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Investigations on the Effect of Amino Acids on Acrylamide, Pyrazines, and Michael Addition Products in Model Systems

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Acrylamide and pyrazine formation, as influenced by the incorporation of different amino acids, was investigated in sealed low-moisture asparagine−glucose model systems. Added amino acids, with the exception of glycine and cysteine and at an equimolar concentration to asparagine, increased the rate of acrylamide formation. The strong correlation between the unsubstituted pyrazine and acrylamide suggests the promotion of the formation of Maillard reaction intermediates, and in particular glyoxal, as the determining mode of action. At increased amino acid concentrations, diverse effects were observed. The initial rates of acrylamide formation remained high for valine, alanine, phenylalanine, tryptophan, glutamine, and leucine, while a significant mitigating effect, as evident from the acrylamide yields after 60 min of heating at 160 °C, was observed for proline, tryptophan, glycine, and cysteine. The secondary amine containing amino acids, proline and tryptophan, had the most profound mitigating effect on acrylamide after 60 min of heating. The relative importance of the competing effect of added amino acids for α-dicarbonyls and acrylamide−amino acid alkylation reactions is discussed and accompanied by data on the relative formation rates of selected amino acid−AA adducts.

KEYWORDS: Acrylamide; asparagine; Maillard reaction; amino acids; proline

INTRODUCTION

Acrylamide, a neurotoxic compound and probable carcinogen found at significant levels in carbohydrate-rich foods, has been shown to originate from the Maillard reaction of the amino acid asparagine with reducing sugars (*1*, *2*) as well as carbonylic compounds deriving from either the Maillard reaction or lipid oxidation processes (*3*−*6*) during heating. The formation of acrylamide in foods has received great attention over recent years, due to its potential adverse health effects; thus, investigations on the routes of formation as well as mitigation strategies have been explored to a substantial extent. Interactions and synergistic effects with other food components, including raising agents (*4*), proteins, or pH modification agents (*7*, *8*), have been shown to influence acrylamide formation. The addition of amino acids has been proposed as a mitigation strategy to reduce the levels of acrylamide in crisps, flat breads, and bread crust (*9*), while glycine has received particular attention as an additive that could potentially reduce acrylamide formation by either competing for available Maillard reaction intermediates or reacting with acrylamide itself through Michael addition type reactions (*10*). A 30 and 70% reduction in the acrylamide content in potato cakes was observed when glycine was added at 0.39 and 1.3% d.w., respectively (*8*), while similar results were also obtained when a glycine solution was sprayed on yeast-leavened breads before heating (*11*) or incorporated into dough formulations (*12*). Lysine and cysteine were also shown to reduce the formation of acrylamide, while alanine had a neutral effect and glutamine increased the yields of acrylamide in sealed aqueous model systems (*13*). This study was conducted to elucidate the effect of amino acids on acrylamide in low-moisture asparagine−glucose model systems and to determine the relative importance of the Michael addition type reactions and competition for available carbonyls on acrylamide mitigation.

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EXPERIMENTAL PROCEDURES

Materials. Asparagine, aspartic acid, cysteine, alanine, glutamine, valine, leucine, tryptophan, proline, glycine, ^{15}N-glycine, phenylalanine, histidine, glucose, fructose ethyl acetate (≥99.9%), sodium thiosulfate, sodium sulfate, potassium bromide, and bromine were purchased from Sigma-Aldrich Ltd. (Poole, Dorset, United Kingdom) and were of ≥99% purity. High-performance liquid chromatography (HPLC) grade methanol, acetic acid, and acetonitrile were purchased from Fisher Scientific (Loughborough, Leicestershire, United Kingdom). 1,2,3-$^{13}C_3$-acrylamide was purchased from Cambridge Isotopes Laboratories Inc. (Andover, MA), while the bromination reagent was prepared as described in ref *14*.

Model System Preparation and Heat Treatment. Aqueous suspensions (5%) of waxy maize starch (WMS) with added reactants (either acrylamide and amino acids or glucose and amino acids) in solution were prepared in a shaking water bath (90 °C) and heated for approximately 5 min and until the internal temperature reached 73 °C. The resulting homogeneous slurry was then immediately cooled in an ice bath, and 6−8 g was transferred to an 18 mL solid-phase microextraction (SPME) vial

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(Chromacol Ltd., Welwyn Garden City, Herts, United Kingdom), frozen at −18 °C overnight, and freeze-dried for 72 h. Model system reactions were performed in a gas chromatography (GC) oven in sealed SPME vials. The initial oven temperature was held at 220 °C for the first 50 s and then set to 160 °C for the remainder of the heating period. This procedure assured a rapid heat transfer with samples reaching the desired core temperature (160 °C) in less than 2 min.

Extraction and Determination of Acrylamide. The determination of acrylamide was performed by gas chromatography−mass spectrometry (GC-MS) after bromination and ethyl acetate extraction of the 2,3-dibromopropanamide using $^{13}C_3$-acrylamide as an internal standard as described previously (*14*). Two microliters of the extract was injected under a pressure pulse (25 psi for 60 s at 250 °C) onto a Varian CP-3800 coupled to a Saturn 2000 mass spectrometer (Varian, Palo Alto, CA) fitted with a DB17-MS capillary column (0.25 mm i.d. × 0.15 μm FT). The initial column temperature was set at 50 °C for 2 min, raised at 20 °C/min to 100 °C, and then at 8 °C/min to 320 °C. The mass spectrometer was operated in the total ion scan mode with a scan range *m*/*z* 50−250, while the transfer line and ion trap temperatures were 260 and 200 °C, respectively. The ion *m*/*z* 155 was used to quantify $^{13}C_3$-2,3-dibromopropanamide, and the ions *m*/*z* 150 or 152 were used to quantify 2,3-dibromopropanamide.

Determination of Pyrazines. A headspace (HS)-SPME/GC-MS method developed previously in our laboratory (*14*) was employed to quantitatively determine pyrazines in model reaction mixtures. Aliquots (0.1 g) of the WMS matrix were accurately weighed, 2 mL of a sodium chloride solution (4.3 M) was added, and the samples were vortex mixed for 10 s. The vials were then placed onto the CTC Combi Pal autosampler (CTC Analytics AG, Zwingen, Switzerland), attached to the Varian GC-MS described above. HS-SPME of the preheated samples (40 °C, 10 min) was performed under agitation for 1 min using a 65 μm PDMS/DVB fiber (Supelco, Bellefonte, PA), followed by desorption (10 min) at 250 °C onto a 60 m DBWAXetr capillary column (0.25 mm i.d. × 0.25 μm FT). The initial oven temperature was set at 40 °C, held for 5 min, increased to 200 at 3 °C/min, and finally to 240 at 8 °C/min. The helium flow rate was maintained constant at a flow rate of 1 mL/min. For quantification purposes, an external calibration method was used by adding known amounts of mixtures of pyrazines to aliquots (0.1 g) of the freeze-dried but unheated WMS matrix. Analysis was carried out in triplicate.

Determination of Acrylamide−Amino Acid Adducts. Aqueous model systems (2 mL) containing 25 μmol of histidine, glycine, cysteine, tryptophan, or proline and 25 μmol of acrylamide were heated at 160 °C in unsealed tubes, and samples were taken over 2 h and every 20 min. The samples were then diluted in 1% formic acid in water (100 mL) and subjected to MS/MS by direct infusion prior to semiquantitative analysis. A LCQ Advantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an electrospray ionization (ESI) source operated in the positive mode was used to identify the presence of the amino acid−AA adducts by direct infusion mass spectrometry. For semiquantitative determinations, aliquots of the samples (1 μL) were injected onto a Discovery HS C_{18} HPLC column (5 cm × 2.1 mm, 5 μm) (Supelco). The mobile phase consisted of 50:50 acetonitrile:water with 1% acetic acid, at a flow rate of 150 μL/min, with a total run time of 5 min. The mass spectrometer was operated at the following settings: capillary voltage of 4.0 kV, source temperature of 280 °C, desolvation temperature of 250 °C, and desolvation gas flow rate of 25 L/h with nitrogen and a helium gas pressure of 2.5 mbar used as the damping and collision activation partner in the mass analyzer cavity. The following ion transitions were used to monitor the formation of the amino acid−AA adducts: Cys-AA, *m*/*z* 193 to *m*/*z* 176; Pro-AA, *m*/*z* 187 to *m*/*z* 128; Try-AA, *m*/*z* 276 to *m*/*z* 188; Gly-AA, *m*/*z* 147 to *m*/*z* 130; and His-AA, *m*/*z* 227 to *m*/*z* 181. Analysis was carried out in duplicate.

Confirmation of the formation of the adducts was established by isotopic dilution reaction mixtures containing 25 μmol of acrylamide, 25 nmol of 1,2,3-$^{13}C_3$-acrylamide, and 25 μmol of the aforementioned amino acids. The samples were heated for 2 h at 160 °C, diluted to 10 mL with 1% formic acid in methanol, and analyzed by direct infusion mass spectrometry.

RESULTS AND DISCUSSION

Effect of Amino Acids on Acrylamide Formation. Acrylamide formation in sealed, low-moisture WMS systems heated at 160 °C was monitored over 60 min and for 10 amino acids, namely, aspartic acid, cysteine, alanine, glutamine, valine, leucine, tryptophan, proline, glycine, and phenylalanine. The effect of amino acids was investigated at two different concentrations, 25 and 75 mmol/kg, while the asparagine and glucose contents of the model systems were 25 and 50 mmol/kg, respectively. An equimolar (50 mmol/kg) asparagine−glucose model system was chosen as a reference, and the results were expressed as acrylamide yield (mmol/mol Asn) rather than net amounts for comparison purposes. The incorporation of other amino acids, with the exception of cysteine, glycine, and aspartic acid, at an equimolar level to asparagine, resulted in an increase of the final yields of acrylamide as compared to the reference system (**Table 1**). The initial rate of acrylamide formation (R_i), as evident from the acrylamide yields at the first 5 min of the reaction, was also greatly enhanced; approximately 2-fold for valine, leucine, and glutamine and by 30% for proline, phenylalanine, and alanine. The above is suggested to be an effect of the increased concentration of Maillard reaction intermediates and in particular glyoxal. An increase in the acrylamide yields as a result of added ammonia in the form of ammonium bicarbonate has been previously established, and mechanistic studies elucidated the importance of ammonia on accelerating sugar fragmentation and the formation of reactive carbonylic intermediates (*4*). Similar results were obtained in our model systems where added ammonia was in the form of amino acids.

Table 1. Acrylamide Yields in Model Systems Containing Asparagine (25 mmol/kg), Glucose (50 mmol/kg), and Amino Acid (25 or 75 mmol/kg)[a]

		heating time (min)					
		5	10	15	20	40	60
amino acid:Asn ratio		mmol AA per mol Asn					
Asn[b]		2.9	6.5	8.0	9.0	9.4	9.8
Gly		1.5	4.1	5.4	6.4	7.2	7.5
Cys		1.2	1.9	3.0	4.0	5.7	6.1
Asp		1.2	3.6	5.5	6.6	8.6	9.8
Try		2.6	6.9	9.0	10.0	11.3	11.5
Ala	1:1	3.8	7.3	8.3	9.7	10.7	11.1
Phe		3.8	7.7	8.7	10.4	11.9	11.8
Pro		3.9	8.2	10.2	11.2	12.8	11.8
Glu		6.3	9.9	10.7	11.7	12.0	11.1
Leu		6.6	10.1	10.4	11.6	12.1	12.5
Val		6.2	10.1	10.6	11.3	12.6	12.2
Gly		1.7	3.1	3.5	3.7	4.5	5.3
Cys		0.5	0.8	1.2	1.5	3.7	4.4
Asp		0.8	2.5	3.7	4.3	6.8	8.6
Try		3.6	5.9	6.5	1.9	2.2	2.2
Ala		5.4	6.9	8.5	8.9	10.4	10.5
Phe	3:1	4.5	6.5	5.6	7.9	7.7	8.5
Pro		1.4	1.9	2.1	2.3	2.5	2.5
Glu		7.0	7.5	7.5	8.2	8.5	7.2
Leu		6.7	8.0	9.1	9.3	10.0	10.7
Val		5.8	9.5	9.8	10.0	9.8	10.6

[a] The typical relative standard deviation calculated by the preparation and analysis of six different model systems (Asn-Gly-glucose) was 6%. All model systems were prepared and analyzed in duplicate. [b] 50 mmol/kg.

In previous investigations on asparagine−sugar WMS model systems (*14*), we showed that the tautomerization of the decarboxylated asparagine−carbonyl Schiff base is a key step in determining the relative yields of pyrazines and acrylamide and that the chemical identity of the carbonyl greatly influences these relative yields. In the above study, glyoxal promoted the formation of the Strecker amine (3-aminopropanamide) and subsequently acrylamide, while, in contrast, methylglyoxal promoted the formation of the Strecker aldehyde and 2,5-dimethylpyrazine. Moreover, acrylamide formation was highly correlated with the formation of pyrazines (up to 20 min of heating at 160 °C), while in

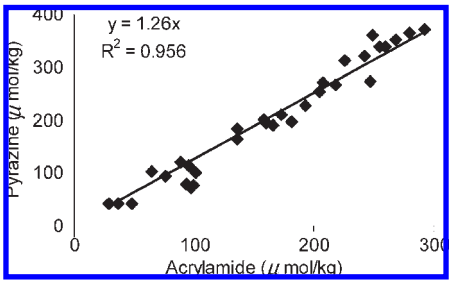

Figure 1. Formation of pyrazine and acrylamide in WMS model systems containing glucose (50 mmol/kg), asparagine (25 mmol/kg), and either cysteine, proline, tryptophan, aspartic acid, phenylalanine, glutamine, alanine, or glycine (25 mmol/kg) and heated up to 20 min at 160 °C.

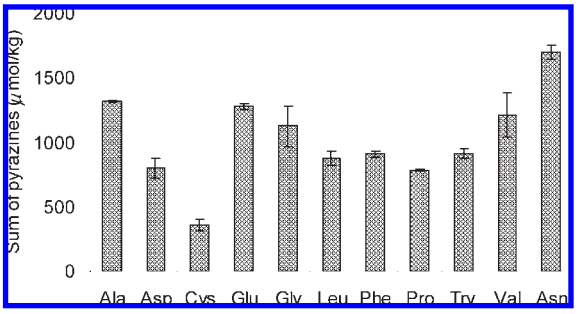

Figure 2. Formation of pyrazines (mean ± standard deviation, $n = 3$) in model systems containing glucose (50 mmol/kg), asparagine (25 mmol/kg), and a second amino acid (25 mmol/kg) and heated in sealed vials at 160 °C for 20 min. The sum of pyrazine, methyl pyrazine, 2,5-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2,3-dimethyl pyrazine, ethylpyrazine, 2-ethyl,6-methyl pyrazine, 2-ethyl,5-methyl pyrazine, and 2-ethyl,3-methyl pyrazine. The reference model system (Asn) with only asparagine and glucose contained 50 mmol/kg of the amino acid.

asparagine−glyoxal model systems, a strong correlation between the acrylamide and the unsubstituted pyrazine was also demonstrated.

In our current study, a strong correlation between acrylamide and the unsubstituted pyrazine was observed irrespective of the type of the additional amino acid, with the exception of valine and leucine (both resulting in slightly higher acrylamide to pyrazine ratios) (**Figure 1**), while correlations with other individual pyrazines (methyl and ethyl substituted) were not evident. Cysteine and glycine were the only effective amino acids in mitigating acrylamide at an equimolar concentration to asparagine (approximately a 40 and 20% reduction, respectively), while the reduced rate of formation in the model systems with added aspartic acid was mostly attributed to pH effects since the pH of this particular model system was 5.5 as compared to 5.9−6.2 for the other systems.

To further investigate the effect of the ratio of other amino acids to asparagine, model systems with an amino acid to Asn ratio of 3:1 at the same glucose concentration (50 mmol/kg) were heated at 160 °C. In these model systems, the effects of the added amino acids were diverse (**Table 1**). The final yields of acrylamide were all lower when the amino acid concentration was increased from 25 to 75 mmol/kg; however, the initial rates of formation, as evident from the acrylamide levels during the first 5 min of the heating period, showed a different pattern with phenylalanine, alanine, and tryptophan, increasing further the rate of acrylamide formation during the early stages of the reaction. Valine, leucine, and alanine resulted in particularly high acrylamide yields at the end of the heating period followed by aspartic acid, phenylalanine, and glutamine. In contrast, the secondary amine containing

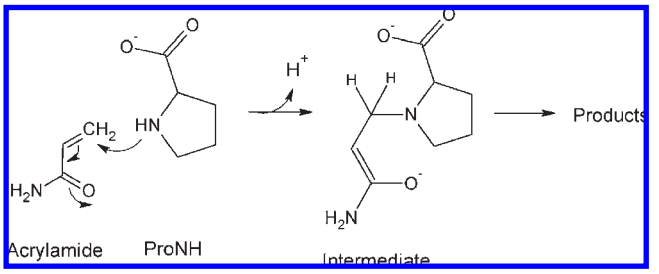

Figure 3. Michael addition reaction of acrylamide with proline.

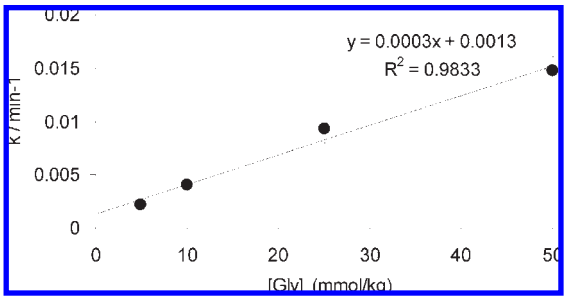

Figure 4. Loss of acrylamide at 160 °C in the presence of different concentrations of glycine.

amino acids, proline and tryptophan, resulted in the lowest final yields followed by cysteine and glycine. Quenching of tryptophan residues by acrylamide is used in modern analytical techniques in fluorescence studies elucidating the structure and function of proteins (*15*), and a noncovalent interaction with acrylamide has been proposed as the main mode of interaction with tryptophan residues of proteins.

We also report the significant mitigating effect of proline, which was the most potent amino acid in reducing the acrylamide levels in sealed low-moisture model systems. Proline is present at significant levels in both wheat and rye flour (*16*) and has been identified as a potent precursor of roasted bread aromas such as 2-acetyl-l-pyrroline and 2-acetyltetrahydropyridine (*17*). At increased concentrations, proline and tryptophan reduced the levels of acrylamide, after 60 min of heating, by approximately 80%, while cysteine and glycine by 55 and 45%, respectively.

Cysteine and glycine have been previously shown to reduce acrylamide levels (*9*, *13*), and results obtained from food systems were also verified in our model reactions. Reaction of the thiol groups of cysteine with α-dicarbonyls has been studied in relation to the formation of *S*-(carboxymethyl)cysteine (*18*). Cysteine has been shown to react with dicarbonyl compounds to form thiol−aldehyde adducts, which were detected by electrospray ionization mass spectrometry. In our investigations, the formation of both pyrazines (**Figure 2**) and acrylamide were suppressed when cysteine was used as the second amino acid, indicating that in the presence of cysteine, α-dicarbonyls were not available to react further with asparagine, which is in line with the reported carbonyl scavenging activity of cysteine (*15*, *18*). Moreover, the sulfhydryl group of cysteine has been implicated in alkylation reactions with acrylamide (*15*, *19*), while other acrylamide−amino acid adducts have also been identified and/or postulated (*10*). Of particular relevance to our results is the alkylation of the secondary amine containing amino acids such as tryptophan, proline, and histidine. The formation of histidine acrylamide adducts via alkylation of either the −NH or the imidazole nitrogen has been previously suggested (*15*). On the basis of our results, we propose the alkylation of the −NH group of both tryptophan and proline as shown in **Figure 3** for proline.

Table 2. Initial Rates, Yields of Acrylamide, and Incorporation of Isotopic Labeling in Pyrazines in Low-Moisture WMS Model Systems with Added Asparagine, Glucose, and ^{15}N-Glycine and Heated at 160 °C[a]

mmol/kg			mean ± SD (mmol/mol Asn/min)	mean ± SD (mmol/mol Asn)	^{15}N	
Glu	Asn	^{15}N-Gly	acrylamide (R_i)	AA yield (60 min)	pyrazine[b] (%)	methylpyrazine[b] (%)
25	25	0	0.47 ± 0.05 c	12.0 ± 0.8 d		
	25	25	0.26 ± 0.02 a	6.2 ± 0.17 a	41	41
	25	50	0.20 ± 0.01 b	5.0 ± 0.05 e	63	54
	50	25	0.26 ± 0.02 a	5.8 ± 0.01 a,b	22	26
	50	50	0.22 ± 0.01 b	4.0 ± 0.20 c	42	38
50	50	0	0.61 ± 0.04 e	9.8 ± 0.01 f		
	25	25	0.42 ± 0.04 c	5.8 ± 0.03 a,b	41	42
	25	50	0.30 ± 0.01 d	3.8 ± 0.09 c	62	55
	50	25	0.46 ± 0.03 c	7.0 ± 0.11 g	20	26
	50	50	0.34 ± 0.02 d	5.4 ± 0.04 b	41	39

[a] Values with the same superscript letter across each column indicate no significant difference at $P \geq 0.05$ ($n = 3$). [b] Calculated at the initial 5 min of the reaction.

Effect of Glycine on Acrylamide Mitigation and Pyrazine Formation. The effect of glycine on acrylamide in the absence of any other interfering compounds was studied in WMS model systems containing acrylamide at a level within the range typically found in foods. In unsealed WMS model systems with only acrylamide added (25 μmol/kg), evaporation and polymerization phenomena at 160 °C were minimal with the final content after 60 min of heating being 23.4 ± 0.4 μmol/kg. The rate of acrylamide loss at 160 °C in the presence of glycine (5, 10, 25, and 50 mmol/kg) was of first order with respect to the concentration of glycine (**Figure 4**), resulting in 10, 25, 43, and 60% reduction in the acrylamide content of the model systems after 60 min of heating. Noteworthy is the fact that glycine was always in excess with the lowest concentration corresponding to a 200-fold excess. Moreover, increasing the glycine concentration from 25 to 50 mmol/kg led to only a further 17% reduction in the acrylamide content of the systems, indicating that the concentration of acrylamide was becoming limiting. In food model systems, acrylamide levels were shown to be influenced by the relative ratio of asparagine to other amino acids (*16*), while the overall loss of acrylamide was influenced by the levels of acrylamide (*20*), which also suggests a concentration-dependent reaction (*5*). The implication of glycine in the Maillard reaction in potato cakes as a function of both flavor formation and acrylamide mitigation (*8*) showed that increasing the glycine concentration by approximately 3-fold (from 52 to 173 mmol/kg dry weight) yielded an approximately 2-fold decrease in acrylamide levels. Moreover, the formation of Strecker aldehydes from various amino acids decreased, significantly indicating the competing effect of glycine for carbonyl intermediates, while on average the total pyrazine content increased with individual pyrazines showing different trends.

To further investigate the competing effect of glycine for reactive α-dicarbonyls, with particular reference to glyoxal, we monitored the formation of pyrazine and methylpyrazine in WMS model reaction systems containing asparagine, glucose, and ^{15}N-glycine at two concentration levels and at eight different combinations. As expected, glycine participated in the formation of the unsubstituted pyrazine, indicating the competition for glyoxal. The ratio of the labeled to the single-labeled pyrazine was found to be dependent on the ratio of glycine to asparagine and was not influenced by the amount of glucose in the systems. In particular, the ratios of the unlabeled to the single-labeled pyrazine were 0.29 ± 0.03, 0.67 ± 0.07, and 1.26 ± 0.08 for the corresponding asparagine glycine ratios of 0.5, 1, and 2, while almost identical results were obtained when the concentration of glucose was doubled to 50 mmol/kg. This leads to the suggestion that the rate of formation of the amino carbonyls deriving from either ^{15}N-glycine or asparagine was the same throughout the heating period and was only influenced by the relative reactivities of the amino acids and the ratio of their initial concentration. An estimate of the relative reactivity of glycine and asparagine toward pyrazine formation was established by calculating the percentage of ^{15}N incorporation in both pyrazine and methylpyrazine throughout the heating process. There were only marginal differences in the ^{15}N (%) content of both pyrazine and methylpyrazine between the initial stages of the reaction and prolonged heating times (20 min). In particular, the % incorporation of labeling was decreased by approximately 10% irrespective of the relative concentration of the amino acids, indicating that glycine was only marginally more reactive, hence consumed faster, in the initial stages of the reaction and/or the above pyrazines were being formed via other pathways, such as the reaction of glyoxal and/or methylglyoxal with ammonia liberated by the deamidation/deamination of asparagine. As illustrated in **Table 2**, although the % incorporation of labeling in the pyrazines was not affected by the levels of glucose in the system, acrylamide formation showed a different pattern. In particular, an interaction between the sugar content and the ratio of amino acids was observed with glycine being more effective in mitigating acrylamide formation at lower sugar content. Thus, on the basis of the above results, it is suggested that the main mode of action of glycine in acrylamide mitigation in low-moisture systems would be via the competing effect for available α-dicarbonyls and in particular glyoxal rather than its participation in alkylation reactions, while other amino acids such as cysteine, proline, tryptophan, and possibly histidine would promote alkylation reactions significantly more due to the increased nucleophilicity of the sulfhydryl group in the case of cysteine and the secondary amine in the case of proline, tryptophan, and histidine.

Relative Amino Acid−AA Formation Rates in Aqueous Model Systems. To further elucidate the relative reactivity of different amino acids toward acrylamide, aqueous model systems of individual amino acids and acrylamide were heated at 160 °C, and the formation of the adducts was monitored by LC-MS/MS over 2 h. An estimate of the relative reactivity of amino acids was obtained by expressing the peak area of selected ion transitions of individual amino acid−AA adducts as the percentage of their peak area at 120 min (**Figure 5**). The presence of the amino acid−AA adducts was further confirmed by mass spectrometry of isotopically diluted reaction mixtures containing both acrylamide and 1,2,3-$^{13}C_3$-acrylamide. Cysteine was the most potent amino acid in forming the Michael addition product of acrylamide in the absence of asparagine and reducing sugars with approximately 50% of the adduct being formed during the first 20 min of the reaction. In contrast, histidine was far less reactive, although a remarkable change in the formation rate was observed after all of

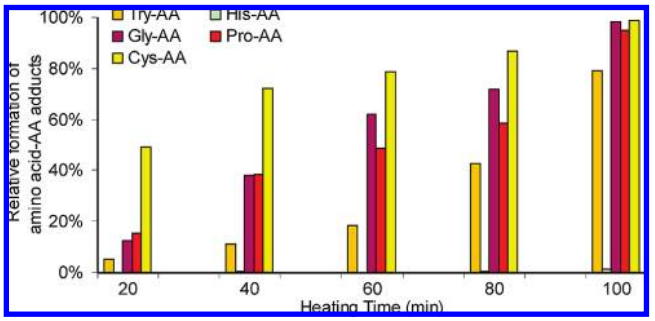

Figure 5. Relative formation of amino acid−AA adducts in aqueous model systems containing 25 μmol of proline, cysteine, tryptophan, histidine, or glycine and 25 μmol of acrylamide and heated at 160 °C for 120 min. The *y*-axis represents the percentage of adduct formed relative to the peak area at 120 min (n = 2).

the water in the system had evaporated (approximately 100 min). Glycine and proline yielded similar results with glycine being slightly more reactive at the intermediate stages of the reaction.

The above contrasts the findings reported earlier on the glucose−asparagine model systems when proline, at increased concentrations, was found to be much more effective in mitigating acrylamide. However, it should be noted that the model systems used in this case were aqueous, and both concentration and temperature effects are likely to have interfered. This is further supported by the fact that all of the secondary amine-containing amino acids were particularly more reactive when most of the water in the systems had evaporated.

Further studies on the formation of amino acid−AA adducts in real food systems would help to identify whether modulation of the amino acid content of bakery products to mitigate acrylamide formation, either through agronomic or processing interventions, presents a realistic opportunity for the food industry. These efforts could particularly address the effect of proline on the acrylamide content and flavor profile of bakery foodstuffs due to both the significant mitigating effect on acrylamide illustrated in this present study and the heat-induced generation of important, proline-derived, character impact compounds such as 2-acetyl-L-pyrroline and 2-acetyltetrahydropyridine. Although numerous investigations have studied the effect of amino acids on acrylamide, an optimized approach having a limited impact on the flavor and color profile of foodstuffs is still to be commercially explored.

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NOTE ADDED AFTER ASAP PUBLICATION

After this paper was published ASAP September 9, 2009, a correction was made to Table 1; the corrected version was reposted September 15, 2009.

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