Heterocyclic Amine Content in Restaurant-Cooked Hamburgers, Steaks, Ribs, and Chicken

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As part of a comprehensive survey of the heterocyclic amine content of foods, beef hamburgers, steaks, and pork ribs were purchased from restaurants, with cooking doneness specified. Samples were pooled by meat type, doneness, and cooking method and analyzed for heterocyclic amine content using HPLC. Results show detectable levels of heterocyclic amines in all samples, ranging from 0.5 ng/g PhIP from a pooled sample of ribs to 20 ng/g total of MeIQx and PhIP from a sample pooled of well-done charbroiled hamburgers. Grilled chicken samples from fast-food restaurant sandwiches or rotisserie-cooked chicken contained MeIQx and PhIP at combined levels of <2 ng/g. Compared to fast-food meat products from this and previous studies, restaurant products are ~10-fold higher in heterocyclic amine content. The amounts of heterocyclic amines measured in these restaurant foods show that samples prepared for laboratory studies are representative of commonly consumed restaurant samples.

Keywords: *Heterocyclic amines; PhIP; MeIQx; DiMeIQx; cooking mutagens*

INTRODUCTION

Our diet exposes us to complex mixtures of organic and inorganic substances that provide sustenance but that can also play important roles in the causation, modulation, and prevention of human disease. One group of chemicals that currently is under scrutiny as having potential dietary carcinogenic activity is the heterocyclic amines, which are often formed during cooking (Sugimura et al., 1988; Hatch et al., 1988). The research emphasis placed on heterocyclic amines results from the findings that they are very potent mutagens in the Ames/*Salmonella* assay (Sugimura, 1982), cause genotoxic effects in other assays (Aeschbacher and Turesky, 1991), and are multisite carcinogens in rodents (Sugimura et al., 1997) and nonhuman primates (Adamson et al., 1990).

As part of a comprehensive study on the occurrence of heterocyclic amines in the diet, we sampled beef hamburgers, steaks, and pork ribs, ordered to various degrees of doneness from restaurants, and pooled samples purchased from multiple visits to the same restaurant chain. Grilled or rotisserie-cooked chicken samples were collected from fast-food restaurants. We used the solid-phase extraction/HPLC method of Gross (1990) and the Ames/*Salmonella* test to analyze each to create a database of foods and heterocyclic amine content.

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EXPERIMENTAL PROCEDURES

The number of stores per restaurant chain, portions purchased, and total foods pooled per sample analyzed are listed in Tables 1−4. A sample combining meat from separate visits to two or four restaurant chains by product and doneness was made by pooling the meat patties, or the meat without the bone, and crumbling finely in a Robot Coupe mixer (Jackson, MS). Cooking methods were separated into griddle-fried and charbroiled categories for steaks and hamburgers and smoked or baked preparation for pork rib samples. Cooking doneness for grilled or rotisserie-cooked chicken samples was not specified. Aliquots were removed and stored at −20 °C until analyzed. One hundred and sixty-eight food portions were purchased and processed for this study.

Chemicals and solvents were of HPLC or analytical grade. Heterocyclic amines, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), were purchased from Toronto Research Chemicals (Downsview, ON) and quantified by molar extinction coefficients as described previously (Knize et al., 1995).

Samples were extracted and analyzed in duplicate, spiked and unspiked, to determine recoveries by HPLC according to the procedures of Gross and Grüter (1992) as described previously (Knize et al., 1995). Results are corrected for recoveries determined from duplicate spiked samples, which varied depending on the meat matrix from 14 to 67% for PhIP to 41 to 83% for MeIQx.

The mutagenic activity of the sample extracts was determined using the standard plate incorporation assay described by Ames et al. (1975), with *Salmonella typhimurium* strain TA98 (a gift of Professor Bruce Ames, University of California, Berkeley) with 2 mg of Aroclor-induced rat liver S9 protein per plate for metabolic activation. Values reported are the

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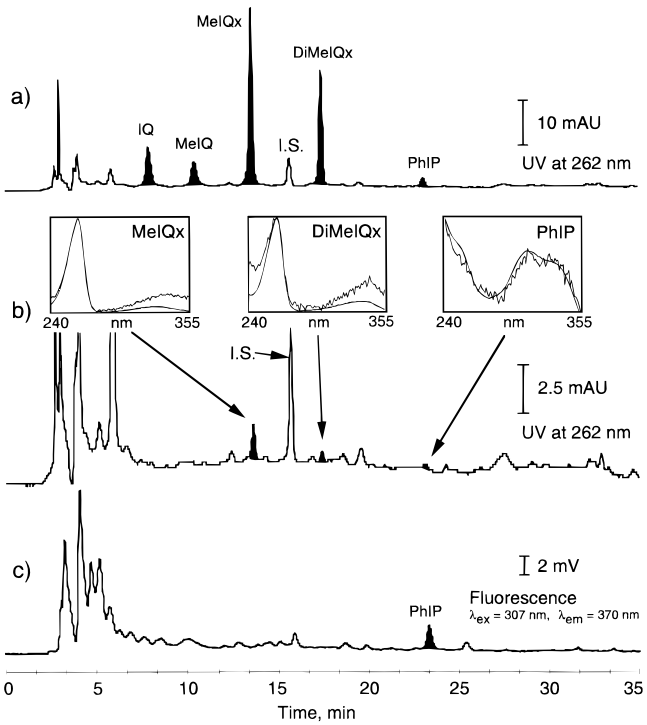

Figure 1. HPLC chromatograms of the extract of a very well-done steak. A sample spiked with five heterocyclic amines before extraction is shown in (a). An unspiked sample showing three heterocyclic amines as shaded peaks is shown in (b). Also shown are superimposed UV spectra from the sample and from standards. The fluorescence chromatogram showing the peak at the retention time of PhIP is shown in (c). I.S. is the internal standard (caffeine, 2.5 ng/μL in methanol/water 50:50).

least-squares fit of the slope from the linear portion of dose−response curves.

RESULTS

Figure 1 shows an example of the UV absorbance chromatograms at 262 nm obtained from the extract of a griddle-fried very well-done steak. A sample spiked before extraction (Figure 1a) shows the five peaks used to calculate recovery. Figure 1b shows an unspiked steak extract chromatogram and the detection of three heterocyclic amine peaks. The UV absorbance spectra are shown for MeIQx, DiMeIQx, and PhIP from the sample, superimposed with reference spectra. The close matching correspondence between the spectra is evidence of identity for the heterocyclic amine. Peaks are not seen for IQ or MeIQ at the appropriate retention time. Figure 1c shows the fluorescence chromatogram further indicating the presence of PhIP. PhIP is the only heterocyclic amine of the five spiked that is fluorescent under these HPLC conditons.

Table 1 shows the results from pooled restaurant samples of beef hamburgers. All samples had detectable heterocyclic amines and more PhIP than MeIQx in all cases. DiMeIQx was detected only in one composite sample, and no IQ or MeIQ was detected in any sample. Mutagenic activity increased generally with cooking doneness, with a ~2-fold increase in potency in a comparison of medium to well-done cooking.

Pooled beef steak samples are shown in Table 2. Like the hamburgers, more PhIP than MeIQx was found in each pooled sample, DiMeIQx was found only in two griddle-fried samples, and IQ and MeIQ were not detected. Mutagenic potency showed an increase with cooking doneness, but the mass amount of heterocyclic amines did not correlate with cooking doneness. Griddle-fried steaks had >3 times the mutagenic potency of the griddle-fried hamburgers cooked to the same specified doneness.

Analysis of pork ribs is shown in Table 3. Only PhIP was found in any sample. No trend of increased heterocyclic amines or mutagenic potency with cooking doneness could be determined from these data.

Table 1. Heterocyclic Amine Content and Mutagenic Activity in Pooled Restaurant Hamburger Samples

method/ doneness	heterocyclic amine content[a] MeIQx, ng/g	PhIP, ng/g	DiMeIQx, ng/g	mutagenic activity, TA98 rev/g
griddle fried				
medium[b]	1.5 ± 0.17[c]	1.9 ± 030	ND[d]	110[e] ± 9[f]
well	1.8 ± 0.33	4.4 ± 0.47	ND	150 ± 11
very well	1.3 ± 0.04	2.6 ± 0.07	0.1 ± 0.05	200 ± 10
charbroiled				
medium	0.2 ± 0.13	5.2 ± 0.20	ND	210 ± 17
well	0.4 ± 0.02	1.8 ± 0.12	ND	230 ± 9.2
very well	1.8 ± 0.30	18.4 ± 7.1	ND	470 ± 28

[a] No MeIQ or IQ was detected in these samples. [b] Two hamburgers were purchased from each of four restaurants, and the resulting eight samples were pooled. [c] Standard deviation of duplicate analyses. [d] Not detected, <0.1 ng/g. [e] From the linear portion of dose−response curves using duplicate plating. [f] Standard error of computer fit line.

Table 2. Sample Source, Heterocyclic Amine Content, and Mutagenic Activity in Pooled Restaurant Beef Steak Samples

method/ doneness[b]	heterocyclic amine content[a] MeIQx, ng/g	PhIP, ng/g	DiMeIQx, ng/g	mutagenic activity, TA98 rev/g
griddle fried				
medium	1.7 ± 0.40[c]	10 ±1.01	0.1 ± 0.09	410[d] ± 20[e]
well	1.8 ± 0.01	6.8 ± 0.39	ND[f]	590 ± 30
very well	2.4 ± 0.08	9.0 ± 1.08	0.4 ± 0.01	720 ± 22
charbroiled				
medium	1.1 ± 0.05	12 ± 0.81	ND	220 ± 32
well	1.6 ± 0.15	15 ± 2.11	ND	270 ± 14
very well	1.2 ± 0.09	5.7 ± 0.16	ND	450 ± 18

[a] No MeIQ or IQ was detected in these samples. [b] Two beef steaks were purchased from each of four restaurants, and the resulting eight samples were pooled. [c] Standard deviation of duplicate analyses. [d] From the linear portion of dose−response curves using duplicate plating. [e] Standard error of computer fit line. [f] Not detected, <0.1 ng/g.

Table 3. Sample Source, Heterocyclic Amine Content, and Mutagenic Activity in Pooled Restaurant Pork Rib Samples

method/ doneness	heterocyclic amine content[a] MeIQx, ng/g	PhIP, ng/g	DiMeIQx, ng/g	mutagenic activity, TA98 rev/g
smoked[b]				
regular	ND[c]	7.4 ± 1.77[d]	ND	53[e] ± 10[f]
well	ND	0.7 ± 0.50	ND	56 ± 8
baked[g]				
regular	ND	0.5 ± 0.01	ND	132 ± 11
well	ND	2.3 ± 0.32	ND	51 ± 5

[a] No MeIQ or IQ was detected in these samples. [b] Two rib portions were purchased from each of four restaurants, and the resulting eight samples were pooled. [c] Not detected, <0.1 ng/g. [d] Standard deviation of duplicate analyses. [e] From the linear portion of dose−response curves using duplicate plating. [f] Standard error of computer fit line. [g] Two rib portions were purchased from each of two restaurants, and the resulting four samples were pooled.

Table 4. Heterocyclic Amine Content and Mutagenic Activity in Grilled Chicken from Sandwiches and Rotisserie-Cooked Chicken

chicken sample	heterocyclic amine content[a] MeIQx, ng/g	PhIP, ng/g	DiMeIQx, ng/g	mutagenic activity, TA98 rev/g
grilled, sandwich				
vendor A[b]	0.72 ± 0.37[c]	0.81 ± 0.11	ND[d]	129 ± 26[e]
vendor B	0.54 ± 0.01	ND	ND	42 ± 8
vendor C	0.38 ± 0.07	0.38 ± 0.04	ND	41 ± 13
vendor D	0.27 ± 0.02	1.44 ± 0.19	ND	ND[f]
rotisserie grilled[f]				
white meat	0.45 ± 0.08	0.75 ± 0.19	ND	44 ± 7
dark meat	0.40 ± 0.06	0.59 ± 0.06	ND	44 ± 4

[a] No MeIQ or IQ was detected in these samples. [b] Two portions were purchased from each of two restaurants, and the resulting four samples were pooled. [c] Standard deviation of duplicate analyses. [d] Not detected, <0.1 ng/g. [e] From the linear portion of dose−response curves using duplicate plating, standard error of computer fit line. [f] Two chickens were purchased from each of two restaurants, and the resulting four samples were pooled.

Table 4 shows the heterocyclic amines in chicken samples taken from grilled chicken sandwiches or chicken cooked on a rotisserie, sold as a chicken quarter, a breast, and wing for the light meat and as a leg and thigh for the dark meat. MeIQx was detected in all samples and PhIP in all but one.

DISCUSSION

After all doneness levels and cooking methods were combined, beef steaks had an average of 11.4 ng of heterocyclic amines/g of sample. Hamburger samples averaged 6.9 ng/g, suggesting steaks contained higher concentrations of heterocyclic amines in general. The composite of well-done charbroiled hamburgers, however, was the sample highest in heterocyclic amines overall. These results represent pooled samples. Individual samples would need to be analyzed to determine the range of heterocyclic amine content within each doneness category. We did not sample meats cooked to a rare level of doneness, believing the greater heterocyclic amine content and greater health risk to reside with medium and well-done samples. The lack of a general correlation of heterocyclic amine content with doneness levels shows that extending the range of cooking to samples cooked to a rare state needs to be done to determine the heterocyclic amines for the full range of samples.

All of the chicken samples had detectable heterocyclic amines, although at levels lower than the those of the hamburgers, steaks, or two of the composite pork rib samples. These heterocyclic amine levels in grilled or rotisserie-cooked chicken were higher than those of the fast-food chicken samples we analyzed previously, in which four of four samples had undetectable levels, analyzed using the same methods and equipment (Knize et al., 1995). It appears that the cooking method (grilling or rotisserie-cooking) and perhaps the preparation (the lack of breading of these samples) make the difference in the heterocyclic amine amounts. The results with grilled chicken were lower in heterocyclic amine amounts than even the least cooked grilled chicken reported by Sinha et al. (1995), which had no MeIQx but 27 ng/g PhIP. Perhaps the grilling temperature, which is unknown for these purchased samples, is the reason.

Mutagenic potency on the Ames test increased with doneness for the hamburger and steak samples in contrast to the heterocyclic amine content measured here. Other mutagens that were not measured in this study could be responsible for this trend in mutagenic potency, or mutagenic potency and doneness could be related just by chance, given the experimental variation in extraction and mutagenic activity testing. For the pork rib samples increased mutagenic potency was not related to increased cooking doneness.

The heterocyclic amine results are in the same range with the few published results for individual restaurant samples, which showed a sirloin steak and a hamburger to have 0.87 and 0.89 ng/g of MeIQx and 13 and 11 ng/g of PhIP, respectively (Knize et al., 1997).

Ribs had undetectable levels of MeIQx. It is unusual to consistently find only PhIP in a cooked meat sample; most samples contain PhIP and MeIQx, although the relative amounts do vary greatly (Skog et al., 1997; Sinha et al., 1995; Johansson and Jägerstad, 1994; Gross et al., 1993; Wakabayashi et al., 1993). Mutagenic potencies of rib samples do not correlate with PhIP levels, suggesting either that known compounds below the limit of detection using these methods are present or that unknown mutagens are present.

The amounts of heterocyclic amines in the restaurant foods are similar to those in beef cooked specifically for experiments in the controlled environment of the laboratory (Sinha et al., 1998; Skog et al., 1995; Johansson and Jägerstad, 1994), indicating that the laboratory cooking procedures do accurately reflect the exposures to the general public. The chicken samples from the fast-food restaurants do differ greatly, being lower in heterocyclic amines than chicken breast meat grilled in the laboratory (Sinha et al., 1995). Perhaps preparation before grilling such as marinating is responsible for these differences (Salmon et al., 1997).

These heterocyclic amine concentrations for restaurant hamburgers are ~10-fold higher than the highest levels in pooled fast-food samples measured in our laboratory (Knize et al., 1995). In these samples the amount of heterocyclic amines detected was <1 ng/g of hamburger. Many fast-food samples had undetectable levels of heterocyclic amines. Fast-food restaurants, which generally prepare food to a standard degree of doneness, usually rapidly, differ from other restaurants in which the food may be cooked-to-order. The short cooking times used in fast-food restaurants may explain the lower level of heterocyclic amines formed in their products.

The meat doneness requested for the restaurant samples appears to have little correlation with the heterocyclic amine content. Analyzing additional samples would be necessary for trend analysis. Repeated analysis of a single sample in a blind study in our laboratory over a 3 year period was shown to have a coefficient of variation of 36% for MeIQx and 24% for PhIP (Knize et al., 1995); thus, we believe the variation seen in Tables 1−4 indicates sample variation and not analysis variation. The mutagenic potency is generally better correlated with the doneness requested for hamburgers and steaks than the heterocyclic amine amounts. MeIQx and DiMeIQx are ~50-fold more potent mutagens than PhIP, so MeIQx, DiMeIQx, and possibly other heterocyclic amines, but not PhIP, are responsible for most of the mutagenic activity we measured.

All samples contained detectable amounts of at least one heterocyclic amine in samples cooked to a medium, well-done, or very well-done state by professional chefs.

There was no clear correlation between increased heterocyclic amine content and increased cooking doneness requested, as is seen in controlled laboratory cooking studies. The content of the heterocyclic amines in restaurant samples is similar to levels found in laboratory cooking studies, showing that meat samples prepared in laboratory studies are good surrogates for meats which North Americans actually consume.

ABBREVIATIONS USED

DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (CAS Registry No. 95896-78-9); IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline (76180-96-6); MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (77094-11-2); MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (77500-04-0); PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5). (CAS Registry No. were supplied by the author.)

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