Heterocyclic Amines: 1. Kinetics of Formation of Polar and Nonpolar Heterocyclic Amines as a Function of Time and Temperature

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ABSTRACT: The influence of time, temperature, glucose, and creatine on the formation of heterocyclic amines (HAs) in beef was investigated. Kinetic models as a function of time and temperature were used to examine the formation of polar and nonpolar HAs, as described by Arrhenius and Eyring equations. The highest amount of MeIQx was 23.93 ng/g at 220 °C for 20 min. However, no formation of 4,8-DiMeIQx was observed at 160 °C, 180 °C, 200 °C, and 220 °C for 5 min. Norharman and 4,8-DiMeIQx were more sensitive to temperature change, whereas MeIQx and AαC were less sensitive. The formation of HAs increased with increasing time and temperature. Glucose and creatine decreased at the same pattern with increasing time and temperature. The formation of HAs and the degradation of precursors were inversely correlated. The results provide valuable information toward modulating and inhibiting the formation of HAs in cooked meats.

Keywords: Heterocyclic amines, Arrhenius, Eyring, glucose, creatine

Introduction

Among the chronic diseases, cancer poses one of the greatest risks for humans. Although cancer incidences have been on the decline, malignant neoplasms are still the number 2 cause of death in the United States, following heart disease. Nutrition and dietary carcinogens are a major factor in causing cancer (Willett 1995; Sugimura 2000). The formation of toxicants is one of the most unfavorable changes during cooking of meat and meat products (Deshpande 2002). Chemical changes during cooking have been increasingly of concern because of their potential mutagenicity/carcinogenicity. Because beef contains abundant nutrients that are susceptible to high cooking temperature, pyrolytic compounds are often formed from free amino acids, protein, and other heat-susceptible nutrients. Most pyrolytic compounds such as heterocyclic amines (HAs) have been shown to be potential mutagens/carcinogens to humans (Sugimura 2000).

HAs were first isolated from charred meat and fish (Nagao and others 1977). Since 1977, more than 20 HAs have been isolated and identified from cooked muscle foods (Ohgaki and others 1991; Wakabayashi and others 1992). HAs have been divided into 2 types, pyrolytic mutagens and thermic mutagens. Pyrolytic mutagens are produced from muscle foods cooked at high temperatures above 300 °C and include Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-1, Phe-P-1, AαC, and MeAαC. Thermic mutagens are commonly formed at normal cooking temperatures below 300 °C and are much more harmful than pyrolytic mutagens (Yoshida and others 1978; Sugimura 1982, 1992; Pearson and others 1992). Thermic mutagens, in particular aminoimidazoazarenes (AIAs), structurally have an imidazo group, which is linked to a quinoline, a quinoxaline, a pyridine, or a furopyridine ring. These compounds are generally classified as imidazoquinolines, imidazoquinoxalines, imidazopyridines, and imidazofuropyridine (Felton and others 1986; Becher and others 1988; Knize and others 1990; Jägerstad and others 1991). The most common and abundant HAs in cooked meats are PhIP, AαC, MeIQx, DiMeIQx, and IQ (Layton and others 1995; Keating and others 1999). These HAs have become of greater concern during the past 3 decades. The International Agency for Research on Cancer (IARC 1993) has recorded some of these HAs in monographs. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is classified as a probable human carcinogen (class 2A) and MeIQ, MeIQx, PhIP, Glu-P-1, Glu-P-2, Trp-P-1, and Trp-P-2 are classified as possible carcinogens (class 2B).

The formation of HAs primarily depends on cooking time, cooking temperature, cooking method, pH value, and type of meat (Pariza and others 1979; Knize and others 1994; Sinha and others 1995; Skog and others 1995). Among these physical factors, time and temperature are the most important (Jackson and Hargraves 1995). The amounts of HAs generally increase with increasing cooking time and cooking temperature (Keating and others 1999). Natural nutrients including amino acids, sugars, creatine, and creatinine are degraded during the cooking of meat, and their reaction products may actively trigger the formation of HAs. Over the years, many researchers have suggested that the formation of HAs is a result of the condensation of free amino acids, creatine, creatinine, monosaccharides, disaccharides, or dipeptides, which all may act as precursors of HAs during cooking at high temperature (Jägerstad and others 1984; Övervik and others 1989; Skog and Jägerstad 1990; Knize and others 1991; Arvidsson and others 1999; Pais and others 1999; Solyakov and others 1999). There could be many different pathways for the formation of HAs during cooking of meats at high temperature. Two possible pathways for the formation of HAs have been postulated: one is that Maillard reaction products (such as pyridines or pyrazines formed through reverse-aldol reaction, enolization, dehydration, and Strecker degradation) and creatinine are condensed by ring closure, dehydration, and desaturation to form HAs (Jägerstad and others 1991; Johansson and others 1995;

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Friedman 1996). Free radicals produced through the Maillard reaction may be involved in this pathway proposed as a result of the types of HAs formed (Pearson and others 1992; Wakabayashi and Sugimura 1998). IQ-type and IQx-type HAs are formed by the reaction of pyridine radicals or pyrazine radicals with creatinine, respectively. The formation of pyridine radicals is dependent on pH. Glyoxal, the precursor for pyridine radicals, is inhibited by acidic condition (Pearson and others 1992). Thus, pH may affect the amount of HAs formed during cooking. The other suggested pathway is that aldehydes and creatinine are directly condensed with pyridines or pyrazines to produce HAs (Jägerstad and others 1991; Abdulkarim and Smith 1998).

The relationship between high consumption of HAs and cancer has become a focus of current epidemiological studies, and the occurrence of HAs in cooked meat has become a public health concern (Willett 1995; Wakabayashi and Sugimura 1998; Sugimura 2000). However, the exact mechanisms on the formation of these mutagens/carcinogens are not clearly understood and have mostly been studied in meat model systems (Yoshida and others 1978; Sugimura 1982; Ohgaki and others 1984; Övervik and others 1989; Arvidsson and others 1997; Arvidsson and others 1999; Borgen and others 2001). Understanding the kinetics of HA formation is essential for preventing their formation and, thus, reducing the health risks from the consumption of cooked meats. The formation of HAs in cooked meat is more complicated and also linked to other complex metabolites. Because many studies with meat model systems have shown that the formation of HAs is related to the concentrations of precursors in meat model systems, a systematic approach to evaluate time, temperature, and precursors is needed to better understand the mechanism of each HA formation in actual cooked meat. Therefore, the objective of this research was to elucidate the effects of time, temperature, and precursors on the formation of polar and nonpolar HAs in cooked beef.

Materials and Methods

Chemicals

Heterocyclic amines (HAs) 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-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 1-methyl-9*H*-pyrido[4,3-*b*]-indol (harman), and 9*H*-pyrido[4,3-*b*]-indol (norharman) were purchased from Toronto Research Chemicals (Toronto, Canada). Solvents used for extraction of HAs, methanol, dichloromethane, toluene, and ammonium solution, were obtained from Fisher Scientific Inc. (Fair Lawn, N.J., U.S.A.). All chemicals and solvents were high-performance liquid chromatography (HPLC) or analytical grade. Bond-Elut propylsulfonic silica (PRS) cartridges (500 mg), C-18 cartridges (100 mg and 500 mg), and Chem-Elut™ containing hydromatrix packing material were purchased from Varian Inc. (Walnut Creek, Calif., U.S.A.). Bond Elut adapters and luer-lock injection needles from Supelco (Bellefonte, Pa., U.S.A.) were used to connect cartridges and reduce flow rate during solvent extraction.

Sample preparation

Freshly ground beef was purchased from a local supermarket. Fat (15.9%) and protein (17.3%) contents were determined by modified Babcock and Kjeldahl methods, respectively. The experiment was designed as a randomized complete block design in a split block arrangement. A ground beef sample (5 g) was transferred into a glass test tube (100 × 13-mm inner dia, 1.0-mm wall thickness) and heated using a thermo-controllable heater set at 160 °C, 180 °C, 200 °C, and 220 °C, which represent low (L), mid-low (ML), mid-high (MH), and high (H) temperatures, respectively, for 5, 10, 15, and 20 min. Surface temperatures were individually recorded using thermocouples. Samples were performed in 4 replicates and analyzed for HAs and precursors, creatine and glucose in duplicate.

Solid-phase extraction

The HAs were extracted and purified using a solid-phase extraction method adopted from Gross and Grüter (1992) with slight modifications. Samples were homogenized with 15 mL of 1 *M* NaOH, using a blender (Fisher Scientific, Pittsburgh, Pa., U.S.A.) for 1 min and mixed with Chem-Elut material. For the determination of recovery, selected homogenate samples were spiked with 250 ng of each HA standard in 50 μL methanol. The mixture was filled into a Chem-Elut column coupled with Bond-Elut propylsulfonic acid (PRS, 500 mg) cartridges for weak-cation exchange. HAs were eluted at 1 mL/min with 50 mL dichloromethane (DCM) containing 5% toluene. The PRS cartridge was serially rinsed at 1 mL/min with 6 mL of 0.1 *N* HCl, 15 mL 40% methanol in 0.1 *N* HCl, and 2 mL water. After discarding the 0.1 *N* HCl eluate, the methanol-HCl and water eluates containing the nonpolar HAs were neutralized with ammonia solution and diluted with water to yield a methanol concentration below 20%. The nonpolar HAs were transferred to a C-18 cartridge (500 mg) that had been conditioned with a mixture of 2 mL methanol and 10 mL water. The C-18 cartridge (500 mg) was rinsed with 2 mL water, dried by positive nitrogen pressure, and then eluted with 1.4 mL methanol/concentrated ammonium solution (9:1). The previous PRS cartridge was coupled to the C-18 cartridge (100 mg), previously conditioned with a mixture of 1 mL methanol and 10 mL water, and rinsed with 20 mL of 0.5 *M* ammonium acetate buffer adjusted to pH 8.0 with ammonia solution. The C-18 cartridge (100 mg) was rinsed with 2 mL water and dried under positive nitrogen pressure. The polar HAs were eluted with 0.8 mL of methanol/NH_4OH (9:1). Nonpolar and polar HAs were concentrated with nitrogen and dissolved in 50 μL (unspiked) and 100 μL (spiked) methanol containing 1 ng/μL caffeine as an internal standard.

Standard curves

Five milligrams each of pure IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, norharman, harman, and AαC were dissolved in methanol and serially diluted to 5.0, 2.5, 1.0, and 0.5 ng/μL. Dilutions and peak areas were used to establish standard curves. Coefficients of determination (r^2) for standard curves were greater than 0.99 for IQ, MeIQ, MeIQx, 4,8-DiMeIQx, norharman, harman, PhIP, and AαC. Heterocyclic amines (HAs) used as standards were very carefully handled using procedures recommended by the Environmental Protection Agency.

Identification and quantification of HAs

HAs were analyzed using reverse-phase HPLC (Perkin-Elmer, Norwalk, Conn., U.S.A.) equipped with UV/Visible detector (263 nm; Perkin-Elmer C-95) and scanning fluorescence detector (Waters 474; Milford, Mass., U.S.A.). A silica-based TSK-gel ODS-80TM (Tosohaas, Montgomeryville, Pa., U.S.A.; 25 cm × 4.6 mm, 5-μL) column with a precolumn (Supelguard LC-18-DB, 2 cm × 4.6-mm inner dia) was used to separate HAs. The mobile phases were as follows: A, 0.01 *M* triethylamine phosphate (TEAP, pH 3.2); B, TEAP (pH 3.6); and C, acetonitrile. The following gradient profile was used (time, %A:%B:%C): initial, 95:0:5; 0 to 10 min, 85:0:15; 10 to 10.1 min, 0:85:15; 10.1 to 20 min, 0:75:25, 20 to 30 min, 0:45:55, and 30 to 35 min, 0:20:80 at a flow rate of 1 mL/min. Peaks were iden-

tified by retention time and spectral shape, which was corrected with library spectra established from standard solutions. The fluorescence detector was programmed to detect other HAs; excitation/emission wavelengths (time) were set at 360/450 (0 to 20 min) for Glu-P-2 and Glu-P-1, 300/440 (20 to 24 min) for norharman and harman, 265/410 (24 to 26 and 28 to 30 min) for Trp-P-2 and Trp-P-1, 315/390 (26 to 28 min) for PhIP, and 335/410 (30 to 35 min) for AαC and MeAαC. The HA concentrations in samples were corrected with extraction efficiency for incomplete recovery and expressed in ng/g.

Creatine analysis

The content of creatine was measured by the α-naphthol-diacetyl method described by Khan and Cowen (1977). A 10-g sample was blended with 50 mL of 10% trichloroacetic acid and centrifuged (3000 × *g*) for 20 min. The supernatant was collected and the residue was washed twice with an additional 50 mL of 10% trichloroacetic acid. The combined extract was collected, diluted to 200 mL with 10% trichloroacetic acid, and then allowed to stand at room temperature for 2 h. One milliliter of the extract was mixed with 5 mL of a NaOH-Na_2CO_3 solution containing 6% NaOH and 16% Na_2CO_3 in distilled water, 3 mL of 1% α-naphthol dissolved in the NaOH-Na_2CO_3 solution, and 2 mL of 0.1% diacetyl solution in distilled water. The mixture was diluted up to 25 mL with distilled water and allowed to stand at room temperature for 20 min. The absorbance was measured at 520 nm using a Spectronic 20 (Bausch & Lomb, Rochester, N.Y., U.S.A.) spectrophotometer. A standard curve was prepared at 2.0, 4.0, 6.0, 8.0, and 10.0 μg/mL creatine treated as described for the sample.

Glucose analysis

Glucose was analyzed from cooked samples according to the methods of Dubois and others (1956) and Somogyi (1945). A 5-g sample was mixed with 100 mL deionized water, placed in a boiling water bath for 1 min, and then 2 mL of saturated lead acetate solution was added to precipitate proteins. After cooling to room temperature, the mixture was diluted to 250 mL with deionized water. The diluted solution was set for 12 h and 100 mL of the supernatant was collected. To remove the Pb^{2+} from the supernatant, 2.5 g of crystallized potassium oxalate was added, followed by centrifugation (1200 × *g*) at 25 °C for 5 min. A mixture of 1 mL of 5% phenol and 5 mL concentrated sulfuric acid was added to 2 mL extract with shaking. After sitting at room temperature for 10 min, the solution was placed in a water bath at 25 °C for 15 min. The absorbance was measured at 490 nm using a Spectronic 20 spectrophotometer. A standard curve was prepared at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 μg/mL glucose in the same manner as described for the sample.

Reaction kinetics

The kinetic parameters for the formation of HAs were analyzed using the Arrhenius equation (Eq. 1) and the Eyring equation (Eq. 4). The effect of temperature can be determined using the Arrhenius equation (Levenspiel 1972):

$$k = A \cdot e^{\frac{-E_a}{R \cdot T}} \quad (1)$$

where *k* is the rate constant (1/min), E_a is the minimum energy required for a reaction (J/mol), R is the gas constant (8.314 J/K/mol), T is the temperature (K), and A is a pre-exponential factor. A plot of ln(*k*) against 1/T is a straight line where the slope is $(-E_a/R)$ and the y-intercept is ln(A). A high activation energy (E_a) means that the reaction is very temperature-sensitive (Levenspiel 1972). The Eyring equation is based on the transition state theory. The formation of HAs is postulated as a 1st-order reaction (Levenspiel 1972; Segel 1976; Arvidsson and others 1999):

$$\frac{C_t}{C_m} = 1 - e^{-k(t-t_0)} \quad (2)$$

where *k* is the rate constant (1/min) for the formation of HAs, C_t is the concentration of HAs at time t, C_m is the estimated maximum concentration (ng/g) of HAs, and t_0 is the lag time (min) for the formation of HAs (Arvidsson and others 1999; Hwang and Ngadi 2002). The transition state is known as an unstable transient at a free-energy maximum (Segel 1976; Tinoco and others 2002). The transition state has a very short life-time, and reactants rapidly reach the equilibrium state. Thus, it is not possible to determine a reactive intermediate. However, energy changes that occur from reactants to transition state to HAs are considered by the Eyring equation. The formation of HAs was explained by the calculated parameters including activation energy, enthalpy, and entropy. These kinetic parameters suggest whether the formation of HAs involves intermediates such as Amadori compounds, Strecker aldehydes, pyridines, pyrazines, and free radicals (Jägerstad and others 1991; Johansson and others 1995; Arvidsson and others 1999). The temperature-dependence is determined using the Eyring equation (Eq. 4).

$$k = \mathrm{k}^{\ddagger}\,\mathrm{K}^{\ddagger} \quad (3)$$

$$k = \left(\frac{k_B T}{h}\right) \cdot e^{-\left(\frac{\Delta G^{\ddagger}}{R \cdot T}\right)} \quad (4)$$

where $k^{\ddagger}$ and $K^{\ddagger}$ are each a universal rate constant and the equilibrium constant between the transition state and reactants, respectively, k_B is the Boltzmann constant (1.381×10^{-23} J/K), h is Planck's constant (6.626×10^{-34} Js), T is the temperature in Kelvin (K), R is the gas constant (8.314 J/mol/K), and $\Delta G^{\ddagger}$ is the free energy of activation.

$$\ln k = \ln\left(\frac{k_B T}{h}\right) - \left(\frac{\Delta H^{\ddagger} + R \cdot T}{R}\right) \cdot \frac{1}{T} \quad (5)$$

$$\mathrm{S}^{\ddagger} = R \cdot \ln\left(\frac{A \cdot h}{k_B T}\right) \quad (6)$$

where $\Delta H^{\ddagger}$ is the activation enthalpy (kJ/mol) and $\Delta S^{\ddagger}$ is the activation entropy (J/mol/K). The activation enthalpy and activation entropy can be calculated from the Eyring equation by nonlinear regression (Levenspiel 1972). A plot of ln(*k*) against 1/T is a straight line with a slope of $[-(\Delta H^{\ddagger} + RT)/R]$ and y-intercept of $\ln(k_B T/h)$. For most reactions, RT is much smaller than $\Delta H^{\ddagger}$. For the evaluation of reaction mechanisms, E_a and ln(A) from the Arrhenius equation (Eq. 1) were compared with $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ from the Eyring equation (Eq. 5). Small values for $\Delta H^{\ddagger}$ indicate fast reaction and negative values for $\Delta S^{\ddagger}$ indicate slow reaction (Tinoco and others 2002).

Statistical analysis

A monomolecular growth model was analyzed using Nonlinear Curve Fitting Function of Microcal Origin® 7.0 (Microcal Software

Inc., Northampton, Mass, U.S.A.). Experimental data on the temperature-dependence was fitted by using the Arrhenius equation and the Eyring equation. All data were analyzed using the Statistical Analysis System software (SAS Inst. 1990). The General Linear Model (GLM) and least significant difference (LSD) procedures were used to compare means. Significant mean differences among treatments were calculated by Fisher's least significant difference (LSD) at $P < 0.05$.

Results and Discussion

Recovery rates of HAs

The recovery rates ranged from 22% to 85% for HAs (IQ, MeIQ, MeIQx, 4,8-DiMeIQx, norharman, harman, PhIP, and AαC) for the solid-phase extraction (Table 1). PhIP showed the lowest recovery rate at all temperatures. Toribio and others (2000) reported that the recovery rates for HAs broadly range from 5% to 98% due to the complexity of the food matrix and the numerous extraction steps. When compared with other HAs, the efficiency of PhIP is relatively lower because the retention of PhIP depends on the concentration of hydrochloric acid during the PRS equilibrium (Gross and Grüter 1992). The efficiency and selectivity of nonpolar HAs such as α-carboline (AαC) and β-carbolines (norharman and harman) vary with ratios of methanol to hydrochloric acid. The extraction rates were used to correct for incomplete recoveries in samples.

Table 1—Extraction efficiency (%) of heterocyclic amines (HAs) at different temperatures

	Temperature[a]		
HA	ML	MH	H
IQ	41.96 ± 4.55	44.36 ± 4.37	48.49 ± 4.09
MeIQ	47.29 ± 8.10	50.48 ± 3.84	47.65 ± 2.13
MeIQx	62.51 ± 6.83	75.24 ± 11.81	61.75 ± 4.58
4,8-DiMeIQx	63.04 ± 2.46	60.36 ± 3.86	57.77 ± 3.57
Norharman	56.93 ± 7.39	63.46 ± 2.65	77.46 ± 4.72
Harman	71.38 ± 5.53	84.98 ± 5.25	80.01 ± 6.41
PhIP	19.80 ± 2.38	21.54 ± 2.49	21.10 ± 3.40
AαC	33.48 ± 10.15	46.37 ± 6.26	62.31 ± 9.55

[a]Mid-low (ML) temperature = 180 °C; mid-high (MH) temperature = 200 °C; and high (H) temperatures = 220 °C.

Table 2—Rate constants (k[a]) for the formation of heterocyclic amines (HAs) at different times and temperatures

	Temperature[b]		
HAs	ML	MH	H
IQ	0.077	0.084	0.136
MeIQ	0.039	0.041	0.049
MeIQx	0.134	0.164	0.157
4,8-DiMeIQx	0.040	0.062	0.108
Norharman	0.054	0.090	0.116
Harman	0.074	0.119	0.110
PhIP	0.082	0.115	0.132
AαC	0.083	0.074	0.103

[a]k = rate constant (1/min).
[b]Mid-low (ML) temperature = 180 °C; mid-high (MH) temperature = 200 °C; and high (H) temperatures = 220 °C.

Effects of time and temperature

The formation of HAs was dependent on heating time and temperature as shown in Figures 1 and 2. HAs were not detected at the low temperature (L = 160 °C) when heated for 15 min or less, while the amount of HAs was increased with increasing heating time and temperature. The formation of HAs showed various patterns as can be seen in Figures 1 and 2. Considerable formation of polar HAs (IQ, MeIQ, MeIQx, 4,8-DiMeIQx, and PhIP) and nonpolar HAs (norharman, harman, and AαC) was observed at mid-low (ML = 180 °C), mid-high (MH = 200 °C), and high (H = 220 °C) temperatures after 20 min. Neither MeIQ nor 4,8-DiMeIQx was detected at ML for 10 min. MeIQ was detected at less than 2 ng/g at all temperatures, except when heated at the highest temperature for 20 min (2.04 ng/g). MeIQx was most predominantly formed throughout the heating time, followed by PhIP and IQ, while MeIQ and 4,8-diMeIQx were formed slowly. Most HAs were quickly formed after 5 min of heating process at all temperatures, although IQ, MeIQ, 4,8-DiMeIQx, harman, and AαC were produced slowly after 5 or 10 min at ML. The highest concentrations of MeIQx were 11.99, 20.50, and 23.93 ng/g at ML, MH, and H temperatures, respectively. Among nonpolar HAs, the amounts of norharman and harman were 10.36 and 10.21 ng/g, respectively, at temperature H after 20 min. The results indicate that the formation of HAs is time- and temperature-dependent (Jackson and Hargraves 1995) and imply that HAs in a complex meat system are differently formed at certain temperature and time points.

Effects of precursors

Creatine and glucose concentrations were measured to determine the relationship between the degradation of precursors and the formation of HAs. The changes of creatine and glucose levels are shown in Figure 3. The creatine concentration in raw ground beef was 3.77 mg/g, which was decreased to 1.51, 1.12, and 0.52 for ML, MH, and H temperatures, respectively, after 20 min. Glucose also decreased from 2.73 to 0.32 mg/g (temp H) with increasing time and temperature (Figure 3). The data from this study did not allow establishment of a stoichiometric relationship between precursors and HA formation. However, the rate constants of a 1st-order reaction were calculated to determine the relationship between the degradation of creatine and glucose and the formation of HAs. The reduction of creatine and glucose was negatively correlated with the formation of HAs. Correlation coefficients were determined between creatine loss and HA formation: IQ ($r = 0.81$), MeIQ ($r = 0.74$), MeIQx ($r = 0.93$), 4,8-DiMeIQx ($r = 0.86$), norharman ($r = 0.84$), and PhIP ($r = 0.87$). The degradation of glucose was also negatively correlated with the formation of MeIQ ($r = 0.84$), MeIQx ($r = 0.87$), 4,8-DiMeIQx ($r = 0.76$), norharman ($r = 0.79$), and PhIP ($r = 0.87$). Some of the HAs (AαC) showed a plateau after 15 min at each temperature (Figure 1 and 2). However, no specific degradation trends of HAs could be observed from Figure 1 and 2, except that MeIQx started decreasing at MH and H after 15 min. The plateaus may be the result of the rapid degradation of glucose (Arvidsson and others 1997) and resulting lack of precursors. Jackson and Hargraves (1995) suggested that MeIQx might convert to melanoidins through Maillard reaction. The imidazoquinoxaline or imidazoquinoline part of the IQ-type HAs could be formed from the degradation of glucose, and the imidazole part could be derived from the degradation of creatine (Jägerstad and others 1984). The result suggests that concentration of precursors present in raw beef is important for HA formation during heating. The complex meat matrix contains various precursors, including amino acids, and the ratio of precursors also plays an important role in the formation of HAs. HAs are formed through complex chemical reactions involving precursors (free amino groups, glucose, creatine, and creatinine) and Maillard reaction products (Amadori compounds, Strecker dehydration, pyridine, and pyrazine) (Jägerstad and others 1984, 1991; Grivas and others 1986; Keating and others 1999). Therefore, the formation of HAs depends on the amounts of precursors and intermediates present in a meat system.

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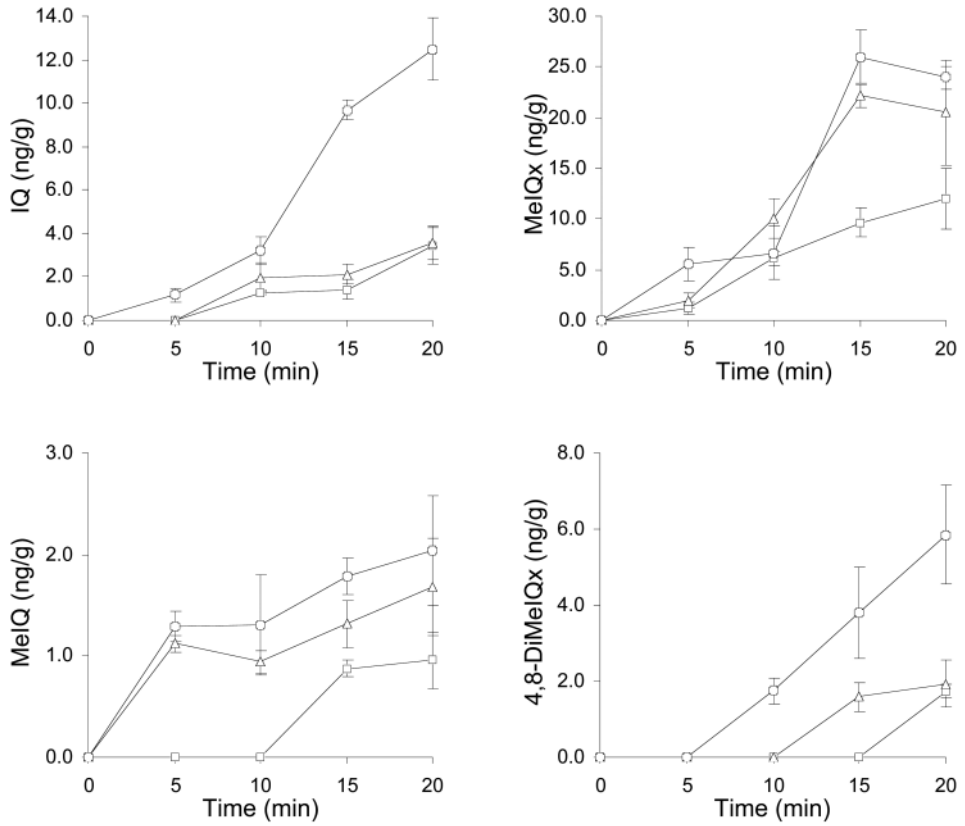

Figure 1—Effects of time and temperature on the formation of IQ, MeIQ, MeIQx, and 4,8-DiMeIQx at 180 (□: ML), 200 (△: MH), and 220 °C (○: H)

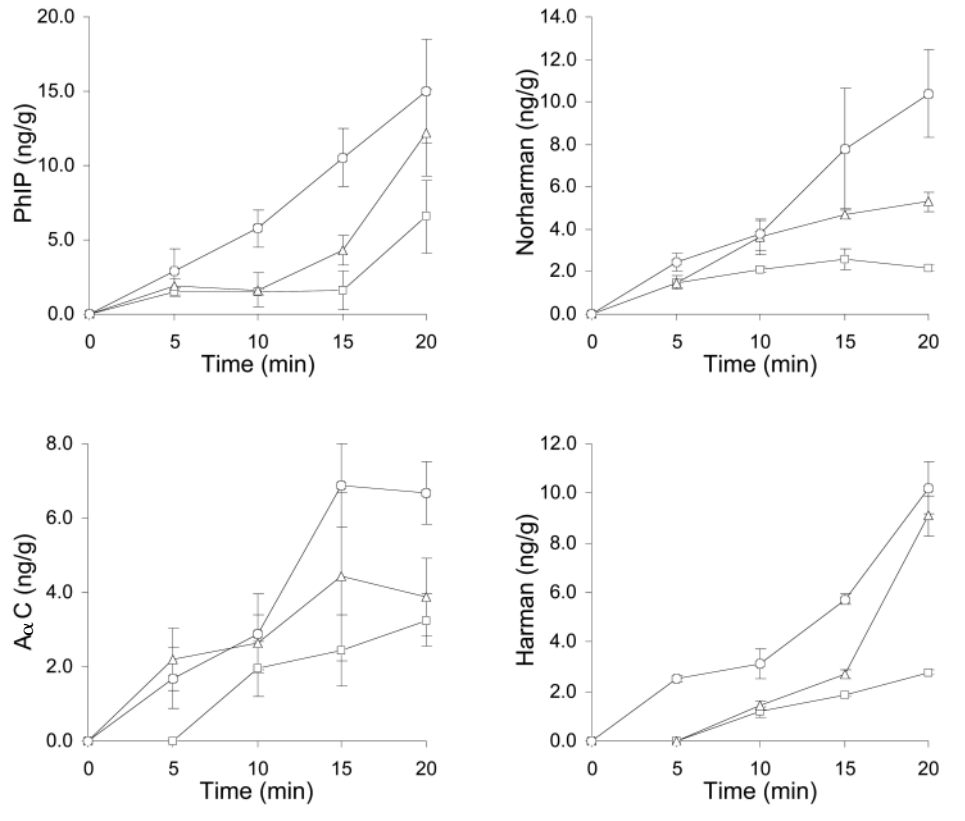

Figure 2—Effects of time and temperature on the formation of PhIP, Aα C, norharman, and harman at 180 (□: ML), 200 (△: MH), and 220 °C (○: H)

Reaction kinetics

Rate constants for the formation of HAs were estimated using the 1st-order reaction model as shown in Table 2. Maximum concentrations increased with increasing time and temperature, while lag times were decreased (data not shown). Arvidsson and others (1997) and Hwang and Ngadi (2002) also reported that the formation rate constant (k) increased and lag time decreased with increasing time and temperature. The temperature-dependence of HA formation was predicted using the Arrhenius and Eyring equations. The activation enthalpy ($\Delta H^{\ddagger}$) is nearly equal to the activation energy (E_a), because the value of $\Delta H^{\ddagger}$ is relatively much larger than that of RT (Tinoco and others 2002). Norharman and 4,8-DiMeIQx showed higher activation energies as compared with other HAs (Table 3). This means that the formation of norharman and 4,8-DiMeIQx is a temperature-sensitive reaction. Thus, the formation of norharman and 4,8-DiMeIQx was much more predominant at high temperature than at low temperature. This suggests that temperature-sensitive HAs are formed at higher temperature and longer heating time. MeIQx had the lowest activation energy (8.80 kJ/mol). Thus, MeIQx, a less temperature-sensitive HA, was extensively formed at all temperatures. The activation entropy ($\Delta S^{\ddagger}$) was estimated to make an assumption about the reaction mechanisms, such as unimolecular ($\Delta S^{\ddagger} > 0$) and bimolecular ($\Delta S^{\ddagger} < 0$) reactions (Arvidsson and others 1997). The calculated activation entropies were less than zero for all HAs (Table 3). The results suggest that the formation of HAs follows a bimolecular mechanism with 1 reactant concentration in large excess. The formation of HAs is a rate-limiting reduction that follows a pseudo-1st-order reaction (Arvidsson and others 1997).

Table 3—The calculated activation enthalpy and the activation entropy

Heterocyclic amines (HAs)	$\Delta H^{\ddagger}$ (kJ/mol)	$\Delta S^{\ddagger}$ (J/mol/K)
IQ	28.32	–207.06
MeIQ	11.45	–249.98
MeIQx	8.80	–245.15
4,8-DiMeIQx	50.93	–161.88
Norhaman	40.10	–183.04
Harman	21.87	–220.64
PhIP	25.07	–213.08
AaC	9.98	–247.32

Conclusions

A kinetic approach was used to investigate the effects of time, temperature, and precursors on the formation of polar and nonpolar HAs in real meat systems. The formation of polar and nonpolar HAs was dependent on time, temperature, and precursors, and followed 1st-order kinetics. The data could be used to predict the relationship between the formation of each heterocyclic amine and the reduction of creatine and glucose as time and temperature change. Therefore, the results provide practical information to modulate the formation of HAs in cooked meat. However, further study is needed to clearly understand the relationship between precursors and formation of HAs.

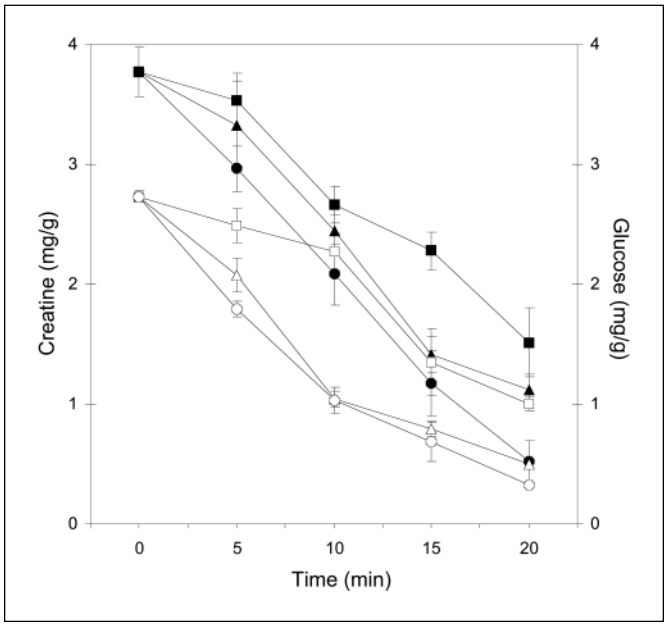

Figure 3—Effects of time and temperature on creatine (■: ML = 180 °C; ▲: MH = 200 °C; ●: H = 220 °C) and glucose (□: ML = 180 °C; △: MH = 200 °C; ○: H = 220 °C) concentrations for 20 min of heating

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Abbreviations

HAs = heterocyclic amines
IQ (CAS Registry nr 76180-96-6) = 2-amino-3-methylimidazo[4,5-*f*]quinoline
MeIQ (77094-11-2) = 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline
MeIQx (77500-04-0) = 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
4,8-DiMeIQx (95896-78-9) = 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline
PhIP (105650-23-5) = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
AαC (26148-68-5) = 2-amino-9*H*-pyrido[2,3-*b*]indole
harman (486-84-0) = 1-methyl-9*H*-pyrido[4,3-*b*]-indol
norharman (244-63-3) = 9*H*-pyrido[4,3-*b*]-indol

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