Effects of Time and Temperature on the Formation of $MeIQ_x$ and $DiMeIQ_x$ in a Model System Containing Threonine, Glucose, and Creatine

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This project studied the effects of processing time and temperature on the formation of $MeIQ_x$ and $DiMeIQ_x$, two of the most common aminoimidazoazaarenes (AIAs) found in food. Each model system containing threonine, glucose, and creatine (2:1:2 molar ratio) was processed for 2 h at a temperature between 125 and 250 °C. Neither $MeIQ_x$ nor $DiMeIQ_x$ was found at processing temperatures lower than 150 °C. At temperatures between 150 and 200 °C, AIA concentrations increased with processing time. However, at 225 and 250 °C the concentrations of the two products increased during the initial 15–30 min of processing and then decreased for the remainder of the processing run. $MeIQ_x$ and $DiMeIQ_x$ did not appear in the reaction mixture until almost all of the creatine was converted into creatinine. This systematic study suggests that maintaining cooking temperatures below 150 °C may reduce the formation of $MeIQ_x$ and $DiMeIQ_x$ in food.

Keywords: *Aminoimidazoazaarene; processing; creatine; creatinine*

INTRODUCTION

During the past decade, considerable efforts have been made to identify precursors, reaction conditions, and mechanisms that lead to the formation of heterocyclic aromatic amines (HAAs) in food (Munro et al., 1993). These compounds have been reported to be mutagenic or carcinogenic. HAAs have been found in a variety of foods such as cooked meats (Sugimura et al., 1988; Gross, 1990; Knize et al., 1994), food-grade meat extracts (Hargraves and Pariza, 1983; Takahashi et al., 1985; Gross et al., 1989), and processed flavors (Jackson et al., 1994). Of the HAAs in cooked food, the majority belong to the chemical class known as aminoimidazoazaarenes (AIAs). These compounds have a 2-aminoimidazo group fused to a quinoline (e.g., IQ and MeIQ), a quinoxoline (e.g., $MeIQ_x$ and $DiMeIQ_x$), or a pyridine (e.g., PhIP) ring (Skog and Jagerstad, 1990). The AIAs are formed during the cooking of meat-containing foods at normal household cooking temperatures (i.e., 150–300 °C). In contrast, the amino acid pyrolysis HAAs (e.g., Glu-P-1, Phe-P-1, and Trp-P-1) form at temperatures above 300 °C but are not commonly found in the Western diet (Felton and Knize, 1990).

From the structure of the AIAs, Jagerstad et al. (1983a) hypothesized that the major precursors are creatine and/or creatinine, amino acids, and reducing sugars. The involvement of these precursors in AIA formation was verified in studies with model systems (Jagerstad et al., 1991). Other studies (Felton and Knize, 1990; Knize et al., 1988; Overvik et al., 1989) found that sugar is not obligatory for the formation of AIAs. Adding small amounts of reducing sugar to model systems (Skog and Jagerstad, 1990, 1991) and meats (Jagerstad et al., 1983b) before cooking resulted in increased yields of mutagens. However, sugars added in amounts greater than half the molar concentration of glycine or creatine inhibited the formation of AIAs in a model system (Skog and Jagerstad, 1990).

The discovery that reducing sugars, amino acids, and creatine are AIA precursors led to speculation that the Maillard reaction plays an important role in the formation of the AIAs. Jagerstad et al. (1983a) hypothesized that the 2-aminoimidazo ring in AIA compounds arises from cyclization of creatine. Strecker degradation products (pyrazines and pyridines) resulting from Maillard browning, along with acetaldehyde, are believed to form the remainder of the molecule.

Factors that affect the formation of AIAs include pH, precursor concentrations, type of amino acid, and processing time and temperature. Of these factors, processing temperature and time are believed to have the greatest effect. Several investigators found that mutagenic activity in cooked meat (Bjeldanes et al., 1982; Overvik et al., 1984; Knize et al., 1985, 1994) and in a model system containing creatine, glucose, and an amino acid (Jagerstad et al., 1983a) increased progressively with increasing cooking temperature. Cooking time is not believed to be as important as temperature for AIA formation. Bjeldanes et al. (1983), Berg et al. (1990), and Sflomos et al. (1989) found that the mutagenic activity in fried meat increased most rapidly during the initial 10 min of cooking and then leveled off or decreased during additional cooking.

Despite previous work, which focused on the effects of time and temperature on mutagenic activity, little is known about how these two factors affect the actual formation of the AIAs. Understanding the reaction conditions for AIA formation is necessary to devise methods for reducing their concentration in foods. This work measured the effects of processing temperature and time on the formation of $MeIQ_x$ and $DiMeIQ_x$, two of the most common AIAs found in foods.

EXPERIMENTAL PROCEDURES

Materials. Glucose, creatine, and threonine were purchased from Sigma Chemical Co. (St. Louis, MO). Standards of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline ($MeIQ_x$, CAS Registry No. 77094-11-2) and 2-amino-3,4,8-trimethylimidazo-[4,5-*f*]quinoxaline ($DiMeIQ_x$, CAS Registry No. 95896-78-9) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). (CAS Registry No. were supplied by the

authors.) *Salmonella typhimurium* strain TA98 was kindly supplied by Bruce Ames (University of California—Berkeley), and Aroclor-induced rat liver S9 was purchased from Organon Teknika (Durham, NC). All reagents were of analytical grade, and solvents were of high-performance liquid chromatography (HPLC) grade.

Safety Precautions. $MeIQ_x$ and $DiMeIQ_x$ are suspected carcinogens and should be handled with caution. Materials containing these compounds should be disposed of as hazardous waste according to the Resource Conservation and Recovery Act issued by the Environmental Protection Agency.

Processing of Model System. Creatine (38.1 mmol), threonine (38.1 mmol), and glucose (19.0 mmol) were dissolved in distilled water, and the solution was brought to a final volume of 500 mL. The reaction mixture was adjusted to pH 6.0 with 0.1 N HCl and then placed in a 1-L stainless steel reaction vessel (Parr Instrument Co., Moline, IL). Each mixture was heated to a processing temperature in the range of 125–250 °C for 2 h with an electric heating mantle. The reaction mixture was agitated at a constant speed and maintained at the desired temperature by a Parr Model 4841 proportional controller. Aliquots of the reaction mixture were removed at 15-min intervals during processing and were analyzed for $MeIQ_x$, $DiMeIQ_x$, creatine, and creatinine concentrations.

HPLC Determination of $MeIQ_x$ and $DiMeIQ_x$. AIA compounds were extracted from the reaction mixture according to the method of Gross (1990) with modifications. Each aliquot (1–2 g) of reaction mixture was dissolved in 10 mL of 1.0 N NaOH. To estimate the percentage of AIAs recovered during the extraction procedures, aliquots of the reaction mixture were spiked with two levels (three replicates per level) of AIA standards. Each solution was adsorbed onto a cartridge containing approximately 20 mL of dry diatomaceous earth (Extrelut, EM Science, Gibbstown, NJ). A Bond Elut propylsulfonic acid silica (PRS) gel column (Varian Sample Preparation Products, Harbor City, CA) was coupled to the bottom of the diatomaceous earth column with adaptor pieces. Dichloromethane (40 mL) was added to the top of the tandem set of columns and allowed to flow through. The PRS column, which contained the adsorbed AIAs, was dried with a nitrogen stream and washed successively with 6 mL of 0.1 N HCl, 15 mL of 0.1 N HCl/methanol (6:4), and 2 mL of distilled water. A Bond Elut (Varian) C_{18} cartridge was coupled to the bottom of the PRS cartridge. The tandem set of columns was washed with 20 mL of 0.5 N ammonium acetate buffer (pH 8.0). The C_{18} cartridge was washed with 1 mL of distilled water. AIAs were eluted from the C_{18} column with 1.0 mL of methanol/ammonium hydroxide (9:1). The solvent was evaporated by using a SpeedVac concentrator (Savant Instruments, Farmingdale, NY), and the residue was dissolved in 100 μL of methanol. A 20-μL aliquot of the extract was used for HPLC determination.

Additional cleanup steps were needed to remove interfering compounds from reaction mixtures that were subjected to temperatures of 200–250 °C. These steps as described by Gross et al. (1992) involved the use of TSK CM-650S gel (TosoHaas, Montgomeryville, PA) and washing steps to selectively remove interfering compounds. A 20-μL aliquot of the extract obtained from these cleanup steps was used for HPLC determination.

A Waters (Milford, MA) high-performance liquid chromatograph equipped with a Model 600E solvent delivery system, a Model 715 autoinjector, and a Model 991 photodiode array detector was used to separate, identify, and quantify $MeIQ_x$ and $DiMeIQ_x$ in the extracts. Separations were carried out on a TosoHaas TSK-gel ODS-80 column (4.6 mm × 25 cm) with an LC-8-DB precolumn (Supelco, Bellefonte, PA) by using a tertiary solvent gradient with a flow rate of 1.0 mL/min. Solvent A was 0.01 M triethylamine adjusted to pH 3.2 with phosphoric acid, solvent B was 0.01 M triethylamine adjusted to pH 3.6 with phosphoric acid, and solvent C was acetonitrile. The gradient program was as follows: 0–10 min, from 5 to 15% C in A; 10–20 min, from 15 to 25% C in B; 20–30 min, from 25 to 55% C in B; 30–35 min, from 15 to 45% B in C; and 35–40 min, from 15 to 0% B in C. The solvent composition changed linearly with respect to time in all cases. Identity of AIA peaks was confirmed by comparing the retention times and UV spectra (200–400 nm) of suspected peaks with those of pure AIA standard peaks.

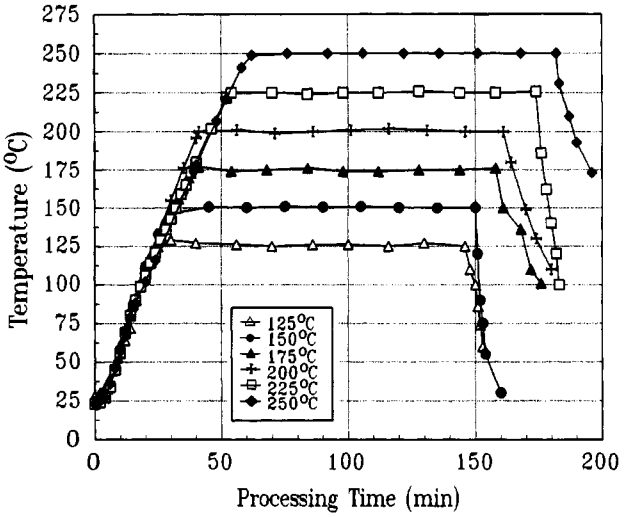

Figure 1. Temperature recorded in an aqueous solution of threonine, glucose, and creatine (2:1:2 molar ratio) during processing. The solution was processed for 2 h at temperatures of 125–250 °C in a 1-L Parr pressure reactor. Triplicate runs were completed for processing temperatures of 150 and 250 °C. One run was completed for other processing temperatures.

In some HPLC runs, 1-min fractions were collected with a Gilson Model 203 (Middleton, WI) fraction collector. The fractions were dried using a SpeedVac concentrator, and the residues were dissolved in DMSO and analyzed for mutagenic activity.

Mutagenic Activity. Mutagenic activity of HPLC fractions was measured according to the standard pour-plate method described by Maron and Ames (1983), using *S. typhimurium* strain TA98 with the addition of Aroclor-induced rat liver S9 protein. It was previously determined (Jackson et al., 1994) that 2 mg of S9 protein was needed per plate to obtain maximum activation of the AIAs. Standard $MeIQ_x$ and $DiMeIQ_x$ were used as positive controls.

Creatine/Creatinine Determinations. Creatine levels were determined by the α-naphthol–diacetyl method described by Khan and Cowen (1977). A Sigma Chemical Co. diagnostic kit (catalog no. 555) was used to measure creatinine content.

Statistical Analysis. Processing runs at 150 and 250 °C were performed in triplicate, but only one run was completed for the other processing temperatures. All determinations of AIA, creatine, creatinine, and mutagenic activity levels were performed in triplicate. The average coefficients of variation (CVs) for the AIA determinations were 10.0 and 8.2% for the model systems processed at 150 and 250 °C, respectively. The average CV for the model system at all processing temperatures was 10%. The average CVs for creatine and creatinine determinations were 6.5 and 4.2%, respectively. Minitab statistical software was used to calculate means and standard deviations and to conduct linear regression analyses for the mutagenicity dose–response curves.

RESULTS AND DISCUSSION

Reproducibility of Processing Runs. Strict control of processing time and temperature is essential for adequate study of how physical parameters affect the formation of AIAs. To determine the variability in the thermal process, runs at 150 and 250 °C were performed in triplicate. The time–temperature profiles for the threonine, glucose, and creatine reaction mixture (Figure 1) indicate that the heating process was reproducible. The variation of temperature at each processing time was small (±3 °C). The reproducibility in the heating process is also shown by the similarities in the initial slopes of the curves (about 5 °C/min).

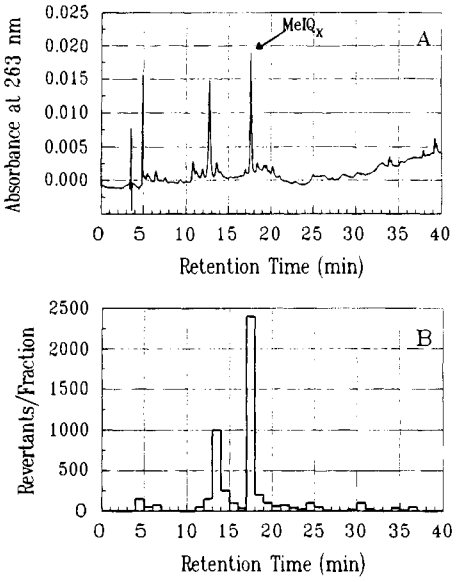

Figure 2. (A) HPLC chromatogram using UV detection at 263 nm for an aqueous solution of threonine, glucose, and creatine (molar ratio of 2:1:2) held at a processing temperature of 150 °C for 120 min. A peak suspected to be $MeIQ_x$ is indicated by the arrow. (B) Mutagenic activity of 1-min fractions obtained during the HPLC separation. The specific mutagenic activity of the suspected $MeIQ_x$ (138 ± 21.7 revertants/ng) was similar to that of a synthetic $MeIQ_x$ standard (119 ± 10.7 revertants/ng).

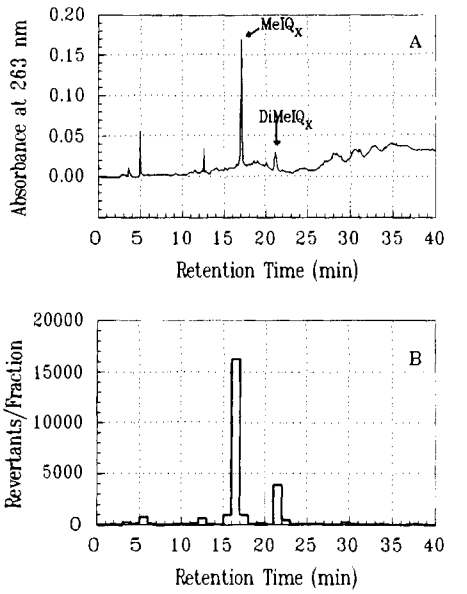

Figure 3. (A) HPLC chromatogram using UV detection at 263 nm for an aqueous solution of threonine, glucose, and creatine (molar ratio of 2:1:2) held at a processing temperature of 250 °C for 15 min. Suspected $MeIQ_x$ and $DiMeIQ_x$ peaks are indicated by arrows. (B) Mutagenic activity of 1-min fractions obtained during the HPLC separation. The specific mutagenic activities of the suspected $MeIQ_x$ (128 ± 19.7 revertants/ng) and $DiMeIQ_x$ (130 ± 20.7 revertants/ng) were similar to those of synthetic $MeIQ_x$ (119 ± 10.7 revertants/ng) and $DiMeIQ_x$ (146 ± 15.7 revertants/ng) standards.

Identification of $MeIQ_x$ and $DiMeIQ_x$ in Processed Model System. Aliquots of the thermally processed threonine, glucose, and creatine model system were analyzed for 8-$MeIQ_x$ and 4,8-$DiMeIQ_x$ according to the method of Gross (1990) or Gross et al. (1992). HPLC chromatograms for the reaction mixture heated at a processing temperature of 150 °C for 120 min and at a processing temperature of 250 °C for 15 min are shown in Figures 2A and 3A, respectively. Also shown are the mutagenic activities (Figures 2B and 3B) of 1-min fractions obtained during these runs.

The chromatogram (Figure 2A) for the purified extract from the system processed at 150 °C had two peaks with substantial mutagenic activity (Figure 2B). The peak at 17.4 min had a retention time that corresponded to that of a synthetic 8-$MeIQ_x$ standard. The amount of 8-$MeIQ_x$ in the fraction corresponding to the peak, uncorrected for recovery, was 17 ng. By using this quantity and the mutagenic activity for the same peak, the specific activity was calculated to be 138 ± 21.7 revertants/ng. This value was in excellent agreement with the specific activity of a pure $MeIQ_x$ standard (119 ± 10.7 revertants/ng).

Although not identified, the peak with retention time of 12.7 min was suspected to be IQ_x. Skog and Jagerstad (1993) reported that IQ_x was produced in an aqueous model system of threonine, glucose, and creatinine that was heated at 180 °C for 15 min. They used identical HPLC conditions and reported chromatograms and relative retention times for IQ_x similar to those shown here.

The HPLC chromatogram for the reaction mixture processed at 250 °C for 15 min (Figure 3A) also had two peaks with mutagenic activity. The retention times of these peaks (16.9 and 21.1 min) were the same as those of $MeIQ_x$ and $DiMeIQ_x$ standards. The quantities of $MeIQ_x$ and $DiMeIQ_x$ in the peaks were 129 and 31 ng, respectively. The calculated specific activities of the suspected $MeIQ_x$ and $DiMeIQ_x$ (128 ± 19.7 and 130 ± 20.7 revertants/ng, respectively) were similar to those obtained for pure $MeIQ_x$ and $DiMeIQ_x$ standards (119 ± 10.7 and 146 ± 15.7 revertants/ng, respectively).

Identities of suspected AIA peaks were tentatively confirmed by comparing the UV spectrum of each peak with spectra of standard AIAs. The UV spectra for the suspected $MeIQ_x$ and $DiMeIQ_x$ peaks (not shown) were identical to the spectra of the pure standards. These data along with the retention times and specific mutagenic activities provide strong evidence of the identities of the major mutagens in the thermally processed reaction mixture.

Effects of Temperature and Time on AIA Formation. Figures 4 and 5 indicate that the formation of $MeIQ_x$ and $DiMeIQ_x$ is highly temperature dependent. $MeIQ_x$ did not form in the reaction mixture until processing temperatures of 150 °C or greater were reached, whereas temperatures of 175 °C or greater were needed to form $DiMeIQ_x$. These findings are supported by others (Rappaport et al., 1979; Laser-Reutersward et al., 1987a; Skog and Jagerstad, 1990) who found that the mutagenic compounds began to form in meats and model systems at temperatures of 150 °C or higher. In contrast, Dolara et al. (1979) and Barrington et al. (1990) reported that the onset of mutagenicity in meat and meat extracts was found at temperatures below 150 °C (i.e., 100 °C). However, whether the source of mutagenicity was due to AIAs is not clear because the authors did not identify the mutagenic compounds.

Figures 4 and 5 indicate that the maximum concentrations of both AIAs increased with processing temperature. This relationship was also reported for the formation of mutagens in ground beef (Commoner et al., 1978; Spingarn and Weisburger, 1979; Bjeldanes et al., 1983; Knize et al., 1985, 1994) and in a model system (Skog and Jagerstad, 1990). Overvik et al. (1984) and Nielsen et al. (1988) measured the formation of mu-

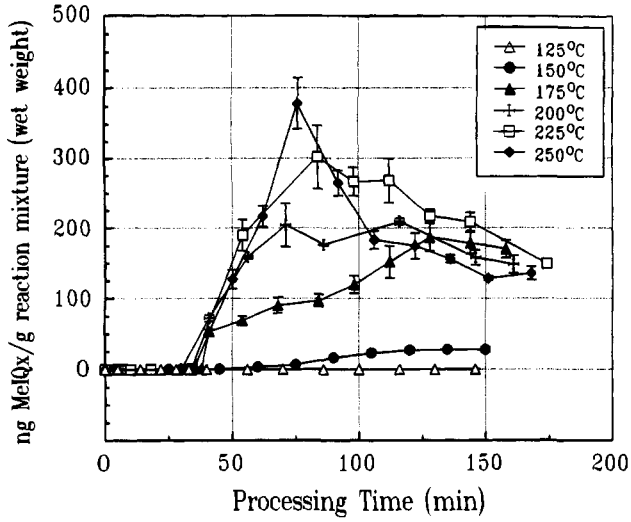

Figure 4. Effects of processing temperature and time on formation of $MeIQ_x$ in an aqueous solution of threonine, glucose, and creatine (2:1:2 molar ratio). Error bars indicate one standard deviation of the mean.

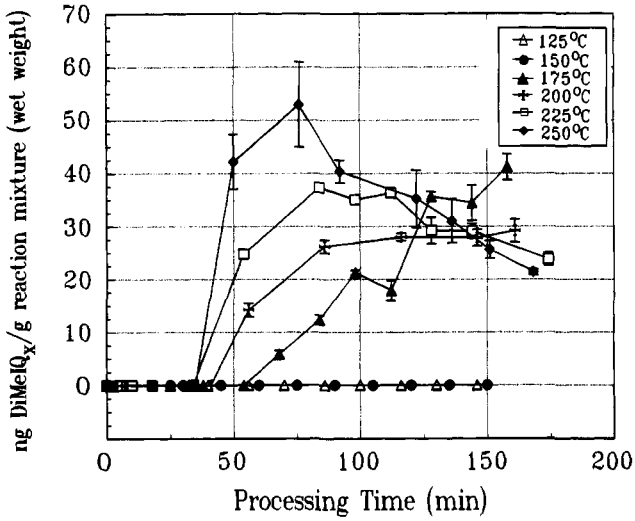

Figure 5. Effects of processing temperature and time on formation of $DiMeIQ_x$ in an aqueous solution of threonine, glucose, and creatine (2:1:2 molar ratio). Error bars indicate one standard deviation of the mean.

tagens when frying lean pork at different temperatures. Both groups showed that an increase in pan temperature from 200 to 250 °C resulted in a doubling of the total mutagenic activity in the fried meat. These results are consistent with the data reported here.

The maximum yields of $MeIQ_x$ and $DiMeIQ_x$, in nanomoles formed per millimole of original creatine, at each processing temperature are shown in Table 1. At all temperatures the yield of $MeIQ_x$ was 5–9 times that of $DiMeIQ_x$. These ratios agree with the ratios of $MeIQ_x$ to $DiMeIQ_x$ found in beef extract and fried meat (Turesky et al., 1988; Gross et al., 1989; Gross, 1990; Knize et al., 1994). In contrast, Skog and Jagerstad (1993) reported that the yield of $DiMeIQ_x$ was 3 times greater than that of $MeIQ_x$ when a model system consisting of water, creatinine, glucose, and threonine was heated at 180 °C for 30 min. Several differences in the experimental procedure may explain these contradictory results. First, the Skog and Jagerstad study used different processing equipment. The model system was processed in stainless steel tubes heated by a thermostatically controlled block. The temperature in the reaction mixture was not monitored during processing, and the contents of the tubes were not mixed. Second, the pressures in the tubes were not reported. In the system studied here, pressures at 125, 150, 175, 200, 225, and 250 °C were 40, 60, 100, 200, 360, and 600 psi, respectively, and remained constant during the run once processing temperatures were reached. The pressure in the reaction vessel may affect yield by influencing reaction pathways. Third, Skog and Jagerstad did not measure or standardize the initial pH of the reaction mixture. Finally, they did not correct their yields for recovery of $MeIQ_x$ and $DiMeIQ_x$.

Table 1. Chemical Yield of $MeIQ_x$ and $DiMeIQ_x$ in the Threonine/Glucose/Creatine Model System Heated at 125–250 °C

	yield,[a,b] nmol/mmol of creatine	
processing temp, °C	$MeIQ_x$	$DiMeIQ_x$
125	0	0
150	1.9	0
175	11.7	2.5
200	12.6	1.8
225	20.1	2.3
250	24.9	3.3

[a] Maximum yield of $MeIQ_x$ and $DiMeIQ_x$ at each processing temperature. [b] Yields were corrected for recoveries.

At all processing temperatures, there was a time lag before AIAs could be detected in the reaction mixture. The lag was in part related to the time required for the reaction mixture to reach processing temperature. Similarly, Pariza et al. (1979) and Spingarn and Weisburger (1979) attributed the 2–4-min lag in the formation of mutagenic compounds in fried beef to the time necessary to raise the temperature of the surface of the meat above 100 °C. However, in the model system studied here, $MeIQ_x$ began to form when the reaction mixture reached a temperature of 150 °C. An explanation for this result is that time was needed for threonine, glucose, and creatine to form AIA intermediates such as creatinine, pyridines, and pyrazines.

Figures 4 and 5 indicate that at 150 and 175 °C AIA levels increased with processing time. However, at processing temperatures of 200 and 250 °C, the concentrations of both AIAs increased during the initial 15 and 30 min of processing and then decreased for the remainder of the run.

These results concur with those of other researchers who studied the effects of cooking conditions on the formation of AIAs and mutagenic activity in model systems and in meat. Gross and Gruter (1992) studied the formation of several HAAs in salmon as a function of cooking time and temperature. In pan-broiled salmon, $MeIQ_x$ and PhIP levels decreased with time. Similarly, Knize et al. (1994) found that the $MeIQ_x$ levels in ground beef increased rapidly during the first 2–4 min of frying at 190 and 230 °C and then plateaued after 6–10 min of frying. Skog and Jagerstad (1990) and Johansson et al. (1993) studied the formation of $MeIQ_x$ and $DiMeIQ_x$ in model systems containing creatine or creatinine, glycine, and various sugars as precursors with a mixture of diethylene glycol and water as the solvent. They reported that mutagenicity in the reaction mixtures plateaued after 10 min at 180 °C. Similarly, Bjeldanes et al. (1983) reported that the mutagenic activity in fried ground beef patties increased rapidly during the first 10 min of cooking at 200 and 250 °C (pan temperatures), but no further increases in mutagenic activity resulted for times up to 20 min. Also, Sflomos et al. (1989) reported that the mutagenic activity in fried lamb

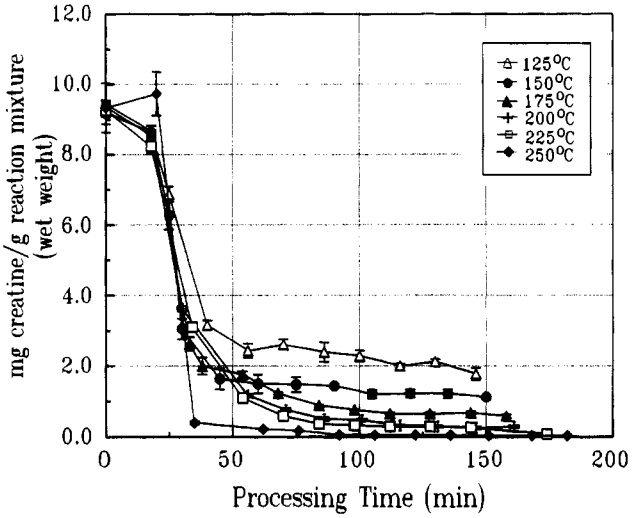

Figure 6. Effects of processing temperature and time on creatine concentration in an aqueous solution of threonine, glucose, and creatine (2:1:2 molar ratio). Error bars indicate one standard deviation of the mean.

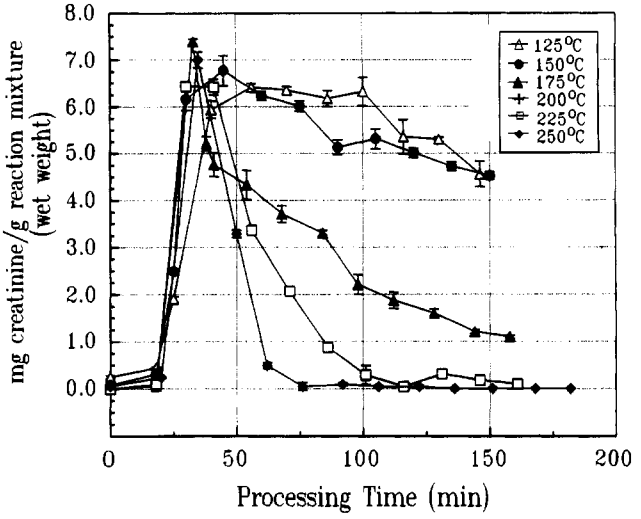

Figure 7. Effects of processing temperature and time on formation of creatinine in an aqueous solution of threonine, glucose, and creatine (2:1:2 molar ratio). Error bars indicate one standard deviation of the mean.

patties increased to a maximum after 10 min of cooking and then decreased during the final 2 min of cooking.

Skog and Jagerstad (1990) found that glucose or Maillard reaction products may combine directly with creatine or creatinine, and the result of this reaction is a decrease in the formation of mutagens. They reported that addition of 5-(hydroxymethyl)-2-furfural to a mixture of glucose, glycine, and creatinine reduced mutagenicity after heating for 10 min at 180 °C. They concluded that at high processing temperatures (>200 °C) competing browning reactions may take place, and these alternative reactions may decrease AIA formation.

A possible explanation for the decrease in AIA levels during processing is that $MeIQ_x$ and $DiMeIQ_x$ may react with other Maillard browning products to form higher molecular weight oligomers known as the melanoidins. This explanation is supported by our observation that at processing temperatures greater than 200 °C a brown precipitate formed in the reaction mixture. Another possibility is that $MeIQ_x$ and $DiMeIQ_x$ decompose at elevated temperatures.

Creatine and Creatinine Concentrations. Creatine and creatinine concentrations were measured to determine if a relationship existed between the concentrations of these compounds and those of AIAs formed in the reaction mixture. For all processing temperatures, creatine levels decreased with processing time (Figure 6), whereas creatinine levels increased to a maximum value and then decreased with time (Figure 7). The extent and rate of the disappearance of creatine and the formation of creatinine depended on processing temperature. Higher temperatures were accompanied by a more rapid decrease in creatine and increase in creatinine. Most of the conversion of creatine to creatinine occurred in the first 40 min of processing. Similar trends were reported by Laser-Reutersward et al. (1987a,b) for cooked bovine tissues (meat, heart, liver, tongue, and kidney). The disappearance of creatinine during processing is likely due to reactions between creatinine and Maillard browning products (Skog and Jagerstad, 1990).

The results of this study did not indicate a linear or a stoichiometric relationship between creatine or creatinine concentrations and AIA formation in the reaction mixture. Similarly, Vikse and Joner (1993) found no correlation between creatine or creatinine concentrations in different meats and formation of mutagenic activity. However, their data suggested that a threshold level of creatine is necessary to produce mutagenic activity. Felton et al. (1994) reported that reducing the concentration of creatine in meat reduced the formation of AIAs in a nonlinear fashion.

In this study AIAs did not appear in the reaction mixture until almost all of the creatine was converted to creatinine. In addition, the appearance of $MeIQ_x$ and $DiMeIQ_x$ coincided with the decrease in creatinine. The shapes of the curves for the formation of $MeIQ_x$ and $DiMeIQ_x$ (Figures 4 and 5) during processing at temperatures greater than 150 °C mirror those for the disappearance of creatinine. These data as well as those reported by Laser-Reutersward et al. (1987a,b) and Taylor et al. (1988) suggest that creatinine is a precursor in AIA-forming reactions.

The results of this study indicate that the decrease in creatinine was far greater than the amount of AIAs formed (Figures 4 and 7; Table 1). Jagerstad et al. (1991) reported that although creatinine is an essential AIA precursor, its concentration is not a yield-limiting factor. Similarly, Laser-Reutersward et al. (1987b) and Skog and Jagerstad (1990) found that only a small amount (fractions of a percent) of creatinine was used up in the formation of AIAs.

Conclusions. Model systems are useful for studying the physical and chemical parameters, kinetics, and reaction mechanisms that describe the formation of AIAs. As shown in this study, processing temperature and time are critical physical parameters in the formation of $MeIQ_x$ and $DiMeIQ_x$. The results suggest that cooking food at temperatures below 150 °C may be an effective method for reducing the level of these AIAs in the diet. Chemical factors such as creatine and/or creatinine concentrations are also important in the formation of AIAs. Our data show that the conversion of creatine to creatinine, which occurs during thermal processing, is an essential step in the formation of $MeIQ_x$ and $DiMeIQ_x$. In addition, the loss of creatinine during processing may be an indicator of AIA formation.

More work is needed to elucidate the kinetics and reaction mechanisms that result in the formation of AIAs. Our finding that $MeIQ_x$ and $DiMeIQ_x$ decrease

in concentration during processing at high temperatures (>200 °C) suggests that a complex set of reactions occurs during processing which result in the formation and decomposition of AIAs. More work is needed to determine if the trends observed in this study agree with those in a more complex model system containing a variety of amino acids, other AIA precursors, and different ratios of precursors.

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