

## Correlation of Acrylamide Generation in Thermally Processed Model Systems of Asparagine and Glucose with Color Formation, Amounts of Pyrazines Formed, and Antioxidative Properties of Extracts

STEFAN EHLING AND TAKAYUKI SHIBAMOTO\*

Department of Environmental Toxicology, University of California, Davis, One Shields Avenue, Davis, California 95616

The relations between the formation of acrylamide and color, pyrazines, or antioxidants in an asparagine/D-glucose browning model system under various conditions were investigated. The highest level of acrylamide was produced in the asparagine/glucose (1:3) system heated at 170 °C for 30 min (2629 µg/g asparagine). Color intensity increased with temperature and heating time. The formation of pyrazines increased steadily with an increase of temperature (140–170 °C) and heating time (15–60 min). Antioxidant formation varied among the samples heated under different conditions. A clear correlation between formation of acrylamide and browning color was obtained. The formation of acrylamide was linearly correlated with the formation of total pyrazines during the initial stages of the Maillard reaction. No obvious correlation between formation of acrylamide and antioxidants was observed. However, excess amounts of asparagine increased the formation of antioxidants, whereas excess amounts of glucose reduced its formation.

**KEYWORDS:** Acrylamide; antioxidants; asparagine; browning color; Maillard reaction; pyrazines

### INTRODUCTION

The recent discovery of acrylamide in cooked foods has raised public concern about food safety because, on the basis of numerous studies, the International Agency for Research on Cancer (IARC) has classified acrylamide as “probably carcinogenic to humans” (1). Consequently, numerous studies to elucidate the formation mechanisms of acrylamide in heat-treated or cooked foods have been performed in order to prevent its formation. Among advanced hypotheses concerning acrylamide formation in cooked foods, the Maillard browning reaction of a sugar and an amino acid had received much attention as the most likely mechanism (2). Significant amounts of acrylamide (221 mg/mol of amino acid) formation were reported in a browning model system that consisted of an equimolar of L-asparagine and glucose heated at 185 °C (2). When asparagine and glutamine, which have the same amide moiety at the end of their molecule, were heated alone at 180 °C for 30 min, 0.99 and 0.17 µg/g of acrylamide were formed, respectively. On the other hand, the addition of glucose to asparagine increased acrylamide formation to 1200 µg/g (3). Also, acrylamide formation increased from 117 to 9270 µg/g with the addition of glucose in a system consisting of asparagine, potato starch, and water (4). These results suggest that asparagine and carbonyl compounds—such as glucose, glyceraldehyde,

and acrolein—play an important role in acrylamide formation in cooked foods (3, 5).

Several factors, such as the initial reactant concentration and ratio, temperature and time of processing, and pH and water activity, have been shown to influence the formation levels of acrylamide in heat-processed foods (6). Certain glycoconjugates have been found to play a major role in acrylamide formation in low moisture Maillard model systems (180 °C, 5 min) by the study based on asparagine, reducing sugars, Maillard intermediates, and sugar degradation products (7). In addition to the chemical reactivity, reaction temperature, and time, the physical state of the ingredients influenced the formation of acrylamide during food processing (8). The complex Maillard reaction is also responsible for the development of desirable food attributes such as color and flavor, as well as antioxidant formation upon heat treatment (9).

In the present study, a Maillard reaction system consisting of asparagine/D-glucose was heated under various conditions to investigate the formation of acrylamide, color, flavor, and antioxidants. The goal was to reveal any existing relevant relationships between these aspects of the Maillard reaction and ways to optimize food processing by heat in order to achieve low levels of acrylamide, good palatability (color and flavor), and potentially nutritional benefits (antioxidants).

### MATERIALS AND METHODS

**Chemicals and Reagents.** L-Asparagine, D-glucose, indole, hexanal, α-tocopherol, and undecane were purchased from Sigma-Aldrich, Inc.

\* To whom correspondence should be addressed. Tel: +1 530-752-4523. Fax: +1 530-752-3394. E-mail: tshibamoto@ucdavis.edu.

(St. Louis, MO). Acrylamide was bought from Bio-Rad Laboratories (Hercules, CA).  $^{13}\text{C}_3$ -Acrylamide was purchased from Cambridge Isotope Laboratories (Andover, MA). Pyrazines were from Organic Chemicals, Ltd. (Brackley, Northamptonshire, United Kingdom). All solvents were from VWR International (Brisbane, CA).

**Sample Preparations for Acrylamide Analysis.** Aqueous solutions (20 mL) containing various molar ratios of asparagine and D-glucose (1:1, 3:1, and 1:3) were heated in a 330 mL swing top glass bottle (Systempack, München, Germany) at various temperatures (140, 150, 160, and 170 °C) and for various heating times (15, 30, 45, and 60 min). All samples were prepared in triplicates.

After 1 mL of internal standard,  $^{13}\text{C}_3$ -acrylamide (200 ng/mL in water) and 3 mL of deionized water were added to 1 mL of reaction mixture, and the reaction solution was purified on an OASIS-HLB solid phase extraction column (Waters Corporation, Milford, MA) by the method previously reported (10). The column was washed with 3.5 mL of methanol followed by 3.5 mL of water prior to use. The sample solution (1.5 mL) was allowed to pass through the column. The column was then eluted with 0.5 mL of water, and the eluate was discarded; subsequently, the column was eluted with 1.5 mL of water, and the eluate was subjected to liquid chromatography–tandem mass spectrometry (LC-MS/MS).

**Measurement of Sample Color.** A reaction mixture (100  $\mu\text{L}$ ) was diluted with 10 mL of purified water, and the absorbance of the resulting solution was measured at  $\lambda = 420$  nm with a Hewlett-Packard 8452A Diode Array Spectrophotometer running UV–Visible Chemstation software (Agilent Technologies, 1995–2000). Water was used as a blank.

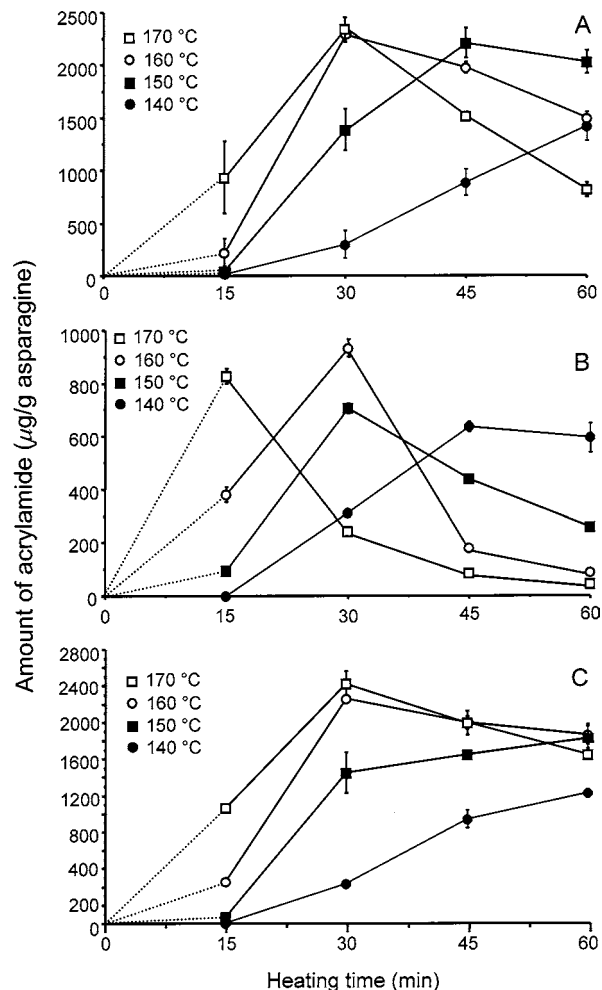
**Sample Preparations for Analysis of Pyrazines.** A reaction mixture (15 mL) was extracted with 25 mL of dichloromethane using a liquid–liquid continuous extractor. After the extract was dried over anhydrous sodium sulfate, it was concentrated to 1–2 mL under a purified nitrogen stream. A 400  $\mu\text{L}$  amount of dichloromethane solution of indole (1 mg/mL) was added to the sample solution as a gas chromatography (GC) internal standard for quantitative analysis of pyrazines prior to GC/MS analysis.

**Antioxidant Activity Test.** The antioxidative activity of the concentrated dichloromethane extract was examined using an aldehyde/carboxylic acid assay (11). Various amounts of extract were added to a 2 mL dichloromethane solution of hexanal (3 mg/mL) containing 200 mg/mL of undecane as a GC internal standard. The oxidation of the sample solution was initiated by heating at 60 °C for 10 min in a sealed vial and storing at room temperature. The headspace of each vial was purged with pure air for 3 s every 24 h for the first 10 days. The decrease in hexanal was monitored at 7 day time intervals for 36 days by GC/flame ionization detector (FID).  $\alpha$ -Tocopherol (100  $\mu\text{g}$ /mL) was used as a known antioxidant. The experiment was replicated three times.

**Instrumentation.** Acrylamide analysis was conducted with a Hewlett-Packard 1100 liquid chromatograph interfaced to an Applied Biosystems API 2000 MS/MS via an atmospheric pressure chemical ionization source operating in the positive ion mode at 475 °C with nitrogen gas. The mass spectrometer was operated in selective ion monitoring mode (SIM) to observe the transition of  $m/z$  72 to  $m/z$  55 for acrylamide and  $m/z$  75 to  $m/z$  58 for  $^{13}\text{C}_3$ -acrylamide. Chromatographic separation was accomplished with a 50 mm  $\times$  2.1 mm Hypercarb column (Thermo, San Jose, CA) with a 5  $\mu\text{m}$  particle size. The mobile phase condition was isocratic at 96/4% aqueous acetic acid (0.1%)/methanol with a flow rate of 400  $\mu\text{L}/\text{min}$ . Under these conditions, acrylamide eluted at 1.4 min.

The quantitative analysis of hexanal in the aldehyde/carboxylic acid assay was conducted using an Agilent model 6890 GC equipped with a 30 m  $\times$  0.25 mm i.d. ( $d_f = 0.25$   $\mu\text{m}$ ) DB-1 bonded phase fused silica capillary column (J & W Scientific, Folsom, CA), and a FID was used. The injector and detector temperatures were 200 and 250 °C, respectively. The oven temperature was programmed from 60 to 120 °C at 10 °C/min and held for 3 min. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 20:1.

Qualitative and quantitative analyses of pyrazines formed in the samples were performed by an Agilent model 6890 GC interfaced to an Agilent 6890 series mass selective detector and Enhanced Chem-



**Figure 1.** Amounts of acrylamide formed in the test system with a 1:1 (A), 3:1 (B), or 1:3 (C) reactant ratio of asparagine and D-glucose.

station G1701BA version B.01.00 (Hewlett-Packard, 1989–1998) software. A 30 m  $\times$  0.25 mm i.d. ( $d_f = 0.25$   $\mu\text{m}$ ) DB-WAX fused silica capillary column (J & W Scientific) was used. The GC oven temperature was held at 60 °C for 5 min and programmed to 120 °C at 3 °C/min and to 220 °C at 20 °C/min and then held for 10 min. The injector and detector temperatures were 250 °C. The linear velocity of the helium carrier gas was 30 cm/s at a splitless mode. Detection of monitoring compounds was performed by SIM of the following fragment ions:  $m/z$  53, 80 (pyrazine);  $m/z$  67, 94 (methylpyrazine);  $m/z$  81, 108 (2,5- or 2,6-dimethylpyrazine);  $m/z$  67, 108 (2,3-dimethylpyrazine);  $m/z$  94, 121 (2-ethyl-5- or 6-methylpyrazine);  $m/z$  81, 122 (trimethylpyrazine); and  $m/z$  90, 117 (indole).

## RESULTS AND DISCUSSION

Acrylamide standard curves showed excellent linearity in the range of 10–100000 ng/mL ( $R^2 = 1.00$ ). The detection and quantitation limits were 50 and 100 pg, respectively, in the present study. These limits were lower than the values obtained by GC/NPD (200 pg for detection and 670 pg for quantitation) previously reported (3).

**Formation Levels of Acrylamide.** The formation levels of acrylamide from asparagine/D-glucose systems with three different reactant ratios are shown in Figure 1A–C. The values are means  $\pm$  standard deviations ( $n = 3$ ).

With a 1:1 reactant ratio (A), a steady increase of acrylamide level was observed at 140 °C. However, at 150 °C, the level of acrylamide reached a peak after 45 min and then reduced. At

higher temperatures (160 and 170 °C), this peak was reached after 30 min, and subsequently, the level of acrylamide reduced considerably (by 35% after 160 °C and 65% after 170 °C). The highest level of acrylamide was obtained at 170 °C for 30 min (2350  $\mu\text{g/g}$  asparagine).

With a 3:1 reactant ratio (B), a slight reduction of acrylamide level was already observed at 140 °C after 45 min. At 150 and 160 °C, the level of acrylamide reached maximum after 30 min and then reduced (by 63 and 90%, respectively). At 170 °C, the reduction of the acrylamide level started after 15 min and continued steadily. The level reduced by 71% after 30 min and by 95% after 60 min. The highest level of acrylamide was obtained at 160 °C after 30 min (932  $\mu\text{g/g}$  asparagine). This level is less than half of the maximum level generated in the asparagine/glucose 1:1 system.

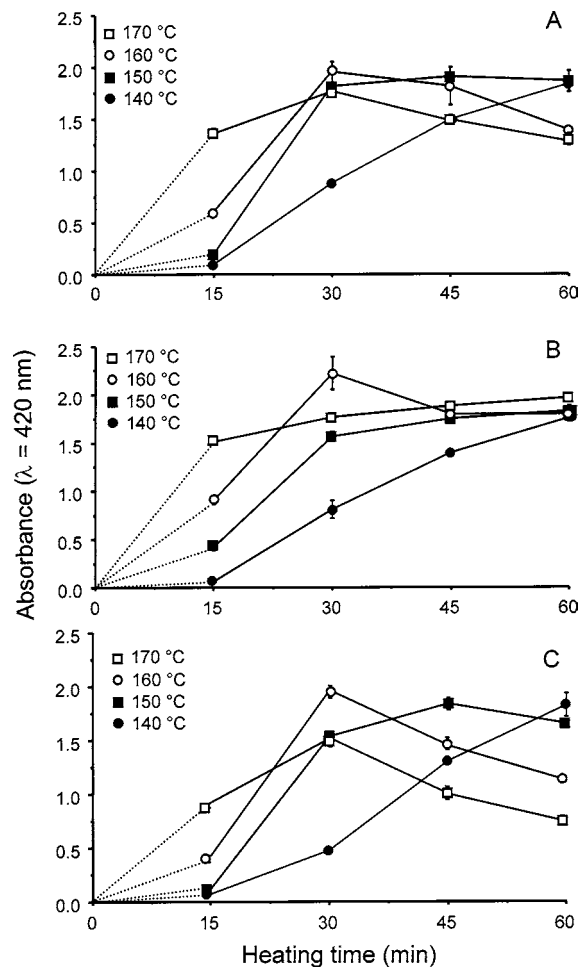
With a 1:3 reactant ratio (C), steady increases of acrylamide level were observed at both 140 and 150 °C, up to and even after 60 min. At 160 and 170 °C, levels of acrylamide reached a peak after 30 min, and then, a modest reduction occurred by 16% at 160 °C and by 30% at 170 °C. The highest level of acrylamide was reached at 170 °C and 30 min (2629  $\mu\text{g/g}$  asparagine). This is the highest level of acrylamide reached among all reaction systems.

There are many reports on the formation levels of acrylamide from asparagine and D-glucose heated with different reactant ratios. When asparagine and D-glucose (1:2.5, w/w) were heated at 180 °C for 30 min, 1200  $\mu\text{g/g}$  asparagine was formed (3). The maximum yield of acrylamide formation was observed at a molar ratio of asparagine/glucose of 0.5–1.0, when they were heated at 175 °C for 10 min or at 155 °C for 20 min (12). It should be noted that the reaction conditions used in these experiments were not the same as those used in this experiment. Therefore, their values reported here are not directly comparable.

In actual foods (e.g., potato and cereal), the asparagine content is always higher than the glucose content. The molar ratio between asparagine and reducing sugars (glucose + fructose) in five potato cultivars ranged from 2.4 to 14.5 (13). Another report indicated that this ratio ranged from 1.0 to 40.2 among 17 potato cultivars (14). The levels of acrylamide in potato chips were strongly correlated with the levels of reducing sugars in potato tubers (15). Generally, the lower this ratio the higher acrylamide formation was observed, which is consistent with the results from the present study.

Generally, the higher the temperature, the sooner the reduction of acrylamide level starts. This suggests that some level of elimination of acrylamide occurs when temperatures exceed certain levels. The conspicuous pattern between the formation and elimination of acrylamide has been reported by several researchers. The elimination of acrylamide generally became predominant at temperatures higher than 160 °C (16). It is reported that the decrease in acrylamide level occurs predominantly due to degradation rather than to polymerization (17). However, the elimination of acrylamide is practically not important because even though the elimination of acrylamide may occur at higher temperatures and over prolonged heating times, the foods produced under these conditions would be unacceptably dark and dry.

At short heating times (15 min) when no acrylamide elimination has yet occurred, the dependence of acrylamide formation on temperature is expressed by a power law (Microsoft Office Excel 2003). The equations obtained are  $y = 3E-47x^{22.186}$  ( $R^2 = 0.99$ ,  $p < 0.005$ ,  $n = 4$ ) for the asparagine/glucose 1:1 system;  $y = 2E-62x^{29.107}$  ( $R^2 = 0.97$ ,  $p < 0.01$ ,  $n = 4$ ) for the asparagine/glucose 1:3 system; and  $y = 37.623x$



**Figure 2.** Brown color intensities formed in the test system with a 1:1 (A), 3:1 (B), or 1:3 (C) reactant ratio of asparagine and D-glucose.

– 3956.3 ( $R^2 = 0.93$ ,  $p < 0.025$ ,  $n = 4$ ) for the asparagine/glucose 3:1 system, which is the closest to a linear relationship.

**Formation of Brown Color.** The wavelength used in the present study ( $\lambda = 420$  nm) has been widely used for assessing the color intensity of browning reaction mixtures (18). The color intensity obtained in the asparagine/D-glucose systems with three different reactant ratios is shown in **Figure 2A–C**. The values are means  $\pm$  standard deviations ( $n = 3$ ). With a 1:1 reactant ratio (A), the color intensity increased steadily at 140 and 150 °C. At 150 °C, it remained fairly constant after 30 min of heating. At 160 and 170 °C, a slight reduction in color intensity occurred after 30 min. The highest intensity (absorbance =  $1.95 \pm 0.10$ ) was obtained from the sample heated at 160 °C for 30 min.

With a 3:1 reactant ratio (B), brown color formation increased steadily when the sample was heated at 140 and 150 °C. On the other hand, when the sample was heated at 160 and 170 °C, the color intensity reached a peak after 30 min and then decreased steadily. The highest absorbance ( $1.96 \pm 0.055$ ) was obtained from the sample heated at 160 °C for 30 min.

With a 1:3 reactant ratio (C), the color intensity increased steadily at 140 °C; however, it decreased slightly at 150 °C after 45 min of heating. At 160 and 170 °C, the color intensity reached a peak after 30 min and then a 50% decrease in intensity occurred. The highest absorbance ( $2.22 \pm 0.172$ ) was also obtained from the sample heated at 160 °C for 30 min; this was the highest intensity among all of the samples tested.



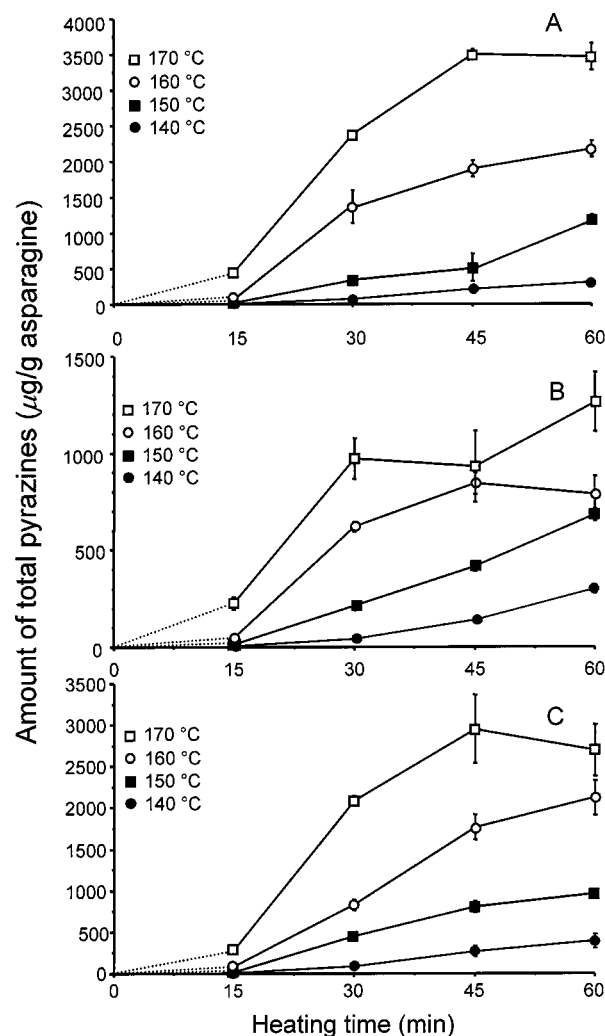
Intensive studies on browning color formation using amino acid/sugar model systems were conducted in the early era of Maillard reaction research. An excess of reducing sugar over amino acid promoted the browning color (19). On the other hand, the concentration of amino acid (glycine) was the factor determining the relative browning rate of a Maillard system containing glucose and fructose. In a leucine/glucose model system, excess leucine was more effective in increasing the browning rate than excess glucose (20). In the present system, excess asparagine slightly promoted browning, while excess glucose did not have any significant effect.

There have been some reports on the role of asparagine in brown color formation in the Maillard reaction (21). It was reported that asparagine, which is the most abundant free amino acid in potatoes, produced the least browning color among amino acids tested in the Maillard reaction systems (22). When potato slices were soaked in amino acid solutions before frying, it found that glutamine was the key amino acid in fry color development, whereas asparagine was responsible for the gloss and quality of the color (23).

**Formation of Pyrazines Associated with Roasted or Toasted Flavor.** Among many chemicals found in Maillard browning model systems, pyrazines are the most abundant flavor chemicals and contribute a roasted or toasted flavor to cooked foods (24). Pyrazines have been analyzed to investigate the relationship between a roasted or a toasted flavor and the level of pyrazines in heat-treated foods, such as French fried and potato chips (25). For example, pyrazines with earthy/musty, roasty/burnt, and woody/papery flavor contributed a characteristic roasted flavor to the espresso coffee (26). Among pyrazines found in roasted peanuts, 2,5-dimethylpyrazine was most highly correlated to roasted peanut flavor and aroma (27). Over 70 pyrazines found in thermally treated foods including cocoa, coffee, barley, popcorn, nuts, bread, potato, and beef, only alkylpyrazines contributed to heated food flavors (25). All of these reports support the relevance of using alkylpyrazines for investigation of roasted or toasted flavor formation in the Maillard model systems. Therefore, the formation of pyrazines was used to investigate the possible relationship between the formation of acrylamide and a roasted or toasted flavor in heat-treated foods.

The total formation of pyrazines in samples heated at different temperatures for different durations is shown in **Figure 3A–C**. **Table 1** shows a typical composition of pyrazines in an extract from the asparagine/glucose 1:1 system heated at 170 °C for 45 min. The values are means  $\pm$  standard deviations ( $n = 3$ ). Pyrazines, which were formed and were used to monitor cooked flavor formation, were pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, and trimethylpyrazine.

The formation patterns of pyrazines were rather simple. The formation of pyrazines increased with increases in temperature and heating time. The greatest total yield of pyrazines was  $3460 \pm 191 \mu\text{g/g}$  asparagine from the asparagine/glucose 1:1 system heated at 170 °C for 60 min (**A**),  $1261 \pm 154 \mu\text{g/g}$  asparagine from the asparagine/glucose 3:1 system heated at 170 °C for 60 min (**B**), and  $2947 \pm 414 \mu\text{g/g}$  asparagine from the asparagine/glucose 1:3 system heated at 170 °C for 45 min (**C**). The formation of pyrazines at temperatures lower than 140 °C was not examined in the present study. However, it has been reported that essentially no pyrazines are produced at temperatures below 100 °C (28). In a previous report, the presence of



**Figure 3.** Amounts of total pyrazines formed in the test system with a 1:1 (**A**), 3:1 (**B**), or 1:3 (**C**) reactant ratio of asparagine and D-glucose.

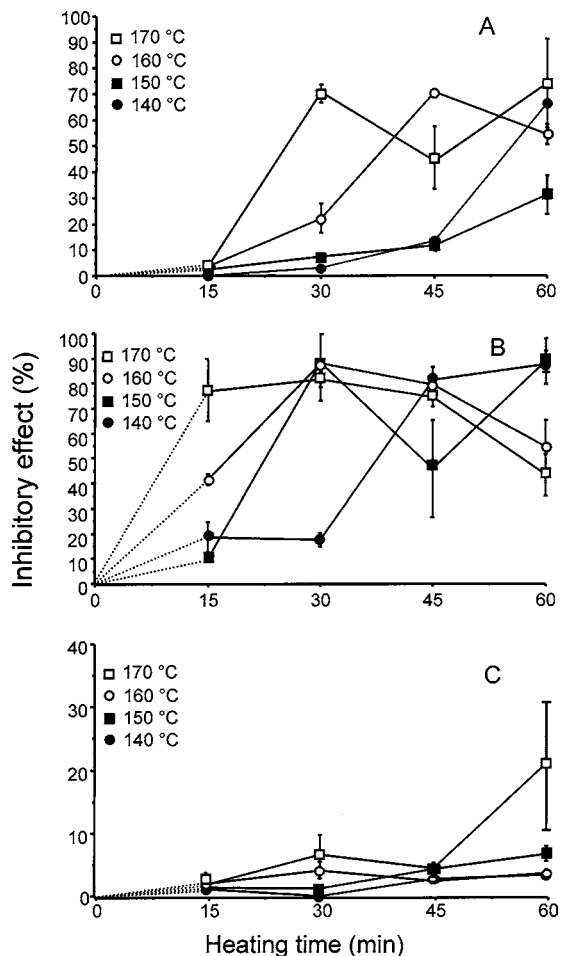
**Table 1.** Composition of Pyrazines in an Extract from the Asparagine/Glucose 1:1 System Heated at 170 °C for 45 min

pyrazines	level ( $\mu\text{g/g}$ asparagine)
pyrazine	$404.6 \pm 3.6$
2-methylpyrazine	$1438.0 \pm 25.8$
2,5-dimethylpyrazine	$484.1 \pm 10.9$
2,6-dimethylpyrazine	$516.1 \pm 12.8$
2,3-dimethylpyrazine	$93.1 \pm 2.1$
2-ethyl-6-methylpyrazine	$255.0 \pm 6.9$
2-ethyl-5-methylpyrazine	$120.4 \pm 2.6$
trimethylpyrazine	$177.9 \pm 4.6$

asparagine produced a higher amount of pyrazines than other amino acids, such as lysine and phenylalanine, did (29).

**Formation of Antioxidants.** The results of antioxidant activity tests on the dichloromethane extract of the asparagine/glucose reaction mixtures are shown in **Figure 4A–C**. The values are means  $\pm$  standard deviations ( $n = 3$ ). This method was validated using the known antioxidant  $\alpha$ -tocopherol, which inhibited hexanal oxidation by almost 100% at a level of 100  $\mu\text{g/mL}$  over 36 days.

With a 1:1 reaction ratio (**A**), almost no antioxidant was produced after 15 min of heating time at any given temperature (the inhibitory effect was less than 5%). Antioxidants formed steadily in the samples heated at 140 and 150 °C over time. The highest levels of inhibitory effect obtained were  $66.4 \pm$



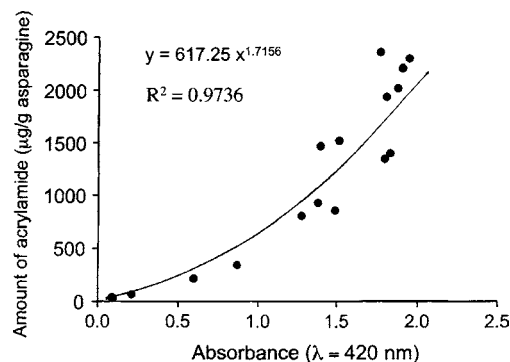
**Figure 4.** Inhibitory effects of the dichloromethane extracts in the test system with a 1:1 (A), 3:1 (B), or 1:3 (C) reactant ratio of asparagine and D-glucose toward hexanal oxidation.

8.4% from the sample heated at 140 °C for 60 min,  $31.1 \pm 7.4\%$  from the sample heated at 150 °C for 60 min,  $70.5 \pm 1.1\%$  from the sample heated at 160 °C for 45 min, and  $73.9 \pm 17.1\%$  from the sample heated at 170 °C for 60 min.

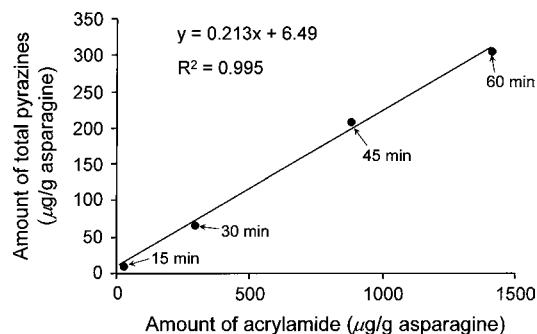
With a 3:1 reaction ratio (B), the formation of antioxidants varied slightly over different temperatures. Only the sample heated at 140 °C exhibited an increase in antioxidant formation over time. The sample heated at 170 °C for 15 min already contained antioxidants, which inhibited hexanal oxidation by  $76.8 \pm 10.9\%$ . However, this decreased after 30 min of heating time. The same trend was observed in the case of the sample heated at 160 °C. The highest inhibitory effect ( $88.7 \pm 9.2\%$ ), which was almost equal to that of  $\alpha$ -tocopherol, was obtained from the sample heated at 150 °C for 60 min.

With a 1:3 reaction ratio (C), only slight formation of antioxidants was observed at any heating temperature or time, except for the sample heated at 170 °C for 60 min, where there was a moderate effect on hexanal oxidation ( $21.1 \pm 9.4\%$ ).

No appreciable relationship between the levels of acrylamide and antioxidants was observed. The results suggest that formation of antioxidants from an amino acid/sugar system is favored by a high amino acid/sugar ratio; higher temperatures and longer heating times were not always associated with a higher formation of antioxidants. This is consistent with results reported previously (30). Also, the antioxidative activity of Maillard reaction products obtained from a lysine/glucose model system was not obviously correlated with reaction conditions, including



**Figure 5.** Power law curve prepared by plotting acrylamide levels vs absorbance at  $\lambda = 420 \text{ nm}$  obtained from the samples with a 1:1 reactant ratio.



**Figure 6.** Linear regression curve prepared by plotting acrylamide levels vs amount of total pyrazines obtained from the samples with a 1:1 reactant ratio heated at 140 °C for 15, 30, 45, and 60 min.

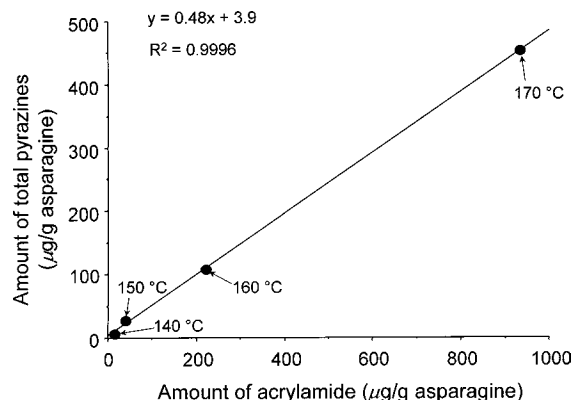
reactant concentration, heating temperature and time, pH, and water activity (31).

**Correlation between Formation of Acrylamide and Color.** Figure 5 shows a typical power law curve prepared by plotting acrylamide levels vs absorbance at  $\lambda = 420 \text{ nm}$  obtained from the sample with a 1:1 reactant ratio. An apparent correlation between formation of acrylamide and color was observed as described by the power law:  $y = 617.25x^{1.7156}$  ( $R^2 = 0.97$ ,  $p < 1\text{E}-12$ ,  $n = 16$ ).

The correlation was also highly significant in the cases of samples with 1:3 ( $R^2 = 0.87$ ,  $p < 1\text{E}-7$ ,  $n = 16$ ) and 3:1 ( $R^2 = 0.76$ ,  $p < 5\text{E}-6$ ,  $n = 16$ ) reactant ratios. The exponent of the power law was highest in the case of a sample with a 3:1 reactant ratio (4.23), followed by the samples with a 1:3 reactant ratio (1.72) and a 1:1 reactant ratio (1.60), suggesting that when excess asparagine is present, acrylamide levels increase at the highest rate with increasing browning.

Good correlations between acrylamide and color have been reported in several actual food systems. Bread crust color was highly correlated with acrylamide content (32). A high level of reducing sugars gave high levels of acrylamide and dark-colored potato chips (15, 33). A relatively strong correlation between browning and acrylamide content exists in small surface products but not in large surface products (17).

**Correlation between Formation of Acrylamide and Pyrazines.** Figure 6 shows a typical linear regression curve prepared by plotting acrylamide levels vs total pyrazine levels formed under the mild heating condition of 140 °C (acrylamide degradation not occurred) from the samples with a 1:1 reactant ratio heated at 140 °C. A clear linear relationship between levels of acrylamide and total pyrazines was observed:  $y = 0.213x + 6.4906$  ( $R^2 = 0.995$ ,  $p < 0.0025$ ,  $n = 4$ ).



**Figure 7.** Linear regression curve prepared by plotting acrylamide levels vs amount of total pyrazines obtained from the samples with a 1:1 reactant ratio heated for 15 min at 140, 150, 160, and 170 °C.

**Figure 7** shows a typical linear regression curve prepared by plotting acrylamide levels vs total pyrazine levels formed from the sample with a 1:1 reactant ratio at the early stage of the browning reaction (15 min heating time). A clear linear relationship between levels of acrylamide and total pyrazines was also observed ( $R^2 = 0.9996$ ,  $p < 0.0025$ ,  $n = 4$ ). An appreciable linear relationship between the levels of acrylamide and total pyrazines was observed in the samples with 1:3 and 3:1 reactant ratios (data not shown).

Acrylamide formation and browning took place in the early stages of the Maillard reaction and were highly correlated. Formation of pyrazines, however, continued well into the later stages of the Maillard reaction and was well-correlated with the level of acrylamide in the initial stages. There is a general lack of correlation of antioxidant level with heating temperature and time. Increasing the asparagine/glucose reactant ratios reduces acrylamide levels, total pyrazine levels, and the retention of browning color and enhances the level of antioxidants but at the expense of reducing total pyrazine formation. Even though it is ideal to eliminate or prevent formation of acrylamide from heat-treated foods, the results suggest that prevention of acrylamide formation can only be achieved at some sacrifice of food palatability, such as preferable flavor and color.

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