods and food flavors. Model systems can also be used to produce heterocyclic amine mutagens that are found in cooked foods but are not fully characterized, as we did in this study with IFP (an aminodimethylimidazofuropyridine with unresolved structure).

To simulate the dry reactions that seem to occur at the meat surface, we developed a model system to mimic these processes. We determined the relative free amino acids, creatine, and glucose concentrations in six meat samples (beef, chicken breast, chicken thigh, turkey breast, pork, and fish) and made a model reaction for each meat type by heating these precursors at the same relative concentrations. Meat pieces were also heated in a laboratory furnace. Triplicate experiments were performed and the heterocyclic amines extracted and quantified to compare the types and amounts produced from the meat (natural precursors) or in our model system. Sixteen mutagenic heterocyclic amines and two comutagenic heterocyclic amines, harman and norharman, were analyzed by solid-phase extraction and HPLC using the standard addition method to determine extraction recoveries. Formation of the heterocyclic amines in the meats, in the corresponding meat drippings, and in the model systems with various precursor compositions was determined.

MATERIALS AND METHODS

Materials. The heterocyclic amines used as analytical standards were purchased from Toronto Research Chemicals (Downsview, ON) and included 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 8-MeIQx, 4,8-DiMeIQx, 2-amino-1,6-dimethylimidazo-[4,5-*b*]pyridine (DMIP), PhIP, 2-amino-6-methyldipyrido[1,2-

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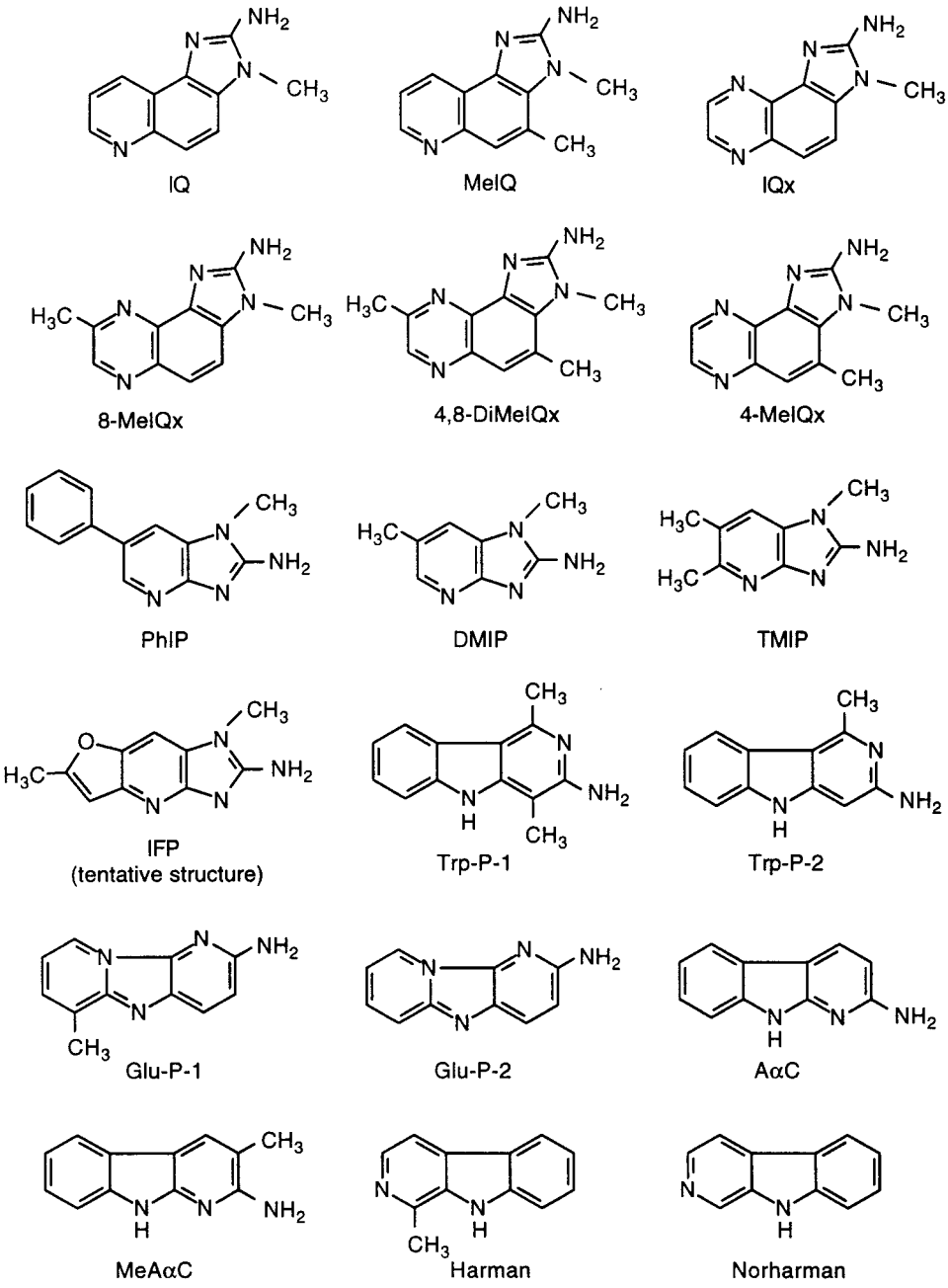

Figure 1. Structures of the 18 compounds studied.

α:3′,2′-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-α:3′,2′-*d*]-imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC). 1-Methyl-9*H*-pyrido[4,3-*b*]-indole (harman) and 9*H*-pyrido-[4,3-*b*]indole (norharman) were from Aldrich Chemical Co. (Milwaukee, WI). 2-Amino-1,5,6-trimethylimidazo[4,5-*b*]pyridine (TMIP) was the kind gift of Dr. Mary Tanga, SRI International (Tanga et al., 1994) and is available from Dr. Harold Seifried (National Cancer Institute, Chemical and Physical Carcinogenesis Branch, 6130 Executive Blvd., EPN/700, Bethesda, MD 20892), and IFP was a natural product isolated from a creatine-added meat mixture (Knize et al., 1990). 2-Amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx) was the kind gift of Dr. Spiros Grivas. 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (4-MeIQx) was synthesized at Lawrence Livermore National Laboratory. All analytes were >98% pure as determined by HPLC and UV detection at 262 nm. The structures of the compounds studied in this work are shown in Figure 1.

Six meat samples [including beef top round steak; boneless, skinless chicken breast, chicken thigh, and turkey breast; pork loin sirloin chops; and cod fish (previously frozen)] were purchased from a local market. Meat pieces ~5.5 × 1.5 cm thick and weighing ~50 g were suspended on a galvanized wire support with 6.3 mm wire spacing over a glass Petri dish cover to collect meat drippings. These were heated in a large capacity laboratory furnace (Thermolyne type F62735 furnace, Barnstead/Thermolyne Corp., Dubuque, IA) at 275 °C for 30 min.

The model systems contained the free amino acids, creatine, and glucose (purchased from major suppliers) combined in the proportions we found in the six different meats (see below). The precursors present in 50 g of meat were mixed together and dry-heated for 30 min at 225 °C in a glass beaker (50 mL) in the same laboratory furnace. The meat and the model system experiments were performed in triplicate.

Instrumentation. Analysis was done on a Millennium 2010 HPLC system with a WISP autosampler, a model 996 diode array detector (Waters Corp., Milford, MA), and a Shimadzu model RF535 fluorescence detector set at excitation 305 nm and emission 370 nm. A TSK-Gel ODS 80T_M column (5 μm, 22 cm × 4.6 mm i.d.) (Toso Haas, Montgomeryville, PA) was used.

Analysis of the Amino Acids, Creatine, and Glucose

Table 1. Concentration (Milligrams per Gram of Meat, Wet Weight) of Free Amino Acids, Creatine, and Glucose in Six Meats

	beef	chicken breast	chicken thigh	turkey breast	pork	fish
L-alanine	0.14	0.21	0.34	0.23	0.28	0.12
L-arginine	1.07	1.19	0.73	0.68	1.80	0.03
L-aspartic acid	0.02	0.13	0.10	0.05	0.04	0.01
L-glutamic acid	0.09	0.23	0.31	0.20	0.13	0.02
L-glycine	0.06	0.08	0.21	0.11	0.10	0.05
L-histidine	0.14	0.18	0.11	0.24	0.22	0.03
L-isoleucine	0.05	0.08	0.13	0.05	0.05	0.02
L-leucine	0.07	0.13	0.14	0.07	0.08	0.02
L-lysine	0.07	0.14	0.25	0.10	0.09	0.18
L-methionine	0.06	0.08	0.10	0.07	0.07	0.04
L-phenylalanine	0.05	0.08	0.09	0.05	0.05	0.01
L-proline	0.10	0.10	0.56	0.17	0.20	0.14
L-serine	0.05	0.12	0.14	0.13	0.07	0.02
L-threonine	0.28	1.63	2.17	2.12	0.47	0.69
L-tyrosine	0.06	0.10	0.09	0.06	0.07	0.03
L-valine	0.06	0.10	0.13	0.08	0.07	0.04
creatine	6.33	3.54	4.44	4.64	4.77	7.06
glucose	7.03	0.47	0.35	0.57	5.15	0.21

in Six Meats. Raw meat pieces were analyzed for creatine, glucose, and free amino acids. The samples were homogenized in distilled water and the supernatants used for the analysis. The creatine content and the glucose content were determined spectrophotometrically according to the method of Wong (1971) and by using glucose kit GAHK-20 (Sigma, St. Louis, MO). The free amino acids were determined by HPLC with fluorescence detection as described previously (Salmon et al., 1997).

Extraction of Heterocyclic Amines. The analysis of the heterocyclic amines was performed as previously described by Gross and Grüter (1992) with some modifications. Briefly, the samples were homogenized in sodium hydroxide and mixed with diatomaceous earth. The amines were eluted from extraction columns, containing the diatomaceous earth mixture, directly to a PRS cartridge using either methylene chloride/toluene or ethyl acetate as described under Results. The PRS cartridges were washed with hydrochloric acid, followed by methanol/hydrochloric acid and water. The latter two washes, which contained the apolar heterocyclic amines, were collected and concentrated using a C18 cartridge. The polar heterocyclic amines were eluted from the PRS using ammonium acetate directly into another C18 cartridge. Finally, the analytes retained were separately eluted with methanol/ammonia. A further cleanup of the heated model system extracts was performed using an SCX cartridge-based procedure (Perfetti, 1996) to confirm the IQ-type compounds (IQ, MeIQ, IQx, 8-MeIQx, and 4,8-DiMeIQx) in these samples by their UV spectra. The final extracts were dissolved in 50 μL of mobile phase. Extraction recoveries were determined by spiking one sample of each meat type, model reaction, or dried meat drippings with each heterocyclic amine and harman and norharman. Amounts reported are corrected for incomplete recoveries. IFP quantification was performed using the extinction coefficient corresponding to PhIP because a synthetic standard is not available.

HPLC Analysis. A 20 μL injection was made with a ternary mobile phase of 0.01 M triethylamine phosphate at different pH values and acetonitrile, operating in a linear gradient, at a flow rate of 1 mL/min as described by Gross and Grüter (1992). Additionally, another mobile phase consisting of triethylamine phosphate at pH 7.0 (Pais and Knize, 1998) and a gradient of 15% acetonitrile for 12 min and 15−50% acetonitrile from 12 to 45 min was used to change the chromatographic selectivity for photodiode array peak matching, which was necessary in some cases.

RESULTS AND DISCUSSION

Composition of Raw Meats. Table 1 shows the composition of free amino acids, creatine, and glucose of the six raw meats we used in our laboratory (analyzed per gram of wet weight). Tryptophan and sulfur-containing amino acids were not detected. The total concentrations of free amino acids in the chicken breast (51.5 μmol/g), chicken thigh (62.9 μmol/g), turkey breast (49.6 μmol/g), and pork (42.5 μmol/g) were about twice the concentrations in beef (26.6 μmol/g) and fish (16.2 μmol/g). The chicken thigh had the highest total concentration of free amino acids, and the fish, the lowest. The chicken breast, chicken thigh, turkey breast, and fish contained threonine as the major amino acid. Arginine was the major amino acid in the beef and the pork. The creatine concentration varied by a factor of 2, whereas glucose varied 35-fold for the six meats. The relative compositions that we determined for the six meats are in accordance with the results reported by other authors (Taylor et al., 1984; Laser-Reuterswärd et al., 1987).

Heterocyclic Amine Extraction and Recoveries. We investigated the presence of 16 heterocyclic amine mutagens and harman and norharman that can be found in cooked meats and have been commonly studied and reported in the literature. These include three heterocyclic amine mutagens not usually investigated in heterocyclic amine analyses of meat, although they have been reported in meats and meat mixtures: DMIP, TMIP, and IFP (Becker et al., 1988, 1989; Felton et al., 1984; Knize et al., 1990). All are mutagens on the Ames/*Salmonella* test, the most potent of which is IFP, estimated to yield 10000 revertant colonies per microgram in strain TA98 (Knize et al., 1990). Other genotoxic carcinogenic properties are not known, but the relationship between aromatic amine mutagens and animal carcinogens suggests they will prove to be animal carcinogens (Hatch et al., 1992). This is the first report of the routine analysis of meats or model systems for these mutagens.

Because of the importance of peak confirmation, the analysis of the heterocyclic amines was carried out under two different HPLC conditions. A ternary mobile phase changing from pH 3.2 to pH 3.5 during the run was used for the routine determination of most of the heterocyclic amines in all of the samples. The HPLC conditions at pH 7.0 were used to confirm and quantify the heterocyclic amines in samples not able to be analyzed with the ternary mobile phase due to interfering peaks, especially at low concentration levels (Pais and Knize, 1998). Furthermore, the IQ-type compounds could not be easily confirmed in the model systems. A further cleanup with an SCX cartridge (Perfetti, 1996) and the use of a mobile phase at pH 7.0 allowed the quantification and confirmation of these compounds.

As an example of the results, Figure 2 shows the UV absorbance chromatograms at 275 and 300 nm and the fluorescence chromatogram corresponding to the beef model system. Parts A and B of Figure 3 show the UV and fluorescence chromatograms corresponding to the beef meat drippings, and parts C and D show the beef meat results. The inset figures show the on-line UV absorbance spectra and the library spectrum of each heterocyclic amine detected at the appropriate retention time. The retention times and the good correspondence shown between spectra is evidence identifying the peaks as heterocyclic amines. For TMIP and DMIP in the meat dripping sample (Figure 3A) and IFP in the beef meat (Figure 3C) further confirmation was performed with

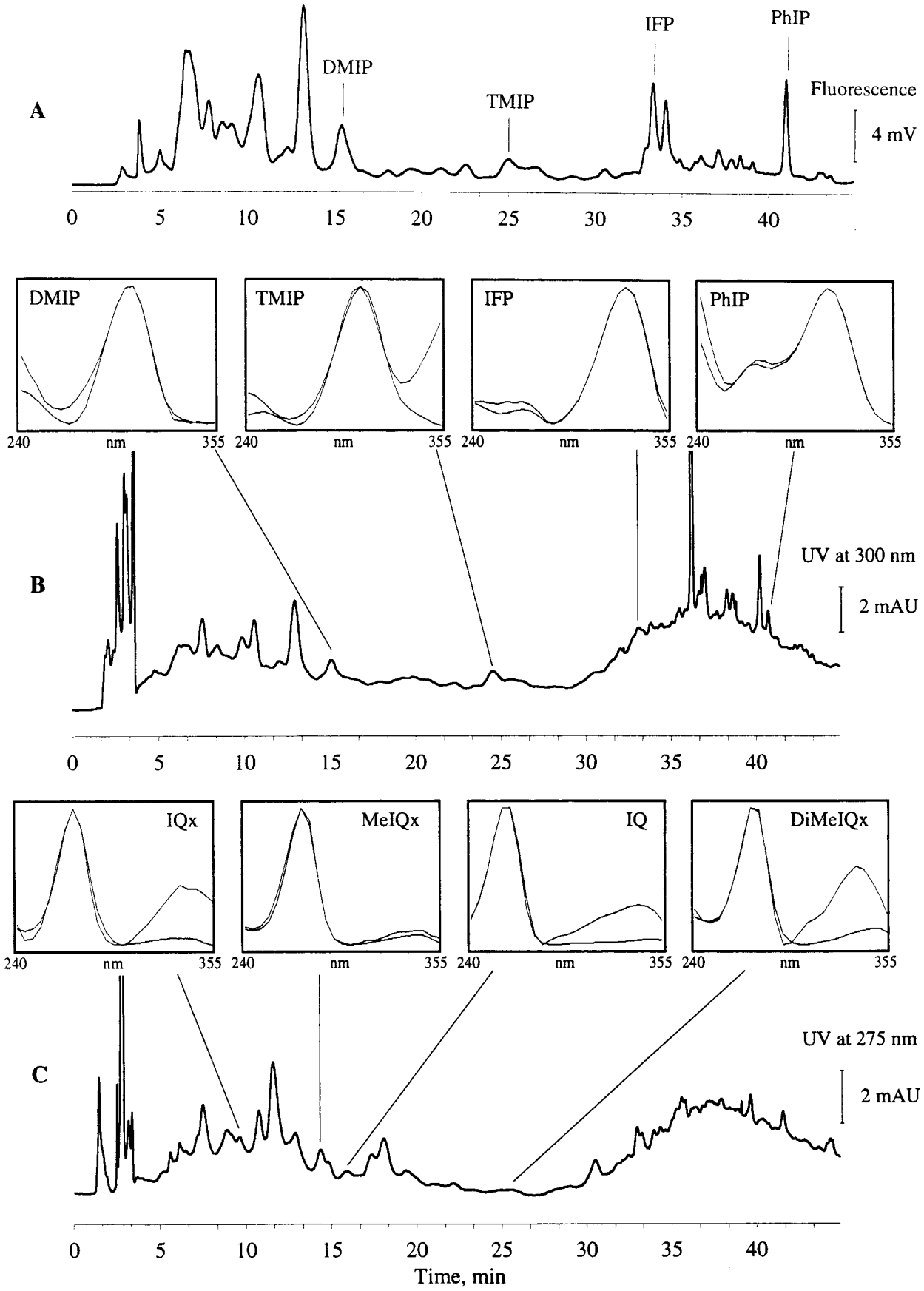

Figure 2. HPLC chromatograms of the polar extract of a beef model system obtained with a mobile phase of triethylamine phosphate at pH 7.0 and acetonitrile: (A) fluorescence detection (sample purified through the PRS-C18 tandem procedure); (B) UV detection at 300 nm (sample purified through the PRS-C18 tandem procedure); (C) UV detection at 275 nm (sample purified through the PRS-C18 procedure tandem and an additional SCX cartridge).

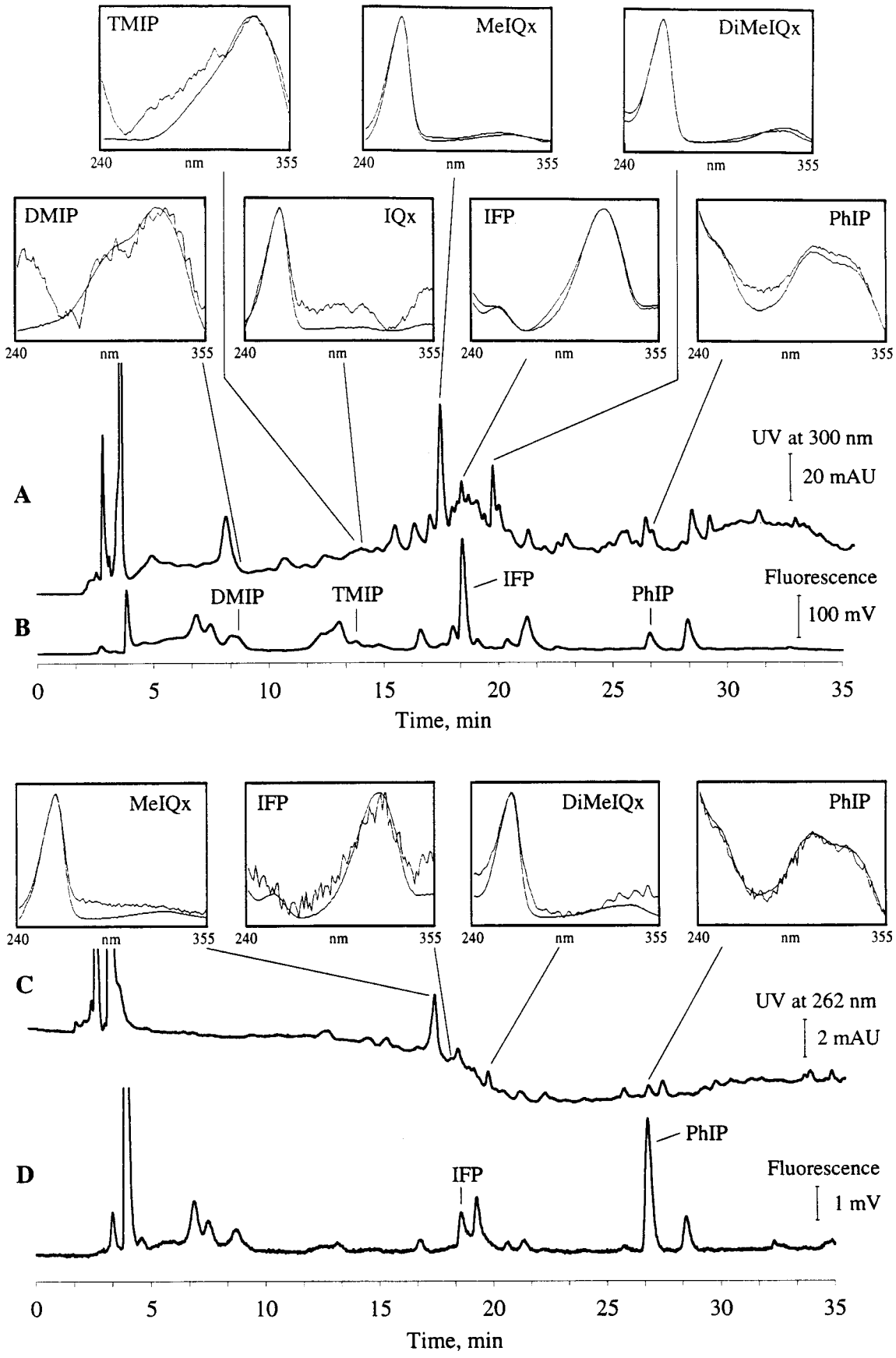

Figure 3. HPLC chromatograms of the polar extracts from the beef meat and drippings obtained with a mobile phase of triethylamine phosphate at pH 3.2 and pH 3.5 acetonitrile: (A and B) chromatograms of the meat drippings (extraction with methylene chloride/toluene); (C and D) chromatograms of the meat itself (extraction with ethyl acetate); (A and C) UV detection; (B and D) fluorescence detection.

the pH 7.0 mobile phase (not shown) due to the low UV absorbance signal for these compounds at low concentrations.

In evaluating the published solid-phase extraction method for new heterocyclic amines from the meats, we determined that DMIP and IFP gave very low recoveries, and TMIP was not recovered at all. Therefore, we modified the extraction procedure to achieve reasonable recoveries for 16 heterocyclic amines and harman and norharman in meat samples. We determined that the extraction performed on the diatomaceous earth support was responsible for the low recoveries from the meat matrix. Ethyl acetate (50 mL) improved recoveries to acceptable levels (10−55%) in the meat samples (except for AαC in the turkey and fish and DMIP in pork); this is similar to the results obtained with methylene chloride/toluene for the model systems and the meat drippings. The recoveries for the model systems and the pan residues did not increase significantly with ethyl acetate, and more interferences were extracted under these conditions. For that reason, methylene chloride/toluene was used for the extraction of these samples. The recovery of 4-MeIQx was confounded by the presence of 8-MeIQx except when analyzed at pH 7. Therefore, not all meat and dripping samples were spiked, but in no case was 4-MeIQx detected in any model system, meat, or meat dripping sample. Extraction recoveries were determined for each of 17 analytes for all six meat types. Because this included analysis in the model systems, in the meats, and in the meat drippings, the total number of recoveries measured was 306.

Recoveries for the model systems were the highest values of the three general kinds of samples studied. They were >40% (52% for the IQ-type compounds after the SCX cleanup) for most of the compounds analyzed in the model systems, except DMIP, TMIP, Glu-P-1, Trp-P-2, AαC, and MeAαC. Recoveries were found to be as low as 19% in a few cases. We were unable to generally improve recoveries for the 17 analytes by changes in extraction volumes from the method used.

The sample matrix greatly influences the extraction processes, and low recoveries were obtained for some mutagens in the meats and their corresponding meat drippings. Most recoveries were >10%, but IQ, MeIQ, DMIP, TMIP, Glu-P-1, Glu-P-2, AαC, and MeAαC were lower in at least one sample. Each of the meats or meat drippings had at least one incidence of a recovery <10%, so a single matrix is not responsible for low recoveries. Prior work in our laboratory showed that samples yielding low recovery give reproducible results, although the recovery-corrected results for these samples are less certain. Our purpose was to survey and compare the meat samples for the 18 analytes using a single extraction method.

Heterocyclic Amine Formation. To control sample heating, which is a difficult variable to regulate in the formation of heterocyclic amines, and to simulate oven-like cooking conditions, a laboratory furnace was used. Preliminary studies determined the temperature needed to form heterocyclic amines for the meats and the model systems. Low temperatures of ∼200−225 °C did not form detectable concentrations of heterocyclic amines in the meats; at higher temperatures (>250 °C), the concentrations of the IQ-type compounds decreased in the model systems. These results concur with those of other authors who found decreased amounts of some heterocyclic amines and the mutagenic activity in model systems and in meats at long cooking times and high temperature (Skog and Jägerstad, 1990; Gross and Grüter, 1992; Jackson and Hargraves, 1995). The cooking conditions were set at a heating time of 30 min and temperatures of 225 °C for the model systems. Meats were heated at 275 °C. These conditions produced 8-MeIQx concentrations in the levels normally found in meats when frying or flame grilling but at conditions we could control to enable our comparison of the different meats. Baking is normally done at lower temperatures. The meats and the corresponding drippings were analyzed separately because of the previous observation that heterocyclic amines are formed in both the meats and meat drippings (Skog et al., 1997). We are interested in the total amount and type of heterocyclic amines produced from the meat precursors.

Model Systems. Nine heterocyclic amines were detected and confirmed in the model system samples. The compounds 4-MeIQx, Glu-P-1, Glu-P-2, Trp-P-1, Trp-P-2, harman, norharman, AαC, and MeAαC could not be found in any model system. The concentrations for the IQ-type compounds found in the model systems were low, between 0.17 and 2.5 ng/g of meat equivalents. The pyridine derivatives (PhIP, IFP, TMIP, and DMIP) were formed in much higher concentrations. Figure 4 shows the results for the six model systems containing precursors corresponding to the levels in the six meats. The error bars correspond to the standard deviations and are ∼10%, indicating the good reproducibility of the model system, the heating conditions, and the analysis method for the three replicate experiments.

IQ was detected in all of the model systems except the turkey, although the concentrations in the beef and both chicken models were very low (<1 ng/g). The highest amount was formed in the fish model. MeIQ was detected in the chicken thigh, pork, and fish model systems, also at very low concentrations (0.3−1 ng/g). Like IQ, the higher concentration was also in the fish model. These results concur with those of Kasai and coworkers (Kasai et al., 1980, 1981), who first isolated IQ and MeIQ from broiled fish. A model system of boiled pork juice heated under reflux formed IQ and MeIQ (Lee et al., 1994). A model system of dry-heated beef juice was also reported to form IQ (Taylor et al., 1986).

IQx was present only in our model system simulating pork and beef, possibly due to the higher arginine content, the major amino acid in both models. IQx has been reported to be formed from arginine in a water-based model system (Johansson et al., 1995).

8-MeIQx and 4,8-DiMeIQx were detected in all of the model systems. Reactions of several single amino acids with creatine and sugars have been reported to form MeIQx and DiMeIQx in various model systems: dry-heating at 180 °C for 2 h; reflux boiling in diethylene glycol/water at 125−128 °C for 2 h; heating in ethylene glycol/water for 10 min in open tubes; or heating in water at 180 °C for 10 min in sealed tubes [see review by Skog (1993); Johansson et al., 1995; Jackson and Hargraves, 1995]. There are no significant differences among the MeIQx concentrations in the model systems, except for the chicken thigh and fish.

Phenylalanine (Övervik et al., 1989; Taylor et al., 1987; Felton and Knize, 1990; Shioya et al., 1987; Skog and Jägerstad, 1991; Johansson et al., 1995), leucine (Övervik et al., 1989), isoleucine (Johansson et al., 1995), and tyrosine (Johansson et al., 1995) have been reported to be the precursor amino acids for the formation of

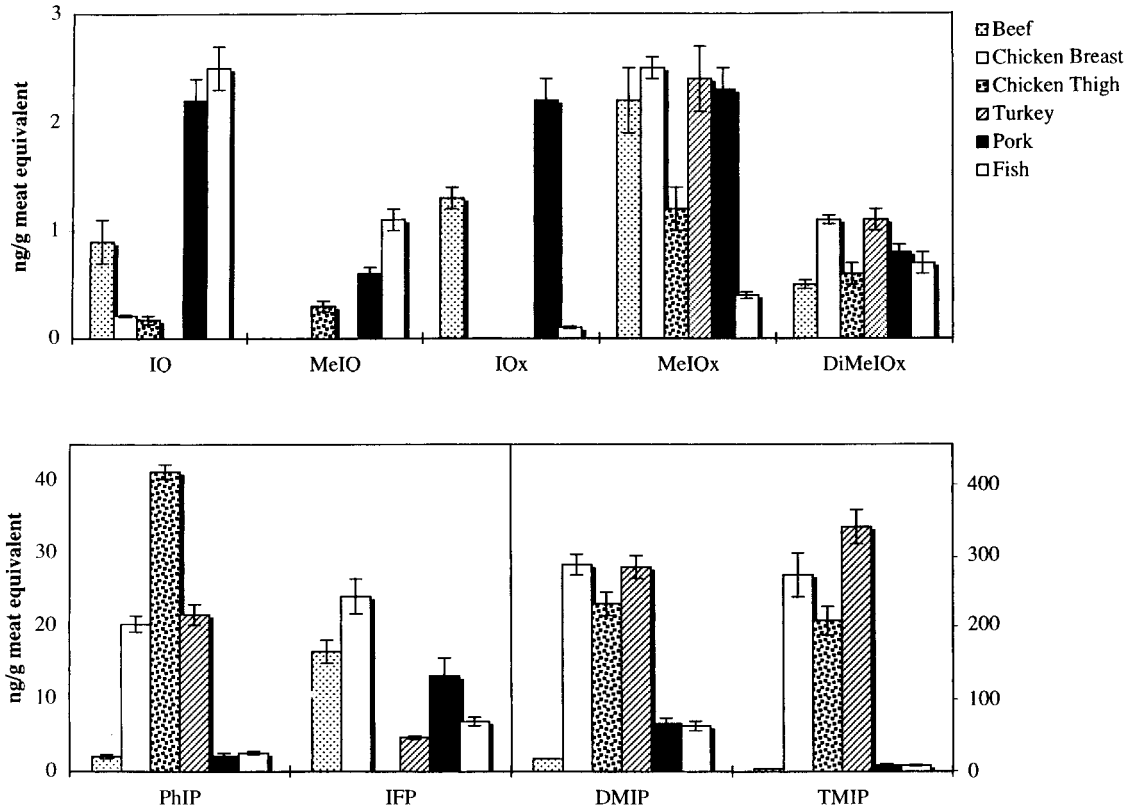

Figure 4. Concentrations of the heterocyclic amines (ng/g of meat equivalent) found in the six model systems. Error bars show the standard deviation of three experiments. Note that three ordinate scales are used.

PhIP. The amounts of these amino acids in the reaction mixtures (Table 1) are highest in the chicken thigh, then in the chicken breast, the turkey, the beef and the pork, and, finally, lowest in the fish. The concentration of PhIP formed in the models corresponds with the content of phenylalanine, leucine, isoleucine, and tyrosine, except for the beef and the pork. The unexpectedly low PhIP concentrations in beef and pork may be explained by the sugar content in these meats. Sugar affects the formation of PhIP, and PhIP's concentration decreases with an increase in the amount of glucose (Taylor et al., 1987). The sugar in the beef and pork model systems is at least 10-fold higher than in the other model reactions, perhaps explaining why the concentration of PhIP is lower than expected from the phenylalanine, leucine, isoleucine, and tyrosine content.

IFP is formed in all of the models except the chicken thigh. The concentrations of IFP (4.6−24 ng/g) are comparable to the PhIP concentrations formed in the models. Taylor et al. (1986) reported the formation of a mutagenic compound with the molecular weight of IFP in a model system of dry-heated beef juice.

Threonine was reported to be the responsible amino acid in the formation of a proposed TMIP in a dry model system (Övervik et al., 1989). The amount of this amino acid in the model system correlates with the concentration of TMIP formed. The formation of DMIP might be explained by the threonine content as well, because TMIP is the methyl analogue of DMIP.

Meats and Meat Drippings. We examined the total amount of heterocyclic amines that could be formed from the precursors in meat, so it was necessary to analyze the meat drippings as well as the meat. Meat drippings are commonly served and consumed along with the meat as gravy. Meat drippings were shown to contain similar amounts of mutagenic activity (Övervik et al., 1987) and heterocyclic amines (Johansson and Jägerstad, 1994) as the meat itself and can therefore be considered to be of equal importance as a source of mutagenic compounds in the diet where drippings are used as a food source. Thus, in our experiments the meats were placed on a metal grate and the meat drippings (a dried residue) were collected in a Petri dish for the analysis of samples separately.

The nine heterocyclic amines present in the model systems were also found in the meats and the corresponding meat drippings. The concentrations (calculated on a nanogram per gram of uncooked weight basis) of these heterocyclic amines present in the meats and the meat drippings are shown in Table 2. 8-MeIQx, 4,8-DiMeIQx, PhIP, DMIP, and IFP were detected in all of the samples. IQ and MeIQ were detected only in the fish meat drippings, and IQx was found only in the beef meat drippings. Of the commonly investigated heterocyclic amines, the concentrations of 8-MeIQx and PhIP in the meats and the corresponding meat drippings were the highest, which concurs with the results found in the literature even though our samples were not cooked in a conventional manner.

Besides these heterocyclic amines, the comutagens harman and norharman were detected in some of the samples. Norharman was found in all meats at concentrations of 11.5 ng/g of beef, 0.27 ng/g of chicken breast, 12.9 ng/g of chicken thigh, 58.3 ng/g of pork, 1.3 ng/g of turkey, and 94.8 ng/g of fish. Harman was detected in all of the meats, except in beef, at concentrations of 5.2 ng/g for chicken breast, 8.3 ng/g for chicken thigh, 16.0 ng/g for pork, 1.9 ng/g for turkey, and 5.0 ng/g for fish. Neither harman or norharman was found in any model system, suggesting they have precursors not included in our model system. Tryptophan has been well established as a precursor to harman and norharman

Table 2. Concentration (Nanograms per Gram) of Heterocyclic Amines Formed in Six Meats and the Meat Drippings

sample	compound[a]	beef	chicken breast	chicken thigh	turkey	pork	fish
meats	MeIQx	1.43 ± 0.08[b]	0.5 ± 0.1	0.02 ± 0.004	1.0 ± 0.5	3.5 ± 1.1	nd[c]
	DiMeIQx	0.20 ± 0.05	0.2 ± 0.08	0.05 ± 0.03	0.19 ± 0.08	0.4 ± 0.3	nd
	PhIP	1.2 ± 0.6	37.5 ± 15.4	8.0 ± 4.7	6.8 ± 3.4	4.7 ± 4.1	3.2 ± 2.3
	DMIP	nd	5.9 ± 5.2	3.1 ± 3.2	nd	37 ± 46	8.9 ± 6.3
	TMIP	nd	2.9 ± 3.5	nd	nd	nd	nd
	IFP	0.2 ± 0.06	7.0 ± 6.6	1.3 ± 1.6	0.9 ± 0.7	2.5 ± 3.9	2.1 ± 2.0
meat drippings	IQ	nd	nd	nd	nd	nd	1.7 ± 0.9
	MeIQ	nd	nd	nd	nd	nd	0.7 ± 0.6
	IQx	0.2 ± 0.03	nd	nd	nd	nd	nd
	MeIQx	6.6 ± 3.4	0.1 ± 0.1	1.9 ± 0.8	0.9 ± 0.4	4.8 ± 0.4	0.2 ± 0.1
	DiMeIQx	1.6 ± 1.5	0.3 ± 0.2	1.9 ± 1.1	1.0 ± 0.5	2.2 ± 0.3	0.04 ± 0.03
	PhIP	5.4 ± 3.0	3.3 ± 1.4	21.8 ± 5.1	4.3 ± 1.6	6.9 ± 3.3	18.1 ± 8.5
	DMIP	13.4 ± 6.7	2.1 ± 0.6	22.8 ± 12.5	6.0 ± 3.1	15.8 ± 4.4	12.8 ± 4.9
	TMIP	0.8 ± 0.3	nd	0.6 ± 0.2	nd	4.0 ± 4.2	nd
	IFP	13.2 ± 8.2	1.8 ± 1.0	6.8 ± 2.8	9.6 ± 5.3	13.4 ± 1.0	0.5 ± 0.3
	Trp-P-1	nd	0.05 ± 0.03	nd	nd	nd	nd

[a] No Trp-P-2, Glu-P-1, Glu-P-2, AαC, MeAαC or 4-MeIQx were found in these samples. [b] Error expressed as standard deviations of triplicate experiments. [c] nd, not detected.

(Sugumura et al., 1977). These two compounds are neither mutagenic nor carcinogenic but are important in studies of mutagenic activity of foods due to their comutagenic behavior. Trp-P-1 was found only in the meat drippings of the chicken breast. This was at extremely low concentrations, averaging 0.05 ng/g for the three samples analyzed.

The formation of heterocyclic amines in the meat drippings corresponding to beef, chicken thigh, turkey breast, pork, and fish was generally much higher than in the meats themselves. In contrast, the concentration of heterocyclic amines formed in the chicken breast meat drippings was lower than in the meat itself. These results are in accordance with the findings of other authors. Gross et al. (1993) found 8-MeIQx, 4,8-DiMeIQx, and PhIP in grill scrapings in concentrations 30−200-fold greater than in the meats, whereas AαC was found only in the meat drippings. In conventional cooking studies heterocyclic amine content in meat drippings is generally comparable to or even greater than the amount of heterocyclic amines present in cooked meat. Johansson et al. (1994) also found amounts of IQ, MeIQ, MeIQx, DiMeIQx, and PhIP in meat drippings equal to or higher than in the meats during frying or oven roasting. The same results were found for MeIQx, 4,8-DiMeIQx, PhIP, MeIQ, harman, and norharman by Skog et al. (1995). These authors later found that only in chicken breast and cod fillet did the meat contain greater concentrations of heterocyclic amines than the meat drippings (Skog et al., 1997). These results concur with our experiments except for the fish. In the fish they found 8-MeIQx, 4,8-DiMeIQx, and PhIP as the major heterocyclic amines in the samples and also detected Trp-P-1, Trp-P-2, harman, and norharman in some of them. None of these authors investigated the presence of DMIP, TMIP, or IFP.

During cooking, meat juice leaks out from the meat slices into the cooking pan. Depending on several factors, such as the muscle tension and direction of the muscle fibers, the amount of meat juice leaking out of the meat slices during cooking varies considerably (Hamm, 1977). This might contribute to the varying heterocyclic amine content of meat drippings from different experiments and, thus, the higher standard deviations of these results than for those of the model systems.

Comparison of Heterocyclic Amines in the Model Systems and the Corresponding Meats. To compare the total formation of heterocyclic amines in the model systems and the six meats, the results corresponding to the meats themselves and the meat drippings were expressed as nanograms of heterocyclic amine per gram of uncooked meat and then combined. These results are shown in Figure 5.

The same nine heterocyclic amines were formed in the model systems and in the meats. As mentioned above, the mutagenic amine Trp-P-1 was detected only in the chicken breast meat drippings and not in the model systems. The new model system developed here forms the nine relevant mutagenic amines that are found in cooked meats. The mutagenic amines 4-MeIQx, Glu-P-1, Glu-P-2, Trp-P-2, AαC, and MeAαC were not found in any of the meat samples or the model systems. The same relative amounts were found for the IQ-type compounds, PhIP and IFP in the meats and the model systems. In contrast, lower amounts of DMIP and TMIP were found in the meats than in the models. The low recovery of DMIP and TMIP in these samples makes comparison with the model system difficult. Still these compounds may play an important role in contributing to the total mass of heterocyclic amines in meats, because they were detected in all of the meats and in all of the model systems.

IQ and MeIQ were formed in the fish model system, and fish was also the only meat where these compounds were detected. The fish precursors and their relative amounts must be important for the formation of IQ and MeIQ. 8-MeIQx and PhIP were present in almost equal amounts in the beef meat and in the model system. However, in the chicken breast and the corresponding model system, the formation of PhIP was much higher relative to MeIQx, as reported in previous work (Wakabayashi et al., 1992; Sinha et al., 1995; Salmon et al., 1997). These results suggest that our new model system is a good surrogate for the heterocyclic amine-forming reactions in cooked meats.

The beef and the pork meats and model systems formed generally the same compounds and in the same relative concentrations. Their amino acid and glucose contents were similar, and therefore the levels of heterocyclic amine formed were comparable.

The good correspondence that has been demonstrated between the heterocyclic amines identified in the various model systems and those found in cooked foods makes analysis for new compounds in model systems worthwhile. This was proven in this work. DMIP, TMIP,

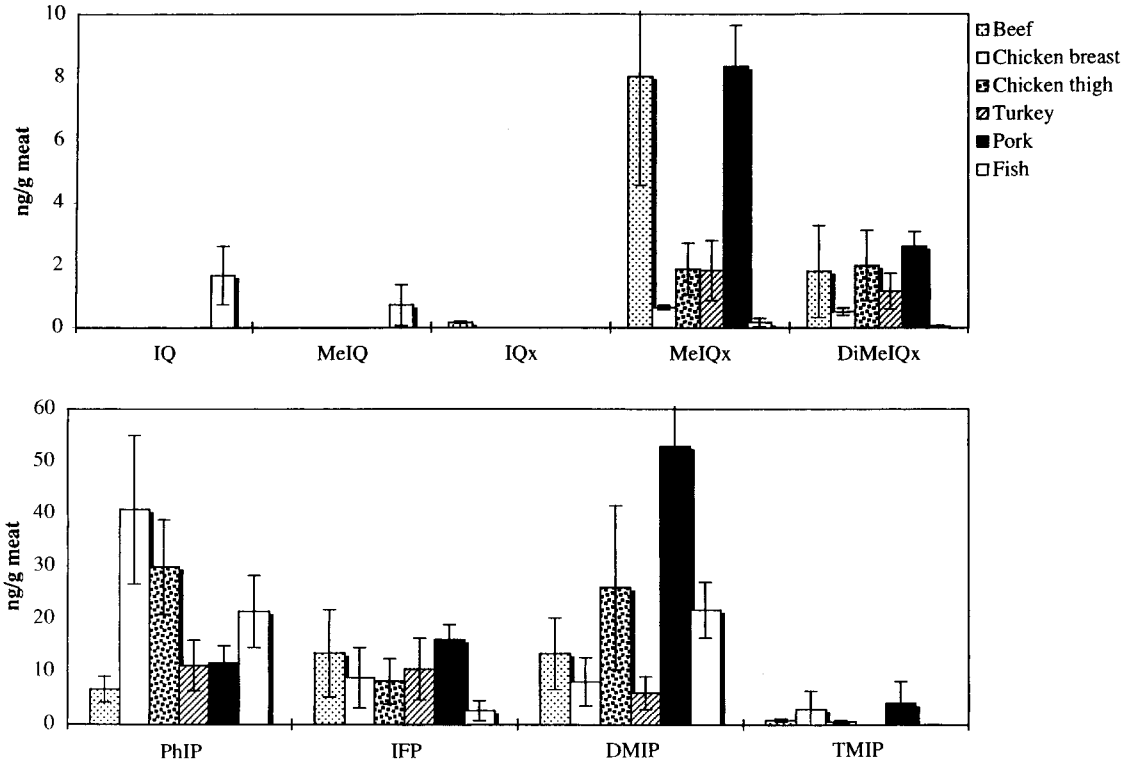

Figure 5. Concentrations of the heterocyclic amines (ng/g of meat) found in the six meats (combined results of the meats and the meat drippings). Error bars show the standard deviation of three experiments. Note that two ordinate scales are used.

and IFP were identified and quantified in the model systems, indicating the likelihood of finding them in the meats. These compounds, for which no analysis method was previously developed, were present in the meat samples. More work needs to be done to determine their abundance in meats prepared for consumption by standard methods. The finding of the new imidazopyridine heterocyclic amines, DMIP, TMIP, and IFP, in the model system and in the meats and meat drippings suggests that these may be significant contributors to the total mass of heterocyclic amines in the diet.

Differences in heterocyclic amine formation between the meats and the model systems may be attributable to other factors such as sample pH or water content. The fat content of the meat also has some influence on heterocyclic amine formation in a model system as Johansson et al. (1993) reported. Other studies have shown lipids to have enhancing effects, no effect, or even inhibitory effects in the mutagenic activity of pork (Nilsson et al., 1986), beef (Barnes et al., 1983; Bjeldanes et al., 1983; Knize et al., 1985), and lamb (Barrington et al., 1990). The greater variation in our meat results suggested by the standard deviations larger than the model results suggests that nonuniformity of the meat pieces and perhaps water, fat, and factors affecting the water and precursor transport should be controlled for future experiments.

In conclusion, the work described here shows that nine mutagenic heterocyclic amines found in meat products are also formed similarly in our dry-heated model system. Besides the IQ-type compounds and PhIP, the heterocyclic amines normally found in cooked meat, three new heterocyclic amines not usually studied, DMIP, TMIP, and IFP, were found to be in these kinds of samples and formed from amino acids, glucose, and creatine. Seven other mutagenic amines were also investigated but were not found in concentrations higher than our limit of detection, ~0.1 ng/g.

The cleanup procedure that permitted the extraction of all 18 compounds studied was a modification of the method developed by Gross and Grüter (1992). Analyte recoveries were improved by using ethyl acetate for extraction, and serious HPLC peak interferences were overcome by changing chromatographic selectivity. This was accomplished by using a mobile phase of pH 7. These changes enabled the routine analysis of samples for 16 heterocyclic amine mutagens, harman, and norharman.

The model system developed here reproduces the heterocyclic amine-forming reactions of cooked meats. Controlled reaction conditions allow studies of the formation and reduction of these compounds and provide a source for the isolation of additional mutagens for which synthetic standards are not available. This work shows that the free amino acid, glucose, and creatine concentrations in meats have a profound effect on the mutagenic products formed upon heating.

ABBREVIATIONS USED

AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole (CAS Registry No. 26148-68-5); 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (95896-78-9); DMIP, 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine; Glu-P-1, 2-amino-6-methyldipyrido[1,2-α:3′,2′-*d*]imidazole (67730-11-4); Glu-P-2, 2-aminodipyrido[1,2-α:3′,2′-*d*]imidazole (67730-10-3); harman, 1-methyl-9*H*-pyrido[4,3-*b*]indole (486-84-0); HPLC, high-performance liquid chromatography; IFP, aminodimethylimidazofuropyridine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline (76180-96-6); IQ-type compounds: IQ, MeIQ, IQx, 8-MeIQx, and 4,8-DiMeIQx; IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (108354-47-8); MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (68006-83-7); MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (77094-11-2); 8-MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (77500-04-0); 4-MeIQx,

2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline; norharman, 9*H*-pyrido[4,3-*b*]indole (244-63-3); PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5); PRS, propylsulfonic acid silica; SCX, benzenesulfonic acid silica; TMIP, 2-amino-1,5,6-trimethylimidazo[4,5-*b*]pyridine; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole (62450-06-0); Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (62450-07-1); UV, ultraviolet. (CAS Registry No. were provided by the authors.)

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