# Mutagenicity and Chemical Analysis of Fumes from Cooking Meat

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Fumes generated during the high-temperature frying of beef were collected using a sampling system consisting of condenser, filter, and adsorbent tubes containing polyurethane foam (PUF) and XAD-4 resin. Condenser, filter, and cooked beef samples were analyzed for heterocyclic amines (HCAs) by HPLC. The PUF and XAD-4 samples were fractionated using supercritical fluid extraction (SFE) and analyzed by GC/MSD. In a modified Ames/*Salmonella* mutagenicity test (TA98 with S9), the mutagenic activity was 30 700 revertants/g in the cooked beef, 10 400 revertants/g in the fume condenser, and 270 revertants/g in the sampling filters. HCAs were found in both the fume condenser (total of 6 ng/g of cooked meat) and the meat (total of 109 ng/g of cooked meat). GC/MSD analysis of the compounds collected on PUF and XAD-4 revealed a variety of aldehydes, ketones, and phenols. Occupational exposure to carcinogens in the fumes may pose a human health risk to food preparers and warrants further investigation.

**Keywords:** *Airborne mutagens, Ames/Salmonella assay, analysis, cooking fumes, fried meat, heterocyclic amines*

## INTRODUCTION

Epidemiological studies have indicated an increased risk of respiratory tract cancer among cooks and bakers (Coggon *et al.*, 1986; Lund and Borgan, 1987). In a cancer mortality study of vegetarians, a lower proportion of deaths was observed from respiratory diseases and lung cancer than in the general population (Kinlen *et al.*, 1983). Dietary chemicals have been shown to play a significant role in the initiation of cancer (Doll and Peto, 1981).

Many genotoxic carcinogens are generated when foods are cooked including polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). HCAs, formed from reactions of creatine or creatinine with other natural meat components during cooking, are among the most potent mutagens/carcinogens discovered to date. They are strongly suspected to be the major mutagenic constituents of fumes from the heat processing of foods rich in protein (Nagao *et al.*, 1977; Rappaport *et al.*, 1979; Felton *et al.*, 1981; Berg *et al.*, 1988). Övervik *et al.* (1989) found that the mutagenicity profiles of meat crust, pan residue, and the aerosol fraction did not change with creatine addition, suggesting that the mutagen-forming reactions are similar in all three fractions. Similar HPLC/mutagenicity profiles of the extracts of meat crust, pan residue, and aerosols from frying pork were observed by Berg *et al.* (1990). While our experiments were in progress, Vainiotalo *et al.* (1993) reported MeIQx (13.7 pg/g of fried meat) and DiMeIQx (7.3 pg/g of fried meat) in fumes from frying meat using a gas chromatography procedure with derivatization, but the specificity of the derivatization method did not allow detection of other mutagens. The goal of the present study is to determine the mutagenicity of fumes generated when beef is cooked as compared to that of the cooked beef and to chemically identify the mutagenic constituents present in the fumes.

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## MATERIALS AND METHODS

**Chemicals.** HPLC grade "resi-analyzed" solvents were obtained from J. T. Baker Chemical Co. For mutagenicity experiments and chemical analysis, benzo[*a*]pyrene was purchased from Aldrich Chemical Co. 2-Amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), [2-$^{14}$C]MeIQx (40 mCi/mmol), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), 2-amino-9*H*-pyrido[2,3-*b*]indole (A$\alpha$C), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were from Toronto Research Chemicals (Downsview, ON). Reference standards were used without further purification. Methanolic solutions of each standard were made and served as stock solutions.

**Adsorbents.** The aerosol fraction of the smoke was collected onto 47 mm diameter Teflon filters with a pore size of 2 $\mu$m (Gelman Sciences, Inc., Ann Arbor, MI). The polyurethane foam (PUF) (Hickory Springs Foam Co., NC) was cut into plugs (50 mm × 21 mm). Teflon filters and PUF plugs were precleaned by sequential 30-min sonication in methanol (3 times) and dichloromethane (3 times) and were dried overnight at 80 °C. XAD-4 resin (Rohm and Haas, Philadelphia, PA) was cleaned by successive washings with 0.5 N HCl, 0.5 N NaOH, and distilled deionized water and then by sequential 30-min sonication in methanol, acetone, ethyl acetate, dichloromethane, and methanol. The resin was dried for 48 h under vacuum at 50 °C. The cleaned and dried Teflon filters, PUF, and XAD-4 were stored in amber glass jars until used.

**Supercritical Fluid Extraction (SFE).** An Isco Model 260D syringe pump (Lincoln, NE) was used for all SFE extractions with supercritical $CO_2$/10% methanol modifier (Scott Specialty Gases, Plumsteadville, PA). The solid matrices were placed in a 5-mL extraction cell (Suprex Corp., Pittsburgh, PA) and introduced into a GC oven (Varian Aerograph) maintained at constant temperature. A 45-cm length of 25 $\mu$m i.d. (375 $\mu$m o.d.) deactivated fused silica capillary (Supelco, Inc., Bellefonte, PA) was used as a depressurizing flow restrictor to maintain supercritical conditions within the extraction cell. The effluent from the outlet of the capillary was directed into a graduated screw-top collection vial fitted with a Teflon septum containing 0.5 mL of methanol. The vial was cooled in a dry ice–acetone bath maintained at −20 to −30 °C. A Pasteur pipet containing a glass wool plug placed through the septum served as a vent.

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The extraction residue was stored at −20 °C in sealed nitrogen-capped vials until analyzed or tested for mutagenicity.

A 50-μL mixture of a stock methanolic solution containing 5 μg/mL of IQ, MeIQ, MeIQx, DiMeIQx, and PhIP was spiked onto either Teflon filters or PUF plugs. After spiking, the extraction cell was kept open to allow the solvent to evaporate. The extraction cell was then sealed, placed in the GC oven, and allowed to equilibrate for 10 min. HCAs were extracted for 2 h using supercritical $CO_2$/10% methanol at 6000 psi and 55 °C. After SFE, an aliquot of the solvent was gently evaporated to dryness with nitrogen, the residue was resuspended with 50 μL of methanol containing 5 μg/mL of caffeine as an internal standard, and 20-μL aliquots were analyzed by HPLC. A duplicate extraction/analysis was made for each solid matrix. Standards were prepared in triplicate from stock solutions, and the solvent was evaporated and dissolved using the same procedure as for the SFE-extracted samples. The extraction recoveries were calculated by comparing the quantitative HPLC analysis of each sample with standards.

**Mutagenic Activity of Heterocyclic Amine Standards.** To attribute mutagenic activity due to each HCA chemically detected in the samples, we first tested standard HCAs for mutagenicity. Dose–response curves were established using both the standard Ames method and the microsuspension procedure. The sensitivities of both tests were compared, and the most sensitive procedure was chosen and applied to the meat and smoke extracts.

*Salmonella typhimurium* strains TA98 and TA100 were kindly provided by Dr. B. N. Ames, University of California, Berkeley. The standard test was conducted according to the revised procedure of Maron and Ames (1983). The microsuspension procedure was used as developed by Kado *et al.* (1983). The S9 and S9 mix were prepared according to the procedure of Ames *et al.* (1975). The S9 from Aroclor 1254 pretreated male Sprague-Dawley rats contained 40.3 mg of protein/mL as determined by the modified biuret method of Ohnishi and Barr (1978).

Briefly, for the standard procedure, bacteria were grown overnight in Oxoid nutrient broth no. 2 (Oxoid Ltd., Hants, England) to approximately 1–2 × $10^9$ cells/mL. The following were added to 13 mm × 100 mm sterile glass culture tubes: 0.5 mL of S9 mix, 50 μL of sample in methanol or dimethyl sulfoxide (DMSO), 0.1 mL of bacteria culture, and 2 mL of molten top agar containing 90 nmol of histidine and biotin. The mixture was gently vortexed and poured onto minimal glucose plates. Plates were incubated at 37 °C in the dark for 48 h, and the bacteria were counted with a BioTran II automated colony counter (New Brunswick Scientific, Edison, NJ). Negative controls were performed with 5 μL of pure methanol and positive controls with 5 μL of benzo[*a*]pyrene diluted in methanol (50 μg/mL). Strain markers were routinely determined for each experiment. Each dose was tested in duplicate, and the number of revertants was calculated from the linear portion of the dose–response curve (four points minimum) according to the method of Moore and Felton (1983).

For the microsuspension procedure, bacteria were grown overnight and harvested by centrifugation (4500*g* at 4 °C for 4 min). Cells were resuspended in ice-cold phosphate-buffered saline (0.2 M PBS, pH 7.4) to a concentration of approximately 1 × $10^{10}$ cells/mL. The following were added, in order, to 13 mm × 100 mm sterile glass screw-top culture tubes kept on ice: 0.1 mL of S9 mix, 5 μL of sample in methanol or DMSO, and 0.1 mL of concentrated bacteria in PBS. The mixture was incubated in the dark at 37 °C with rapid shaking for 90 min. The tubes were then placed in an ice bath and taken out one at a time just before 2 mL of molten top agar containing 90 nmol of histidine and biotin (Ames *et al.*, 1975) was added. The combined solutions were vortex-mixed and poured onto minimal glucose plates. The plates were incubated and counted as previously described.

**Cooking and Air Sampling Procedure.** The apparatus used for cooking and smoke collection is shown in Figure 1. An electric hot plate (A) was used to heat a Teflon-coated aluminum pan (30-cm diameter) (B). The pan temperature was monitored by using a thermocouple (3) kept on the surface of the pan by an aluminum holder (C). This temperature-measuring device included a meat thermocouple (2), which allowed us to continually measure the temperature in the center of meat. The fume

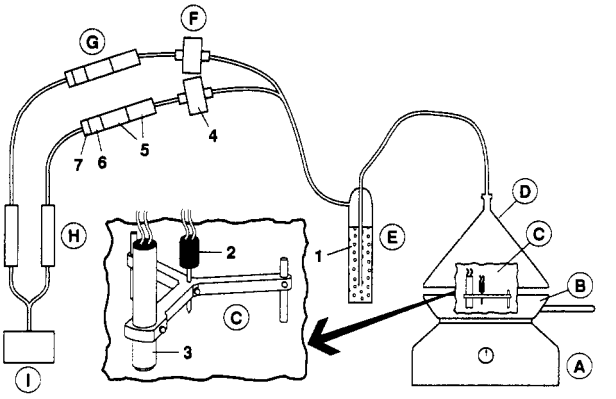


**Figure 1.** Sampling device used for smoke collection: (A) electric plate; (B) frying pan (Teflon-coated aluminum); (C) thermometers; (D) collection funnel (glass); (E) bead trap (glass bubbler); (F) filter holder (PTFE); (G) adsorbent tube; (H) flow meter; (I) vacuum pump; (1) meat and (2) pan thermocouples; (3) 4-mm glass beads; (4) 47-mm PTFE filter; (5) 2 PUF plugs; (6) 5-mL XAD-4 bed; (7) glass wool plug.

produced by cooking the meat was collected through a glass funnel (30-cm diameter) (D) and directed through Teflon tubing into a glass bubbler (E) containing about 600 g of glass beads (4-mm diameter) (1) kept in an ice bath. Since the vapors were not completely adsorbed, the bead trap was followed by two identical parallel sampling trains. Each train contained a 47-mm Teflon filter (4) in a Teflon holder (Pallflex Products Corp.) (F) and a 16 × 135 mm open-ended glass tube (G) containing two PUF plugs (5) and one 5-mL XAD-4 bed (6), held in place by precleaned glass wool plugs. The flow rate was created by a vacuum pump (I), regulated at 20–25 L/min in each train by a 25-L flow meter (H).

Ground beef (2 kg, sold as 15% fat) was purchased locally. Fresh patties (100 g each) were fried, three at a time for 6 min on each side. The average cooking temperatures were 277 °C at the surface of the pan and 70 °C in the center of the meat. The maximum temperature reached in the center of the meat was 92 °C. After cooking, the total fried meat weight was 1.019 kg. The smoke condensed in the bead trap formed a liquid which was emptied for every six patties cooked. All of the fractions were pooled in 1-L precleaned amber glass jars stored at −20 °C. The Teflon filters were changed for every six patties cooked and were stored at −20 °C. The same PUF plugs and XAD-4 resin were used for the entire sampling period. After sampling, the glass tubes containing the adsorbants were plugged and stored at −20 °C until SFE.

**Extraction and Purification Procedures.** The fried meat sample was prepared according to the solid-phase extraction method of Gross (1990). Briefly, 4-g samples were homogenized in 1 N NaOH and mixed with Chem Elut diatomaceous earth (Varian Sample Preparation Products, Harbor City, CA) to form a free-flowing powder. The mixtures were poured into Extrelut (EM Science, Gibbstown, NJ) extraction columns coupled to Bond Elut PRS (propylsulfonic acid–silica gel, 500 mg) solid-phase extraction columns (Varian). HCAs were extracted to the PRS columns with 40 mL of 5% toluene in dichloromethane. For mutagenicity testing, the samples were eluted from the PRS cartridge with 2 mL of methanol/ammonium hydroxide (9:1), evaporated under a stream of nitrogen, and resuspended in 200 μL of methanol. For the HPLC analysis, the sample retained by the PRS cartridge was processed according to the procedures of Gross and Grüter (1992). Further sample cleanup including a separation of polar (i.e., IQ, MeIQx, DiMeIQx, PhIP) and nonpolar (Trp-P-1, Trp-P-2, AαC) HCAs was performed using the method of Gross *et al.* (1992). Replicate meat samples were unspiked or spiked with IQ, MeIQx, DiMeIQx, PhIP, Trp-P-1, Trp-P-2, and AαC. After purification, the samples were gently evaporated to dryness and resuspended with 100 μL of methanol containing 5 μg/mL of caffeine as an internal standard.

Fifty-milliliter samples of the condensed liquid from the bead trap (total volume 920 mL) were either spiked with standards or left unspiked and acidified to pH 2 with 0.25 N HCl and then

**Table 1. Supercritical Fluid Extraction Recoveries of Heterocyclic Amines (250 ng) Spiked onto Teflon-Coated Filters and Polyurethane Foam (PUF)**

| | % recovery (% ± SD)[a] | |
|---|---|---|
| | filter | PUF |
| IQ | 95.3 ± 2.6 | 90.5 ■ 17.3 |
| MeIQ | 93.3 ± 1.6 | 102.0 ± 6.3 |
| MeIQx | 83.2 ± 1.4 | 87.0 ± 0.3 |
| DiMeIQx | 82.6 ± 2.7 | 89.5 ± 2.1 |
| PhIP | 1.2 ± 18.5 | 22.1 ■ 27.4 |

[a] Standard deviations are based on two separate extractions using supercritical $CO_2$/10% MeOH at 55 °C, 6000 psi, and 0.929 g/mL for 2 h.

extracted twice with 50 mL of dichloromethane in a separatory funnel. The organic phase was neutralized with 0.25 N NaOH, and the solution was applied to an XAD-2 Amberlite resin column (1 × 10 cm). The sample was desorbed from the XAD-2 column with 50 mL of methanol/acetone (1:1) and the solvent evaporated under a nitrogen stream. For mutagenicity testing, the sample was resuspended in 100 μL of methanol. After purification, the samples were gently evaporated to dryness and resuspended with 50 μL of methanol containing internal standard. Further cleanup of the bead trap was required for confirmatory UV spectra and was done on TSK-gel CM-650 according to the method of Gross *et al.* (1992).

The Teflon filters from the smoke sampling were pooled in a sonication bath and extracted twice during 30 min with 20 mL of methanol/ammonium hydroxide (9:1), pH 11. The extract was evaporated with nitrogen to a volume of about 1 mL. Then, 50 mL of deionized water was added, mixed, and poured onto Bond-Elut $C_{18}$ columns previously conditioned with 2 mL of methanol and 10 mL of water. The adsorbed residue was desorbed with 1.2 mL of methanol/ammonium hydroxide (9:1). For mutagenicity testing, the solvent was removed with nitrogen and the residue was resuspended with 200 μL of methanol and stored at −20 °C until tested.

The SFE of PUF and XAD-4 was adapted from Wong *et al.* (1991) and Kado *et al.* (1992). The four PUF plugs were pooled and extracted in a 10-mL extraction cell that was sealed, connected to the SFE apparatus, equilibrated at 55 °C for 10 min, and dynamically extracted for 2 h with $CO_2$/10% methanol at 6000 psi. The effluent was collected and stored as previously described until tested and analyzed. The same procedure was followed for XAD-4 resin.

**HPLC Analysis.** A Millenium 2010 HPLC system (Millipore Corp., Milford, MA) equipped with a diode array detector (Model 996) was used to analyze 20-μL samples. The fluorescence detector was programmed to 265/410 nm (excitation/emission) for Trp-P-2, 306/371 nm for PhIP, and 335/410 nm for AαC. The separations were accomplished using a Toyo Soda TSK-gel ODS80TM column (250 mm × 4.6 mm i.d.) with a mobile phase of 0.01 M triethylamine phosphate, pH 3.6 (A solvent), and acetonitrile (B solvent). A linear gradient (5–15% B, 0–10 min; 15–25% B, 10–20 min; 25–55% B, 20–30 min) was used.

**GC/MS Analysis.** The PUF and XAD-4 methanolic extracts were analyzed using a Hewlett-Packard Model 5890 gas chromatograph using the splitless mode on a 30 m × 0.25 mm i.d. DB-5 capillary column (0.25-μm film thickness; J&W Scientific, Rancho Cordova, CA), with an injection port temperature of 250 °C. The column temperature was maintained at 40 °C for 4 min and programed at 5 °C/min to 200 °C and then at 20 °C/min to 300 °C and held at this temperature for 9 min. Helium was used as carrier gas at 1 mL/min. The GC was interfaced to a Hewlett-Packard Model 5970 quadrupole mass selective detector (MSD) maintained at 250 °C. The MSD was equipped with an electron impact positive 70-eV ion source interfaced with a Hewlett-Packard G1030A ChemStation and a NIST/EPA/MSDC mass spectral data base for data processing and library searches. The MSD was autotuned using perfluorotributylamine standard. The programmed solvent delay was 2.5 min, and scans were made from 35 to 500 mass units.

**Quality Assurance and Control.** All solvents and precleaned adsorbant extracts were tested by GC/MSD, HPLC, and bioassays for possible contaminants. Mutagenicity assays were performed with both negative and positive controls. A field blank sample was collected from the raw meat, extracted, and analyzed using the same conditions as for the cooked beef sample.

## RESULTS

**Supercritical Fluid Extraction of Heterocyclic Amines.** We first optimized the SFE procedure with [2-$^{14}$C]MeIQx (100 ng) spiked onto Teflon filters and PUF (data not shown). Extraction with supercritical $CO_2$ alone at 6000 psi and 55 °C recovered less than 5% of the radioactivity initially spiked after 4 h. On the contrary, with $CO_2$/10% methanol we measured extraction recoveries higher than 90% after 1.5 h. Thus, the efficiency of methanol as a modifier was clearly shown. This result has been confirmed by spiking both Teflon filters and PUF plugs with a mixture of five HCAs and by extracting during 2 h with supercritical $CO_2$/10% methanol at 6000 psi and 55 °C. As shown in Table 1, the recoveries calculated with HPLC analysis were greater than 90% with spiked quinolines (IQ, MeIQ), and 80–90% with quinoxalines (MeIQx, DiMeIQx). Recoveries for PhIP were less than 23%. Excluding the data obtained with PhIP, there was an overall variability of only 4.3%.

**Table 2. Mutagenic Activity of Heterocyclic Amines in the Microsuspension and the Standard Plate Incorporation Procedures in TA98 and TA100 (+S9)**

| | | revertants/μg[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | TA98 | | | TA100 | | |
| compd | solvent | micro | standard | increase[b] | micro | standard[c] | increase[b] |
| IQ | methanol | 985 000 | 308 000 | 3.2 | 6 350 | 4 000 | 1.6 |
| | DMSO | 672 000 | 179 000 | 3.8 | | | |
| MeIQ | methanol | 5 210 000 | 898 000 | 5.8 | 57 400 | 63 000 | 0.9 |
| | DMSO | 2 640 000 | 572 000 | 4.6 | | | |
| MeIQx | methanol | 954 000 | 63 000 | 15.0 | 8 900 | 8 540[d] | 1.0 |
| | DMSO | 628 000 | 72 900 | 8.6 | | | |
| DiMeIQx | methanol | 1 900 000 | 435 000 | 4.4 | 27 700 | 11 200[d] | 2.5 |
| | DMSO | 1 210 000 | 253 000 | 4.8 | | | |
| Trp-P-2 | methanol | 3 890 000 | 38 300 | 101.6 | 25 200 | 1 490[e] | 16.9 |
| | DMSO | 2 520 000 | 28 900 | 87.2 | | | |
| AαC | methanol | 385 | 345 | 1.1 | | 40[f] | |
| | DMSO | | | | | | |
| PhIP | methanol | 233 000 | 3 520 | 66.2 | 13 300 | 140 | 95.0 |
| | DMSO | 217 000 | 3 330 | 65.2 | | | |

[a] Calculated from the linear portion of the dose–response curve (four points minimum) from replicate platings by the method of Moore and Felton (1983). [b] Fold increase in sensitivity of microsuspension over the standard plate incorporation procedure. [c] From Felton and Knize (1990). [d] From Knize *et al.* (1987). [e] From Hatch *et al.* (1984). [f] From Peters *et al.* (1981).

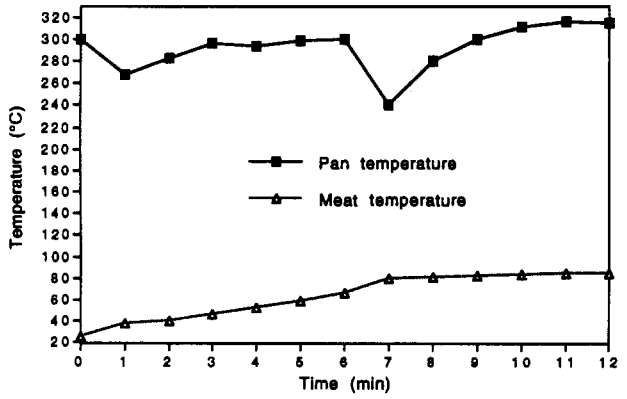


**Figure 2.** Pan and meat temperatures during frying of three beef patties, 6 min on each side.

**Mutagenic Activity of Heterocyclic Amine Standards.** The mutagenic activity of standard HCAs in strains TA98 and TA100 is shown in Table 2. MeIQ had the highest mutagenic activity, while AαC had the lowest. In both TA98 and TA100, the mutagenic activity for each compound was much higher using the microsuspension procedure compared to the standard plate test. A 100-fold increase in sensitivity was observed with Trp-P-2 in *Salmonella* strain TA98 using the microsuspension method. For TA100, almost the same order of magnitude of increased sensitivity was observed with PhIP. AαC had low activity in TA98 using either procedure. The ranking of each compound varied with the procedure used. A mixture of pure IQ, MeIQ, MeIQx, DiMeIQx, and PhIP at 5 μg/mL was also tested. By both procedures, the slope of the dose–response curve of the mixture was equivalent to the sum of those of individual compounds (data not shown). The HCAs tested were 1.1–2 times more mutagenic under identical experimental conditions when they were dissolved in methanol rather than DMSO.

**Cooking Procedure.** Pilot experiments were performed by sampling fumes directly onto Teflon filters and solid adsorbants connected to the top of the glass funnel. Because of the large amount of grease and water generated during cooking, this system did not allow enough sampling capacity, suggesting the need for a grease/water trap. Thus, a bead trap condenser was installed and was followed by two parallel trains, each one containing a 47-mm Teflon filter, two PUF plugs, and a 5-mL XAD-4 resin bed. This sampling device allowed for high flow rates and a total sampling of fumes.

The meat and pan temperatures recorded during the cooking of three beef patties are presented in Figure 2. The pan temperature was initially calibrated at 300 °C. When the fresh meat was dropped into the pan, the temperature decreased and then reached a constant level of about 295 °C until the patties were turned over. The average pan temperature was 277 °C. The meat temperature increased constantly and reached 80–85 °C after 7 min of cooking.

**Mutagenic Activity of Fried Meat and Smoke Samples.** The fried meat extracts were mutagenic in the microsuspension procedure and had 30 700 *S. typhimurium* TA98 revertants/g of fried beef (Table 3) or 16 800 revertants/g of fresh beef. The fumes produced by frying 2 kg of beef were condensed mainly onto the bead trap as 920 mL of a brown-gray liquid. This condensate was tested before purification and contained an activity of 10 400 revertants/g of fried meat, whereas the activity dropped to 4440 revertants/g after purification. The filter sample had a much lower mutagenic activity in TA98. The undiluted PUF and XAD-4 extracts were toxic to TA98.

**Table 3. Mutagenic Activity of Fractions from a Beef Frying Experiment in the Microsuspension Procedure with TA98 (+S9)**

| sample | revertants/g of cooked food[a] |
|---|---|
| fried meat | 30 700 |
| neat condensate | 10 400 |
| purified condensate | 4 440 |
| filters | 270 |
| PUF | toxic |
| XAD-4 | toxic |

[a] Calculated from the linear portion of the dose–response curve (four points minimum) from replicate platings by the method of Moore and Felton (1983).

**Table 4. Concentration of Heterocyclic Amines in the Fried Meat and Smoke Condensate Samples**

| | ng/g of fried beef | |
|---|---|---|
| compd | meat | purified condensate |
| MeIQx | 16.4 | 0.971 |
| DiMeIQx | 4.5 | 0.147 |
| PhIP | 67.5 | 1.47 |
| AαC | 21.0 | 3.48 |

After purification with a $C_{18}$ column according to the procedure described for the filter extraction, the extracts remained toxic. Dilutions of the extracts were also tested and were either toxic or not mutagenic.

The activity due to the heterocyclic amines found either in the meat sample or in the purified bead trap sample was calculated by multiplying the weight of each HCA detected in the sample by its corresponding mutagenic activity in the microsuspension assay (Table 2) and by summing the individual activities. The total activity calculated for the meat sample was 39 900 TA98 revertants/g of fried beef (30 700 measured) and for the bead trap sample was 1550 TA98 revertants/g of fried beef (10 400 measured). Using the standard Ames test, the calculated activities were 3250 and 130 TA98 revertants/g for the fried beef and bead trap samples, respectively, resulting in a comparable ratio.

**Chemical Analysis of Fried Meat and Smoke Samples.** HPLC chromatograms and the UV absorbance spectra of the meat and bead trap condensate are shown Figures 3 and 4, respectively. After extraction, complex chromatograms are obtained; peak confirmation using ultraviolet absorbance spectra from a photodiode array detector (shown in panels A–D), comparing the sample spectrum (jagged line) and reference spectrum (smoother line), is required. Quantitative results are presented in Table 4. PhIP accounted for 62% of the total weight of the HCAs found in the fried meat, while AαC accounted for 57% in the bead trap condensate. PhIP and AαC were the two most abundant compounds in both samples. MeIQx and DiMeIQx were detected, but IQ and Trp-P-2 were not. The overall recoveries of spiked HCAs were MeIQx 63%, DiMeIQx 68%, IQ 63%, PhIP 31%, Trp-P-2 51%, and AαC 33% for the meat sample and MeIQx 81%, DiMeIQx 81%, IQ 82%, PhIP 75%, Trp-P-2 31%, and AαC 11% for the bead trap sample.

The PUF and XAD-4 fractions extracted with supercritical $CO_2$/10% methanol were analyzed by GC/MSD as shown by the total ion chromatograms in Figure 5. The main peaks listed in Table 5 were tentatively identified by searching the mass spectral library. Total ion chromatograms were similar for both PUF and XAD-4 samples, but more peaks were observed after 25 min in the PUF chromatogram compared to that of XAD-4. The chemicals identified include alkanes, alcohols, aldehydes, ketones,

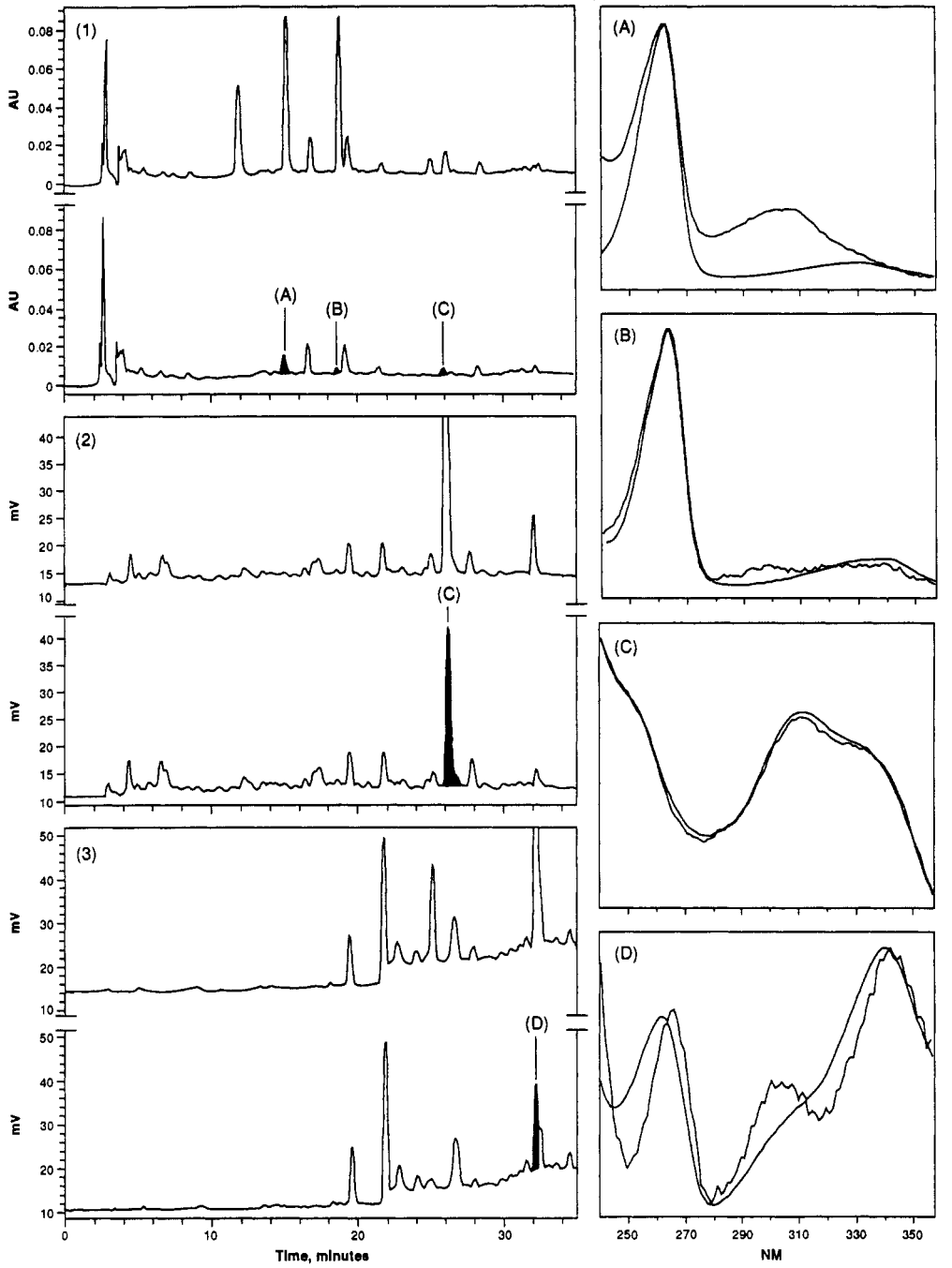


**Figure 3.** Chromatographic analysis of fried beef patties: UV chromatograms (262 nm) of spiked (upper) and unspiked polar extract (1); fluorescence chromatograms of spiked (upper) and unspiked extracts of the polar (2) and apolar (3) extracts; (right) UV absorbance spectra of peaks from the samples and standards, (A) MeIQx, (B) DiMeIQx, (C) PhIP, (D) A$\alpha$C.

phenols, and one acid. The most abundant chemicals were aldehydes, common to both PUF and XAD-4 fractions.

## DISCUSSION

**Method Development.** Supercritical $CO_2$ was inefficient in extracting HCAs spiked onto a solid matrix, whereas supercritical $CO_2$/10% methanol at 6000 psi and 55 °C resulted in good recoveries of quinolines and quinoxalines spiked onto Teflon filters and PUF. However, the recovery of PhIP was poor and requires further investigations to optimize SFE recovery. We chose identical SFE conditions for the fried beef fumes. SFE is an efficient and reliable technique that presents certain advantages over conventional liquid solvent extraction (Hawthorne, 1990). It allows for the extraction and concentration of volatile compounds in one step, thereby minimizing potential loss of the compounds, and provides a methanolic extract that can be directly analyzed by GC/MSD or bioassay. However, one disadvantage is that the flow restrictor is subject to plugging when the samples are wet or contain high amounts of extractable material and particulate matter. Therefore, the bead trap condensate and the filters were extracted with conventional liquid solvents.

The microsuspension procedure used to evaluate the mutagenic activity of standard HCAs was 5–100 times more sensitive than the standard plate incorporation assay with strain TA98. However, the microsuspension procedure did not increase the sensitivity of TA98 for A$\alpha$C, whereas the larger increment of sensitivity was observed with another aminocarboline compound, Trp-P-2. Our study shows the mutagenic activities to be higher when

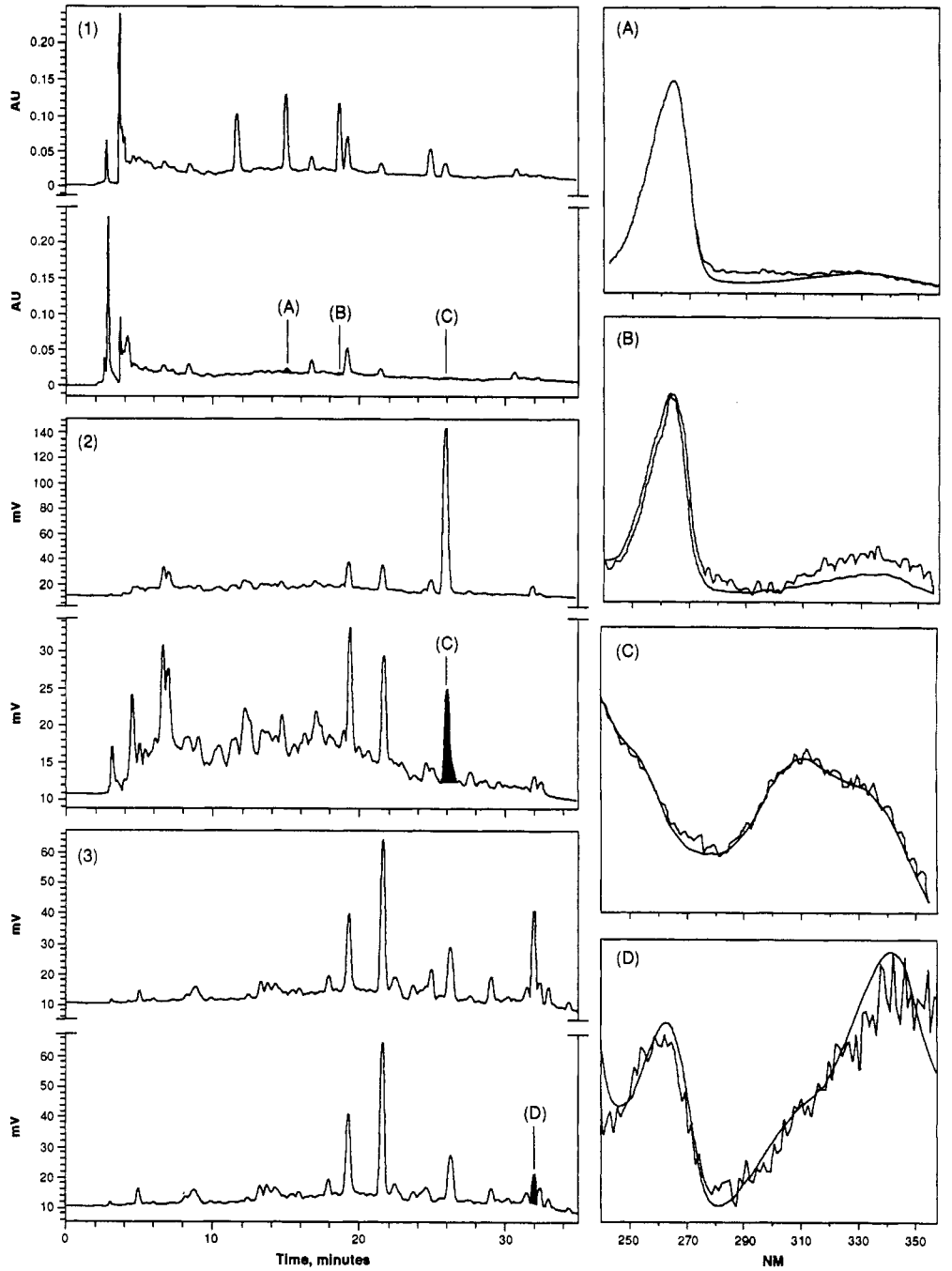


**Figure 4.** Chromatographic analysis of bead trap condensate. UV chromatograms (262 nm) of spiked (upper) and unspiked polar extract (1); fluorescence chromatograms of spiked (upper) and unspiked extracts of the polar (2) and apolar (3) extracts; (right) UV absorbance spectra of peaks from the samples and standards, (A) MeIQx, (B) DiMeIQx, (C) PhIP, (D) Aαc.

HCAs were dissolved in methanol rather than in DMSO. DMSO is compatible with the *Salmonella* microsome test (Ames *et al.*, 1975; Maron *et al.*, 1981; Mori *et al.*, 1985) but may inhibit the mutagenicity of certain compounds, such as *N*,*N*-dimethylnitrosamine (Yahagi *et al.*, 1977) and dimethyl- and diethylcarbamyl chloride (Hermann *et al.*, 1978). This solvent or its derivatives may be toxic to TA98 and TA100 (Vaughan *et al.*, 1989). Methanol can also be toxic at high concentrations (Arimoto *et al.*, 1982). However, it is more volatile than DMSO and may evaporate quickly during the preincubation or incubation steps, thereby reducing its toxicity. Methanol was easier to use in resuspending the purified samples, as well as to dissolve the meat, bead trap, and filter extracts.

Few studies concerning the sampling and analysis of fumes from cooking are reported in the literature. The aerosol fraction alone was investigated by Nagao *et al.* (1977) and Rogge *et al.* (1991). Other investigators have examined the aerosol and volatile fractions using filters and solid adsorbents (Teschke *et al.*, 1989; Berg *et al.*, 1988, 1990; Övervik *et al.*, 1989; Löfroth *et al.*, 1991; Vainiotalo *et al.*, 1993; Vainiotalo and Matveinen, 1993). Several investigators used condensation traps filled with solvents (Rappaport *et al.*, 1979; Felton *et al.*, 1981). To avoid sampling problems due to the large amount of water and grease contained in the fumes, we constructed a sampling device using a condensation trap followed by two parallel sampling trains.

**Mutagenic Activities and Chemical Analysis of Fried Meat and Smoke Samples.** The mutagenic activity of the fried meat extract was 30 700 TA98 (+S9) revertants/g, while the total activity extracted from fume samples was 10 700 TA98 revertants/g of fried meat. Thus, the fume activity represented 35% of the fried meat

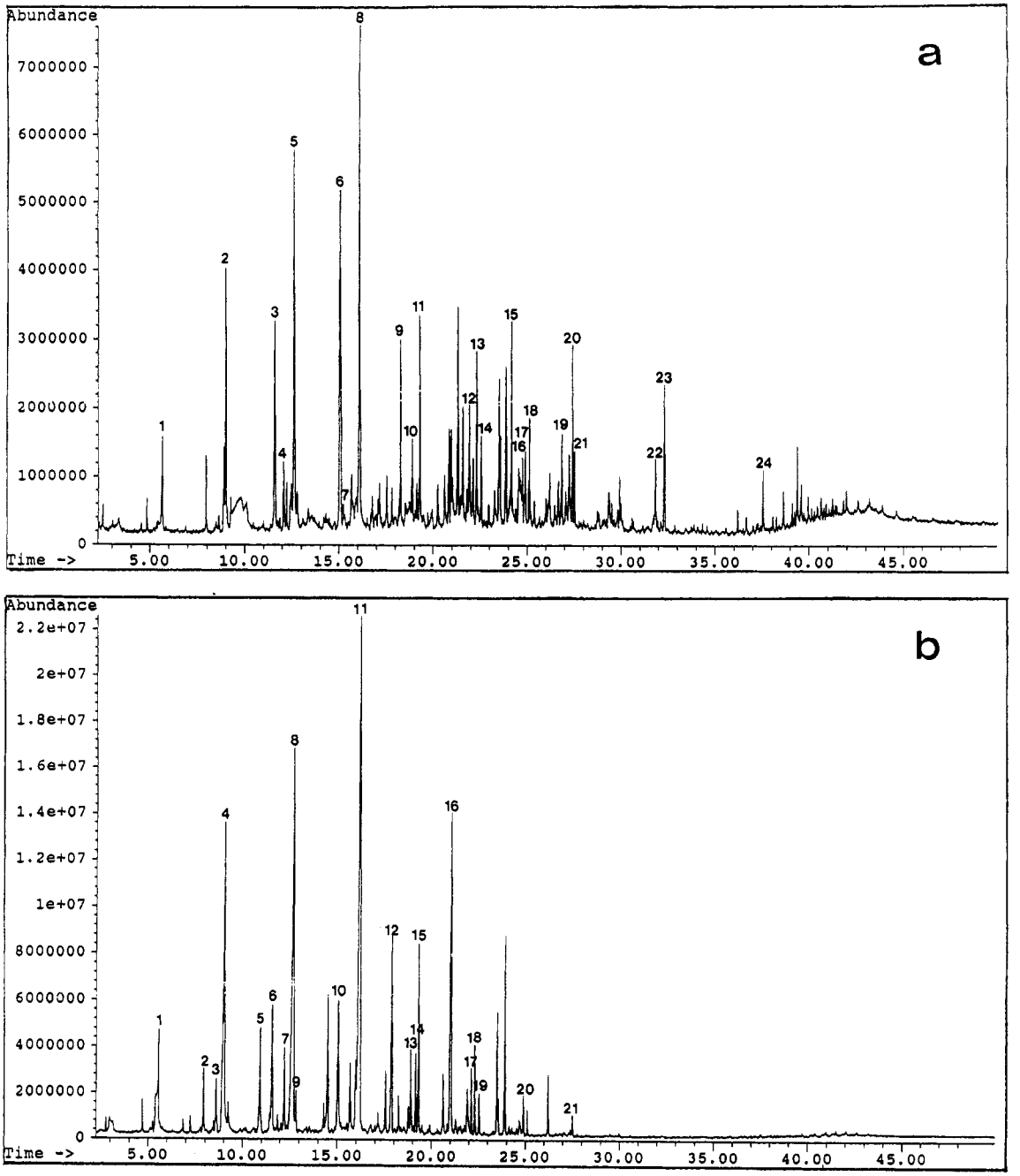


**Figure 5.** Total ion chromatograms of PUF (A) and XAD-4 (B) fractions extracted by supercritical $CO_2$/10% methanol.

activity. Only 42.6% of the total mutagenic activity found in the bead trap remained after purification for HCA analysis. Little activity was extracted from the filters (0.9%), while the PUF and XAD-4 fractions were toxic to TA98. Similarly, Berg *et al.* (1988) found that after broiling pork at 215 °C for 10 min and analyzing with the standard Ames test, the aerosol fraction represented 18% and the volatile fraction 1.2% of the meat crust activity. With the same kind of meat fried at 225 °C for 10 min and using the microsuspension procedure, Löfroth *et al.* (1991) found that the activity of aerosol particles represented 43% of the surface food activity.

HPLC analysis was used to quantify PhIP, AαC, DiMeIQx, and MeIQx in both the meat and the smoke extracts. PhIP and MeIQx were present in the meat at levels of 67.5 and 16.4 ng/g of fried beef, respectively. These two promutagens each contributed about 15 700 TA98 (+S9) revertants/g. The contribution of DiMeIQx (4.5 ng/g) was half as low, while AαC, as abundant as MeIQx in mass (≈20 ng/g), contributed only 8 revertants/g. In the smoke condensate, MeIQx (0.971 ng/g) was the most potent HCA analyzed and contributed about 930 TA98 revertants/g of fried meat. The amount of PhIP (1.47 ng/g) was 10 times higher than DiMeIQx, but its mutagenicity contribution was similar (about 300 revertants/g). The most abundant HCA found in the smoke condensate was AαC, at a concentration of 3.48 ng/g. Its mutagenic contribution was negligible (≈1 revertant/g). Among the quantified HCAs, AαC seems to be the most volatile since its level in the smoke condensate reached 16% of its level in the meat, while the other HCA levels were less than 6% of the meat content. IQ and Trp-P-2 were not detected in the meat or the fume samples.

One recent study reported the presence of 0.0137 ng/g MeIQx and 0.0073 ng/g DiMeIQx in cooking fumes as analyzed by GC/MSD from filters and sorbent tubes after 1440 g of minced beef/pork was fried at 210 °C (Vainiotalo *et al.*, 1993). We found these same quinoxaline derivatives at concentrations of 0.971 and 0.147 ng/g of fried beef, respectively. Our results were 10–20 fold higher and were

**Table 5. Tentative Identification by GC/MSD of Volatile Compounds Adsorbed from Fumes on PUF and XAD-4 and Extracted with Supercritical $CO_2$/10% MeOH**

| | PUF | | XAD-4 | |
|---|---|---|---|---|
| compd | peak no. | RT (min) | peak no. | RT (min) |
| alkanes | | | | |
| dodecane | | | 14 | 19.2 |
| tridecane | 12 | 22.1 | 17 | 22.1 |
| pentadecane | 21 | 27.5 | 21 | 27.5 |
| nonadecane | 17 | 24.9 | | |
| 1-tetradecane | 25 | 39.4 | | |
| eicosane | | | 20 | 24.9 |
| C5-cyclopropane | 6 | 15.1 | 10 | 15.1 |
| C8-cyclopropane | 9 | 18.3 | | |
| cyclododecane | 19 | 26.8 | | |
| alcohols | | | | |
| 1-hexanol | | | 2 | 7.9 |
| 1-heptanol | 3 | 11.6 | 6 | 11.6 |
| 1-dodecanol | 15 | 24.2 | | |
| 1-octadecanol | 22 | 31.8 | | |
| phenol | 4 | 12.1 | | |
| 4-methylphenol | 7 | 15.2 | | |
| aldehydes | | | | |
| hexanal | 1 | 5.6 | 1 | 5.5 |
| heptanal | 2 | 9.0 | 4 | 9.0 |
| octanal | 5 | 12.6 | 8 | 12.7 |
| nonanal | 8 | 16.1 | 11 | 16.2 |
| decanal | 11 | 19.3 | 15 | 19.3 |
| dodecanal | 13 | 22.3 | 18 | 22.3 |
| tridecanal | 18 | 25.1 | | |
| (*Z*)-2-heptenal | | | 5 | 10.9 |
| (*Z*)-2-nonenal | | | 12 | 17.9 |
| (*E*)-2-decenal | | | 16 | 21.0 |
| (*E*,*E*)-2,4-heptadienal | | | 9 | 12.8 |
| (*E*,*E*)-2,4-decadienal | 14 | 22.6 | 19 | 22.5 |
| ketones | | | | |
| 5-methyl-2-hexanone | | | 3 | 8.5 |
| 6-methyl-2-heptanone | | | 7 | 12.2 |
| 2-decanone | 10 | 18.9 | 13 | 18.9 |
| 2-dodecanone | 16 | 24.7 | | |
| 2-tridecanone | 20 | 27.4 | | |
| 2-pentadecanone | 23 | 32.3 | | |
| acid | | | | |
| hexadecanoic acid | 24 | 37.5 | | |

probably due to the experimental conditions: type of meat, frying temperature, sampling system, and extraction and analysis procedures.

The GC/MSD analysis of PUF and XAD-4 fractions showed that alkanes, alcohols, aldehydes, ketones, phenols, and acids were the main volatile compounds generated during the frying of beef. Their presence may explain the bacterial toxicity we observed for *Salmonella* TA98 in the PUF and XAD-4 SFE fractions. Among these classes of compounds, linear aldehydes from $n$-$C_6$ to $n$-$C_{13}$ were widely represented, with nonanal as the most abundant. However, not all of the chemical groups previously reported, such as terpenoids, esters, furans, lactones, amides, nitriles, and steroids, were detected (Urbach and Stark, 1975; Shibamoto *et al.*, 1981; Alenca *et al.*, 1983; Ohnishi and Shibamoto, 1984; Rogge *et al.*, 1991; Um *et al.*, 1992; Vainiotalo and Matveinen, 1993). They may be formed by the oxidation of fat or from sugar degradation. No polycyclic aromatic hydrocarbons (PAHs) were detected. These compounds are generated by heating fat at high temperatures, but only low amounts benz[*a*]pyrene, chrysene, and dibenzanthracenes have been detected in fumes from frying meat experiments (Larsson *et al.*, 1983; Um *et al.*, 1992; Vainiotalo and Matveinen, 1993).

This study shows that the mutagenic activity of the fumes generated during cooking of meat represents 35% of the cooked meat activity. The meat mutagenicity could be mainly attributed to the HCAs analyzed since the total theoretical activity represents 130% of the observed activity. The level of these chemicals in fumes appears to be much lower than that in the cooked meat. They seem to be responsible for only 15% of the fume mutagenic activity. The presence of HCAs and other mutagenic compounds in fumes of meat cooking may pose human health risks, especially to food processing workers, and warrants further investigation and assessment.

## ABBREVIATIONS USED

A$\alpha$C, 2-amino-9*H*-pyrido[2,3-*b*]indole (CAS Registry No. 261148-68-5; Registry No. were supplied by the author); B(*a*)P, benzo[*a*]pyrene; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (95896-78-9); DMSO, dimethyl sulfoxide; GC, gas chromatography; HPLC, high-pressure liquid chromatography; HCAs, heterocyclic amines; MSD, mass spectrometry detector; 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); PAHs, polycyclic aromatic hydrocarbons; PBS, phosphate-buffered saline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5); PRS, propylsulfonic acid silica; PUF, polyurethane foam; SFE, supercritical fluid extraction; Trp-P-2, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (62450-10-3); UV, ultraviolet.

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