JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Acrylamide and Pyrazine Formation in Model Systems Containing Asparagine

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The effect of different sugars and glyoxal on the formation of acrylamide in low-moisture starch-based model systems was studied, and kinetic data were obtained. Glucose was more effective than fructose, tagatose, or maltose in acrylamide formation, whereas the importance of glyoxal as a key sugar fragmentation intermediate was confirmed. Glyoxal formation was greater in model systems containing asparagine and glucose rather than fructose. A solid phase microextraction GC-MS method was employed to determine quantitatively the formation of pyrazines in model reaction systems. Substituted pyrazine formation was more evident in model systems containing fructose; however, the unsubstituted homologue, which was the only pyrazine identified in the headspace of glyoxal−asparagine systems, was formed at higher yields when aldoses were used as the reducing sugar. Highly significant correlations were obtained for the relationship between pyrazine and acrylamide formation. The importance of the tautomerization of the asparagine−carbonyl decarboxylated Schiff base in the relative yields of pyrazines and acrylamide is discussed.

KEYWORDS: Acrylamide; Maillard reaction; pyrazines; solid phase microextraction; glyoxal

INTRODUCTION

The formation of acrylamide, a neurotoxic compound and possible carcinogen, in heated foodstuffs has been an issue of ongoing investigations for the past 5 years. Asparagine, through its participation in the Maillard reaction, has been identified as the major precursor of acrylamide (*1, 2*), and heat-treated products containing relatively high amounts of asparagine have been shown to yield correspondingly high acrylamide concentrations (*3*). The levels of acrylamide in foodstuffs and/or in model systems were also shown to depend on pH (*4*), the temperature and duration of heat treatment (*5, 6*), the presence of food additives (*7, 8*), initial moisture (*9, 10*), and sugar content (*11, 12*).

Acrylamide formation has been studied in relation with other Maillard reaction products that are often used as product quality indicators. Monitoring of flavor volatiles has been the subject of investigations on acrylamide mitigation in food model systems using citric acid and/or glycine (*13*). Investigations on color and acrylamide formation have led to highly significant correlations (*14*) illustrating the relationship between the formation of melanoidins and acrylamide, whereas, in aqueous model systems, highly significant correlations between acrylamide and pyrazines were also observed (*15*). Such correlations illustrate that acrylamide formation is interrelated with quality attributes of heated foodstuffs that derive via the involvement of common Maillard reaction intermediates.

Although the participation of asparagine in acrylamide formation is well established, the relative importance of different sugars and/or carbonyls is still a matter of debate, and there is a plethora of quite often contradictory information, which is mostly attributed to the type of model system as well as the conditions employed. It is suggested that both molecular mobility and sugar reactivity would determine the relative effect of sugars on acrylamide formation (*16*), whereas temperature may also play an important role in determining these relative reactivities as discussed by Becalski et al. (*17*). Kinetic approaches have much to offer in studying acrylamide formation because they provide a better understanding of the processes involved, especially when complemented with data on the formation of intermediates and/or Maillard reaction end products. This study was carried out to elucidate some of the effects of sugars, glyoxal, and methylglyoxal on the kinetics of formation of both acrylamide and flavor volatiles, namely, pyrazines, in low-moisture, starch-based model reaction systems.

EXPERIMENTAL PROCEDURES

Materials. Asparagine, glucose, fructose, maltose, and maleimide were purchased from Sigma-Aldrich Co., Ltd. (Poole, Dorset, U.K.) and were of ≥99% purity. Hexane (GC grade), glyoxal trimer dihydrate (≥97%), methylglyoxal (5.5 M), hydrochloric acid (10 M), pentafluorobenzyl hydroxylamine hydrochloride (PFBHA), 2-fluorobenzaldehyde

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10.1021/jf703744k CCC: $40.75
Published on Web 07/15/2008

(≥98%), 1,2-dichlorobenzene (99%), ethyl acetate (≥99.9%), sodium thiosulfate, sodium sulfate, potassium bromide, and bromine were also purchased from Sigma-Aldrich. HPLC-grade methanol was purchased from Fischer Scientific (Loughborough, Leicestershire, U.K.). ($^{13}C_3$)Acrylamide (99% isotopic content) was purchased from Cambridge Isotopes Laboratories Inc. (Andover, MA). All pyrazine reference compounds were purchased from Sigma-Aldrich, and they were of ≥98% purity. Waxy maize starch (WMS) was obtained from Roquette S.p.A (Cassano Spinola, Italy). The bromination reagent (1 L) was prepared with potassium bromide (400 g), hydrobromic acid (20 mL), saturated bromine solution (320 mL), and deionized water.

Model System Preparation and Heating Regimen. A suspension of WMS in deionized water (5 g/100 mL) with added reactants in solution was heated in a shaking water bath (90 °C). The resulting homogeneous slurry (pH 6.1) was rapidly cooled, and aliquots (6−8 g) were transferred to 18 mL SPME vials (Chromacol Ltd., Welwyn Garden City, U.K.) and frozen at −18 °C overnight before freeze-drying to a final moisture content of 4−6%. Model systems with added sugars and asparagine were heated in a GC oven in sealed SPME vials with metal caps and 1.5 mm silicone/PTFE septa. The initial oven temperature was held at 220 °C for the first 50 s and then set to 160 °C for the remaining of the heating period. This procedure assured a rapid heat transfer with samples reaching the thermal equilibrium in approximately 2 min. Samples were heated for 2.5−60 min and then rapidly cooled in an ice bath. Model systems with added glyoxal were sealed and heated at 80, 100, 120, and 160 °C, whereas in all cases the heating regimen was standardized so that the samples reached the desired thermal equilibrium in approximately 2 min. Wet model systems with added WMS (0.2 g), glucose or fructose (50 μL, 100 mmol/L), asparagine (50 μL, 100 mmol/L), and distilled water (100 μL) were also prepared, vortex mixed, and heated at 160 °C in unsealed vials as described previously.

In Situ Trapping of Carbonyls and Determination of Glyoxal. Aliquots of WMS (0.2 g), glucose or fructose (50 μL, 50 mmol/L), asparagine (50 μL, 50 mmol/L) or distilled water (50 μL), and PFBHA (100 μL, 20 mg/mL) were added into the SPME vials, vortex mixed, sealed, and heated for up to 15 min in a GC oven at 160 °C. Model systems were prepared in triplicate. After cooling, fluorobenzaldehyde [1 mL of 1 μg/mL solution in 1:1 (v/v) aqueous methanol], HPLC water (5 mL), and PFBHA (0.5 mL, 20 mg/mL) were added to the matrices, and the samples were vortex mixed and left to further derivatize at ambient temperature overnight. The pentafluorobenzyl derivatives were extracted by adding 1 drop of HCl (10 M) followed by washing with 2 mL of hexane and concentration of the organic phase to approximately 0.5 mL. GC-MS analysis was performed on a Varian CP-3800 coupled to a Saturn 2000 mass spectrometer (Varian, Palo Alto, CA). Aliquots of the extracts (1 μL) were injected onto a DB 17 MS (30 m × 0.25 mm i.d. × 0.15 μm film thickness) in the split mode (20:1) at 250 °C. The initial temperature of the oven was set at 120 °C followed by an increase at 8 °C/min to 190 °C and then at 15 °C/min to 300 °C. The MS was operated in the EI mode with the ion trap and transfer line temperature set at 200 and 260 °C, respectively. The glyoxal and methylglyoxal content of freeze-dried samples containing carbonyls was also determined using the above protocol to account for any losses during sample preparation. Values presented in the paper indicate the amount of carbonyls in the freeze-dried material.

Extraction and Determination of Acrylamide. Aliquots of the pulverized matrices (0.2−0.3 g) were accurately weighed, ($^{13}C_3$)-acrylamide (500 μL, 2.4 μg/mL in 0.2 mmol/L formic acid) and deionized water (7 mL) were added, and the samples were stirred for 25 min. The extracts were then centrifuged (10000 rpm, 15 min), the solid residues were re-extracted with aqueous methanol (6 mL, 2:1 v/v), and the combined extracts were derivatized with the bromination reagent (5 mL) overnight before neutralization with sodium thiosulfate (3−4 drops, 1 M). The samples were subsequently extracted with ethyl acetate (2 × 4 mL) and concentrated over anhydrous sodium sulfate in a vacuum evaporator to approximately 1 mL. Aliquots of the ethyl acetate extracts (2 μL) were injected onto a DB 17 MS (30 m × 0.25 mm i.d. × 0.15 μm film thickness) in splitless mode at 250 °C; the splitter opened after 60 s. A pressure pulse of 25 psi was employed for the first 60 s of the run with a helium flow rate of 1 mL/min at 50 °C. The oven temperature was set at 50 °C for 2 min and 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, whereas the transfer line and ion trap temperatures were 260 and 200 °C, respectively. The ion *m/z* 155 was used to quantify brominated ($^{13}C_3$)acrylamide, and the ions *m/z* 150 and 152 were used to quantify brominated acrylamide. Maleimide was also identified in the ethyl acetate extracts and was quantified by comparison of the molecular ion (*m/z* 97) with the brominated ($^{13}C_3$)acrylamide internal standard as described above. All analyses were performed in duplicate.

Table 1. Method Validation Data for the Determination of Pyrazines by HS-SPME

compound	LRI[a]	*m/z*[b]	LOD[c] (μmol/kg)	range of linearity[d] (μmol/kg)
pyrazine	1230	80	1	3.0−2000
methyl-	1283	94	1	3.0−2000
2,5-dimethyl-	1339	108	0.2	0.5−1500
2,6-dimethyl-	1345	108	0.2	0.5−1500
ethyl-	1350	107	0.2	0.5−1500
2,3-dimethyl-	1364	108	0.2	0.5−1500
2-ethyl-6-methyl-	1400	121	0.2	0.5−1500
2-ethyl-5-methyl-	1407	121	0.2	0.5−1500
2-ethyl-3-methyl-	1420	121	0.2	0.5−1500
acetyl-	1651	94	2	3.0−1500

[a] Calculated linear retention index values. [b] *m/z* used for quantitation. [c] Limit of detection (signal-to-noise ratio of 3:1) based on 0.1 g of sample weight. [d] Lower and upper limits based on 0.1 and 0.05 g of sample weight, respectively. Lower limit of linearity set at 5:1 signal-to-noise ratio.

Determination of Pyrazines. Aliquots (0.05−0.1 g) of finely ground samples were accurately weighed in 18 mL SPME vials, 1,2-dichlorobenzene [50 μL of 1.2 μg/mL solution in 1:1 (v/v) aqueous methanol] and 2 mL of a sodium chloride solution (4.3 M) were 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. Headspace SPME (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. Chromatography was performed on a DB-WAXetr (60 m × 0.25 mm i.d. × 0.25 μm film thickness), with the initial oven temperature set at 40 °C, held for 5 min, and increased to 200 °C at 3 °C/min and finally to 240 °C at 8 °C/min. The external calibration method was used for quantification, in which aliquots (50−100 μL) of dilutions of a mixture of pyrazines, dissolved in 1:1 (v/v) aqueous methanol, were analyzed by SPME in the presence of 0.1 g of the finely ground unheated matrix and 2 mL of sodium chloride solution. Analysis was carried out in triplicate, and calibration curves were prepared for each batch of samples. Selected ions were used for quantification of the individual components (**Table 1**).

RESULTS AND DISCUSSION

Development of an HS-SPME Method for the Quantitative Determination of Pyrazines in Model Systems. The potential of SPME to obtain quantitative results on the formation of pyrazines in WMS model reaction systems containing sugars and amino acids was explored. Parameters investigated included temperature and time of incubation, extraction and desorption time, sample weight, type of fiber, and effect of methanol and sodium chloride. The final parameter settings are reported under Experimental Procedures. Validation of the SPME method for the quantification of pyrazines formed in WMS reaction model systems containing sugars and amino acids revealed relative standard deviations of 0.7−13% and low limits of detection (0.2−2 μmol/kg based on 0.1 g of sample weight) for individual compounds (**Table 1**). Moreover, there was a linear relationship between sample weight (0.01−0.1 g) and the peak areas of pyrazines, which indicated that less sample could be used for

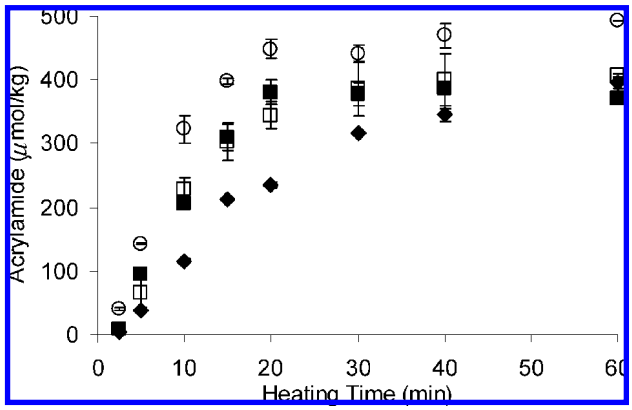

Figure 1. Formation of acrylamide at 160 °C in sealed, low-moisture WMS model systems containing equimolar amounts of asparagine and sugar (50 μmol/g); glucose (○), fructose (■), tagatose (□), maltose (◆).

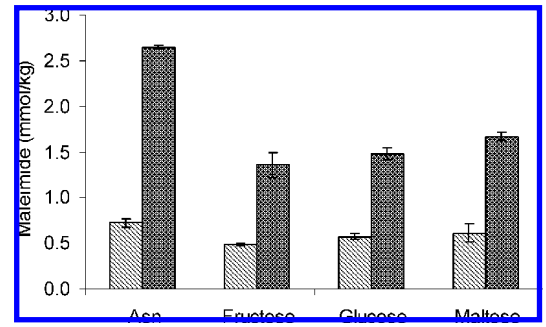

Figure 2. Formation of maleimide in asparagine−sugar (50 μmol/g, equimolar) WMS model systems heated at 160 °C for 5 min (lighter bars) and 15 min (darker bars).

model systems with high pyrazine content, thus extending the upper limit of linearity without affecting the partitioning of the compounds in the headspace. Trimethylpyrazine was found to coelute with 2-ethyl-3-methylpyrazine using the chromatographic conditions employed, and differentiation of the two was achieved by using *m/z* 121 for the quantification of the latter pyrazine.

Effect of Sugars on Acrylamide Formation. Different kinetic behaviors were observed in model systems containing different sugars with respect to acrylamide formation (**Figure 1**). Glucose was more effective than fructose, tagatose, or maltose, leading to higher formation rates and final yields, the initial acrylamide formation rates being 38.4 ± 0.5, 27.8 ± 0.04, 26.2 ± 0.3, and 14.2 ± 0.6 μmol kg^{-1} min^{-1} for glucose, fructose, tagatose, and maltose, respectively. Fructose and tagatose, an isomer of fructose, resulted in almost identical yields of acrylamide throughout the heating period (2.5−60 min), whereas maltose, despite being initially much less reactive, resulted in final acrylamide yields similar to those provided by the ketoses.

The significant correlations found between sugar and acrylamide content (*12, 18*), as well as the higher acrylamide yields of asparagine glycoconjugates compared to binary mixtures of asparagine and sugars or carbonyls (*19*), suggest that the formation of the Schiff base could be rate limiting. To further explore this suggestion, we attempted to monitor the formation of maleimide, a decomposition product of asparagine deriving through an intramolecular cyclization reaction that prevents the formation of the Schiff base leading to acrylamide at high temperatures (*20*). Maleimide was identified in the present study in model systems heated at 160 °C, and semiquantitative data were obtained throughout the heating period (2.5−60 min). Quantitatively, the formation of maleimide was investigated at two different time points and in model systems with added asparagine (50 μmol/g of WMS) either alone or in the presence of fructose, glucose, or maltose (50 μmol/g of WMS) (**Figure 2**). At the initial stages of the reaction (5 min), the yields of maleimide were significantly different ($p < 0.05$) in the fructose−asparagine and asparagine-only model systems. At 15 min, and in the absence of sugars, maleimide corresponded to approximately 5% (molar ratio) of the initial asparagine concentration, whereas in the presence of either glucose or fructose the maleimide yields were significantly lower with no significant differences between the two sugars. However, when maltose was used as the carbonyl source, the yields of maleimide were significantly higher ($p < 0.05$) compared to the monosaccharides, although, quantitatively, the differences were small. These results suggest that, at the conditions employed, the competing reaction of the formation of maleimide could not explain the differences in the acrylamide formation potential between glucose and fructose, whereas it could partially explain the lower amounts of acrylamide formed in the model systems with added maltose.

In an attempt to investigate the participation of sugar fragmentation products in the relative reactivities of sugars toward acrylamide formation, wet model WMS systems (50% moisture) with added glucose or fructose (12.5 μmol/g of dry weight) and with or without asparagine (12.5 μmol/g of dry weight) were heated at 160 °C in the presence of PFBHA, a carbonyl trapping agent. Model systems with only the sugar and in the presence of PFBHA revealed the formation of trace amounts of glyoxal (0.2 mmol/mol of sugar after 12.5 min), and there were no significant differences between the two sugars. Glyoxal formation was much greater in model systems containing glucose or fructose and asparagine. The rate of glyoxal formation in model systems containing glucose, in the presence of the amino acid, was approximately 3 times faster compared to fructose, the yields after 15 min being 9.3 and 4.0 mmol/mol of sugar for the glucose and fructose model systems, respectively (**Figure 3a**). This contrasts with the study of Amrein et al. (*8*), who reported that both sugars resulted in similar amounts of glyoxal in aqueous model systems heated at 120 °C for 30 min. However, the type of model system as well as the temperatures employed could influence the relative glyoxal yields. A discrepancy in the general trend found at 2.5 min (**Figure 3a**) could further support this suggestion because the temperature of the wet systems may have been significantly lower at this early stage of the reaction due to water evaporation. Acrylamide formation in unsealed asparagine−glucose or asparagine−fructose (25 μmol/g of dry weight, equimolar) model systems of the same water content (50%) without the incorporation of the derivatization reagent revealed that glucose was almost twice as reactive as fructose, but only after the first 10 min of heating (**Figure 3b**).

To evaluate the effect of evaporation of volatile reactive intermediates, low-moisture model systems with fructose or glucose and asparagine (50 μmol/g, equimolar) were heated in unsealed SPME vials. In both systems the initial rate of acrylamide formation (2.5−15 min) was 1.5 times lower compared to the corresponding sealed low-moisture systems. However, in the glucose−asparagine reactions the maximum yields (60 min) were similar, whereas when fructose was used as the reducing sugar, the final yields decreased by 30%, suggesting that the sugar fragmentation intermediates formed from fructose were either more volatile and/or less reactive toward acrylamide formation.

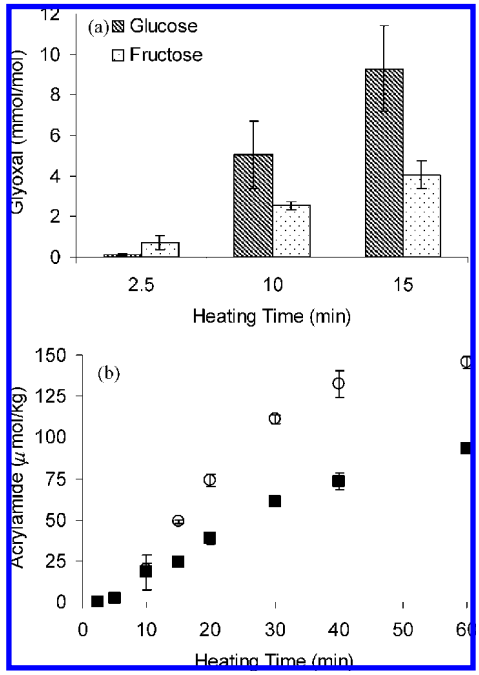

Figure 3. (**a**) Formation of glyoxal at 160 °C in sealed (50% moisture) model systems containing equimolar amounts (12.5 μmol/g of dry weight) of asparagine and either glucose or fructose ($n = 3$). (**b**) Formation of acrylamide at 160 °C in equimolar (25 μmol/g of dry weight) glucose−asparagine (○) or fructose−asparagine (■) wet (50% moisture) model systems heated in unsealed vials ($n = 2$).

Table 2. Formation of Acrylamide (AA) in Glyoxal−Asparagine Model Systems

Asn[a]	glyoxal[a]	temperature (°C)	time (min)	AA (μmol/kg)	yield[b]
50	150	80	120	5.2	0.10
50	150	100	120	29.9	0.60
25	7.5	160	5	30.4	1.22
25	7.5	160	10	39.4	1.58
25	7.5	160	20	33.4	1.33
25	75	160	5	142.8	5.70
25	75	160	10	156.6	6.26
25	75	160	20	124.7	4.99

[a] Reactant concentration in micromoles per gram. [b] Millimoles of AA per mole of Asn.

Acrylamide Formation in Glyoxal−Asparagine Model Systems. The importance of glyoxal as a key dicarbonyl implicated in acrylamide formation has been previously established (*8, 21*). Model systems with added glyoxal and asparagine were heated at various temperatures to obtain an estimate of the extent of the reactivity of glyoxal (**Table 2**). Acrylamide formation was evident at temperatures as low as 80 °C, although the yields were very low and the heating time had to exceed 90 min for detectable amounts to be obtained. At 160 °C acrylamide formation was instant, reaching a maximum at 10 min, whereas there was some decline thereafter. Kinetic data for the glyoxal−asparagine model systems were obtained at 120 °C and at three different concentrations of glyoxal monomer (7.5, 15, and 30 μmol/g of WMS), whereas the asparagine concentration remained constant at 25 μmol/g of WMS (**Figure 4**). At this temperature the formation of acrylamide was linear with time, and there was a strong dependence on the amount of glyoxal added ($R^2 = 0.992$). The yields of acrylamide after 80 min of heating at 120 °C were 2.2, 4.0, and 7.6 mmol/mol of Asn, for glyoxal concentrations of 7.5, 15, and 30 μmol/g, respectively. Experiments in low-moisture WMS model systems with added methylglyoxal (5.5, 9.5, or 18.7 μmol/g) and asparagine (25 μmol/g) were also conducted. However, the relative yields of acrylamide were low (0.37−0.59 mmol/mol of Asn after 80 min at 120 °C), whereas a correlation with the initial methylglyoxal concentration was not as evident. Amrein et al. (*8*) reported acrylamide yields of 3.70 and 0.03 mmol/mol of Asn in the respective asparagine−glyoxal or asparagine−methylglyoxal aqueous solutions (pH 7.0, 25 mM, equimolar) heated at 120 °C for 10 min, which is in agreement with the findings of our study.

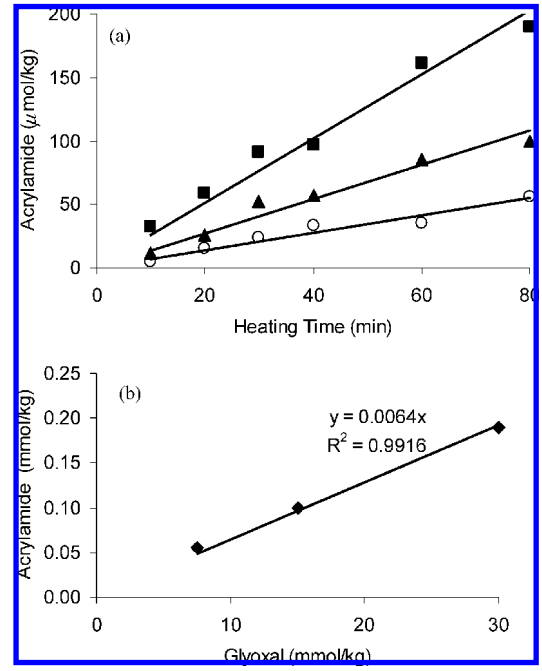

Figure 4. (**a**) Kinetics of acrylamide formation in model systems containing asparagine (25 μmol/g) and glyoxal [7.5 (○), 15 (▲) and 30 (■) μmol/g)] heated at 120 °C ($n = 2$). (**b**) Correlation of acrylamide formation at 80 min with initial glyoxal concentration.

Pyrazine Formation and Correlation with Acrylamide. As expected, the unsubstituted homologue was the only pyrazine identified in the headspace of model systems containing glyoxal and asparagine, heated at 120 °C. A strong correlation between the formation of acrylamide and pyrazine was observed, irrespective of the concentration of glyoxal, with an acrylamide to pyrazine molar ratio of approximately 1:2 (**Figure 5a**). However, when glyoxal was replaced with methylglyoxal, the formation of pyrazines was much favored compared to the formation of acrylamide, with yields (80 min) of 32.9−47.6 mmol/mol of Asn, whereas 2,5-dimethylpyrazine was the major pyrazine formed (**Figure 5b**).

Granvogl et al. (*22*), in reaction systems with added methylglyoxal and various amino acids, showed that both the Strecker amine and the Strecker aldehyde are formed; however, the yields of the aldehyde were always much higher compared to the amine. The results obtained in our experiment suggest that, when glyoxal is used as the carbonyl source, the tautomerization of the decarboxylated Schiff base leading to the formation of the Strecker amine of asparagine, 3-aminopropanamide (3-APA), and acrylamide is greatly favored (four molecules of the α-aminoketone are formed for every molecule of acrylamide). In contrast, in the case of methylglyoxal, formation of the tautomer is not favored and the reaction shifts toward the Strecker aldehyde route, which is in agreement with the findings of Granvogl et al. (*22*). However, as discussed later, glyoxal could also form the α-aminoketone by directly reacting with

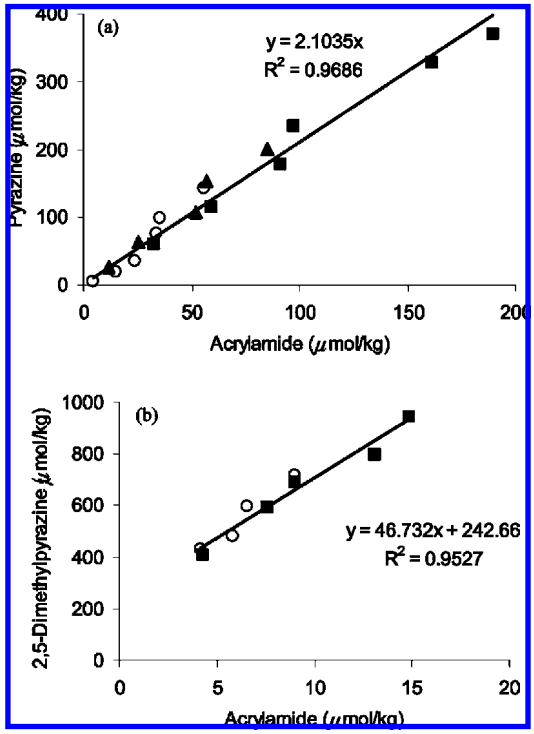

Figure 5. Correlation of acrylamide with pyrazine or 2,5-dimethylpyrazine formation in sealed model systems heated at 120 °C (10−80 min) containing asparagine (25 μmol/g) and (**a**) glyoxal [7.5 (○), 15 (▲), and 30 (■) μmol/g] or (**b**) methylglyoxal [5.5 (○) and 9.5 (■) μmol/g].

ammonia released from either the deamination of 3-aminopropanamide or the deamidation/deamination of asparagine. Therefore, a series of experiments using isotopically labeled asparagine would be required to prove whether both the amine and amide nitrogens were incorporated in the pyrazine molecule and to what extend, so that stoichiometric calculations could be further validated.

Determination of the residual amounts of α-dicarbonyls in the heated asparagine−glyoxal and asparagine−methylglyoxal matrices revealed that after 80 min of heating, 12% of the initial glyoxal (7.5 μmol/g) was still present in the matrix, whereas residual methylglyoxal corresponded to only 1% of its initial concentration (5.5 μmol/g). Similar differences were also obtained for the model systems with higher initial α-dicarbonyl concentrations. Hydrolysis of the tautomer leading to the formation of the Strecker amine regenerates the carbonyl (*22*), whereas preferential formation of the Strecker aldehyde and pyrazines, such as in the case of methylglyoxal (**Figure 5b**), would lead to a much faster decrease in the carbonyl pool. Therefore, the concentration of carbonyls and asparagine, relative to the formation of acrylamide, would be inherently more likely to be limiting in systems with high methylglyoxal/glyoxal concentration ratios because a large proportion of both the amino acid and the carbonyl would be consumed via the Strecker aldehyde route. This is suggested to be even more profound in unsealed model systems where evaporation effects would add to the overall depletion of carbonyls. Thus, the above implications could partly explain the differences in acrylamide yields between sealed and unsealed systems as well as between different sugars observed in this study.

HS-SPME analysis of the heated reaction matrices containing sugars and asparagine revealed the presence of a number of pyrazines including pyrazine, methylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, and acetylpyrazine. Most of these pyrazines have been previously identified in model systems containing asparagine and glucose (*15, 23*) with acetylpyrazine being the exception. Alkylpyrazines are formed via the condensation of two α-amino carbonyls, which are the products of the transamination of dicarbonyls or hydroxyl carbonyls formed in the Strecker degradation of amino acids (*24*). The presence of the above pyrazines suggests that C_2 dicarbonyls (glyoxal), C_3 dicarbonyls (methylglyoxal), and C_4 dicarbonyls were all formed as sugar fragmentation products. The relative proportions of the dicarbonyls produced are reflected in the relative amounts of the pyrazines found, and these were greatly influenced by the type of sugar (**Table 3**). Model systems with added aldoses, namely, glucose and its disaccharide maltose, resulted in particularly high yields of the unsubstituted pyrazine, which is formed from the α-amino carbonyl from glyoxal (*24, 25*). As discussed above, glyoxal was formed more readily from glucose than from fructose.

The rates of formation of pyrazines and acrylamide in model systems with added maltose were lower than for glucose. The formation of the Amadori compound in maltose−glycine model systems has been found to be slower than the corresponding systems with added glucose (*26*), implying the reduced reactivity of the disaccharide. This suggestion was further supported in our study by the increased amounts of maleimide found in the maltose−asparagine compared to the monosaccharide model systems. It is also interesting to observe that, in the maltose−asparagine systems, the yield of the unsubstituted pyrazine (1195 μmol/kg) was >60% of the total pyrazines formed during 60 min of heating, and this was greater than the other sugars. This implies that maltose produces more C_2 (glyoxal) and fewer C_3 and C_4 dicarbonyls. Nevertheless, the total pyrazine and acrylamide formation rates were lower for maltose than for the other sugars because overall there were fewer carbonyls available to react with asparagine. The formation of the unsubstituted pyrazine through glyoxal could arise from routes other than the Strecker degradation pathway (*27*). Binary mixtures of glucose and ammonium chloride were shown to yield low amounts of pyrazines (*23*); however, the unsubstituted homologue corresponded to approximately 75% of the total pyrazines formed. In our laboratory, dry model systems with added glyoxal and ammonium bicarbonate, heated at 160 °C in sealed vials, also yielded the unsubstituted homologue. Moreover, experiments using isotopically labeled glutamine (^{15}N amide) have shown that more than half of the amounts of pyrazines are derived through deamidation (*28*). Therefore, the presence of high amounts of the unsubstituted pyrazine in both the glucose and maltose model systems could be partly attributed to the reaction of glyoxal with ammonia released from asparagine deamidation and/or deamination processes (*29*). This could be especially true in the case of maltose, because the reduced reactivity of the disaccharide will favor the parallel deamination and dehydration of asparagine, leading to the formation of maleimide. Another possible pathway could be via a retro-aldol type reaction of the imine formed from glucose and ammonia as proposed by Amrein et al. (*8*), where instead of being oxidized, the enaminol intermediate could form the α-amino carbonyl, leading to pyrazine.

Fructose resulted in the formation of higher quantities of pyrazines compared to glucose after 60 min of heating at 160 °C, although this was not the case at shorter heating times (<20 min). Methylpyrazine and the 2,5- and 2,6-dimethyl homologues accounted for most of the quantitative differences observed, indicating the formation of more methylglyoxal than glyoxal,

Table 3. Formation (Means ± SD in Micromoles per Kilogram)[a] of Pyrazines in Low-Moisture, Asparagine−Sugar WMS Model Systems (50 μmol/g, Equimolar) Heated at 160 °C

		heating time							
		2.5 min	5 min	10 min	15 min	20 min	30 min	40 min	60 min
pyrazine	maltose	nd	114 ± 6	360 ± 18	581 ± 5	808 ± 43	992 ± 64	1047 ± 54	1195 ± 34
	fructose	nd	16.2 ± 0.6	88.4 ± 5.1	153 ± 7	187 ± 9	237 ± 12	334 ± 22	368 ± 30
	tagatose	nd	32.1 ± 4.0	178 ± 9	256 ± 8	352 ± 11	427 ± 11	573 ± 50	606 ± 41
	glucose	32.2 ± 2.4	209 ± 11	477 ± 10	639 ± 28	734 ± 9	888 ± 84	921 ± 49	993 ± 13
methyl-	maltose	nd	6.4 ± 0.6	41.4 ± 1.8	107 ± 0.8	165 ± 9	269 ± 19	372 ± 18	516 ± 11
	fructose	13.8 ± 1.2	161 ± 11	504 ± 31	774 ± 21	1091 ± 67	1276 ± 57	1727 ± 95	1888 ± 165
	tagatose	3.9 ± 1.1	96.7 ± 11.0	404 ± 13	607 ± 37	813 ± 20	993 ± 43	1235 ± 107	1384 ± 120
	glucose	11.5 ± 0.6	114 ± 7	364 ± 5	567 ± 20	723 ± 33	879 ± 62	1016 ± 54	1171 ± 26
2,5-dimethyl-	maltose	nd	nd	nd	nd	1.5 ± 0.1	2.4 ± 0.1	4.1 ± 0.4	6.1 ± 0.5
	fructose	3.9 ± 0.1	48.4 ± 2.8	94.3 ± 4.2	133 ± 6	206 ± 15	234 ± 11	318 ± 22	347 ± 42
	tagatose	nd	7.8 ± 1.0	28.1 ± 1.3	45.6 ± 2.3	59.7 ± 1.7	71.8 ± 3.7	85.8 ± 8.2	101 ± 10
	glucose	3.2 ± 0.2	27.6 ± 1.5	77.8 ± 1.3	113 ± 5	138 ± 8	164 ± 12	185 ± 8	212 ± 5
2,6-dimethyl-	maltose	nd	nd	nd	2.6 ± 0.1	4.4 ± 0.1	8.5 ± 0.6	13.1 ± 0.6	20.0 ± 0.9
	fructose	2.2 ± 0.3	37.3 ± 4.3	98.1 ± 5.3	160 ± 6	266 ± 20	322 ± 17	454 ± 26	504 ± 60
	tagatose	nd	7.2 ± 0.9	34.5 ± 1.6	61.3 ± 4.1	84.3 ± 1.7	106 ± 6	134 ± 14	160 ± 19
	glucose	nd	1.1 ± 0.2	5.7 ± 0.3	10.7 ± 0.8	16.4 ± 0.8	21.7 ± 2.1	29.8 ± 1.6	37.8 ± 0.1
ethyl-	maltose	nd	1.7 ± 0.1	9.5 ± 0.5	23.1 ± 0.5	33.5 ± 2.0	50.9 ± 3.1	69.7 ± 3.8	93.9 ± 3.4
	fructose	nd	3.3 ± 0.4	15.0 ± 0.3	28.4 ± 0.9	42.5 ± 3.0	60.5 ± 2.3	86.2 ± 5.2	105 ± 10
	tagatose	nd	5.6 ± 0.3	31.8 ± 1.0	56.9 ± 3.1	79.9 ± 1.4	106 ± 4	132 ± 12.4	160 ± 14
	glucose	nd	1.6 ± 0.2	9.6 ± 0.1	19.6 ± 0.4	28.6 ± 1.8	38.2 ± 3.2	50.8 ± 1.5	65.5 ± 0.3
2,3-dimethyl-	maltose	nd	nd	10.0 ± 0.4	19.9 ± 0.9	25.0 ± 1.6	33.2 ± 2.3	45.8 ± 3.1	61.0 ± 3.6
	fructose	nd	2.6 ± 0.2	10.2 ± 0.6	20.3 ± 0.8	31.7 ± 3.1	46.4 ± 1.5	69.4 ± 4.4	88.9 ± 8.8
	tagatose	nd	2.5 ± 0.5	13.9 ± 1.1	25.4 ± 1.9	35.7 ± 0.8	47.7 ± 3.2	63.5 ± 5.1	76.2 ± 6.8
	glucose	0.9 ± 0.1	6.7 ± 0.9	20.0 ± 0.7	29.2 ± 0.9	37.4 ± 1.9	43.4 ± 3.7	53.0 ± 3.4	65.2 ± 1.6
2-ethyl-6-methyl-	maltose	nd	nd	nd	nd	2.6 ± 0.1	4.8 ± 0.4	9.4 ± 0.4	14.4 ± 0.2
	fructose	nd	2.0 ± 0.2	8.6 ± 0.4	18.4 ± 0.7	29.9 ± 1.6	46.8 ± 2.3	70.9 ± 2.7	88.9 ± 10.6
	tagatose	nd	1.0 ± 0.1	8.5 ± 0.5	17.9 ± 1.0	26.7 ± 0.8	39.0 ± 1.1	47.3 ± 5.3	61.9 ± 4.9
	glucose	nd	0.6 ± 0.04	4.3 ± 0.1	8.8 ± 0.3	13.2 ± 0.7	17.5 ± 1.5	24.0 ± 1.1	31.8 ± 0.4
2-ethyl-5-methyl-	maltose	nd	nd	nd	nd	0.7 ± 0.1	1.1 ± 0.1	1.9 ± 0.04	2.9 ± 0.2
	fructose	nd	2.5 ± 0.3	6.9 ± 0.2	11.9 ± 0.3	19.1 ± 1.2	25.2 ± 1.5	34.9 ± 1.2	40.6 ± 4.4
	tagatose	nd	1.1 ± 0.1	5.2 ± 0.2	10.5 ± 0.7	14.0 ± 0.3	18.3 ± 0.7	21.4 ± 1.9	25.5 ± 2.1
	glucose	nd	0.6 ± 0.05	2.8 ± 0.2	5.0 ± 0.5	7.1 ± 0.5	9.0 ± 0.8	11.1 ± 1.0	13.9 ± 0.2
2-ethyl-3-methyl-	maltose	nd	nd	nd	nd	1.4 ± 0.1	2.5 ± 0.2	4.8 ± 0.3	7.6 ± 0.1
	fructose	nd	0.8 ± 0.1	3.3 ± 0.2	6.5 ± 0.4	10.1 ± 0.5	16.0 ± 0.6	25.7 ± 1.6	34.4 ± 4.3
	tagatose	nd	0.6 ± 0.03	3.7 ± 0.2	7.0 ± 0.4	10.5 ± 0.2	15.6 ± 0.6	19.2 ± 1.6	26.2 ± 1.9
	glucose	nd	0.4 ± 0.01	2.2 ± 0.1	4.3 ± 0.4	6.2 ± 0.3	8.1 ± 0.7	11.4 ± 0.7	14.9 ± 0.1
acetyl-	maltose	nd	nd	nd	nd	nd	nd	15.2 ± 0.6	20.3 ± 0.6
	fructose	nd	nd	nd	nd	nd	10.6 ± 0.5	14.9 ± 2.5	17.4 ± 1.3
	tagatose	nd	nd	nd	nd	10.6 ± 0.5	15.4 ± 1.1	21.9 ± 2.1	24.1 ± 4.5
	glucose	nd	5.5 ± 0.3	18.8 ± 1.4	32.1 ± 1.1	40.7 ± 1.2	54.7 ± 4.2	63.4 ± 0.4	73.4 ± 4.7
total pyrazines	maltose	nd	122 ± 7	421 ± 21	733 ± 5	1043 ± 55	1364 ± 89	1583 ± 80	1937 ± 53
	fructose	19.9 ± 1.5	274 ± 18	829 ± 46	1306 ± 41	1883 ± 121	2275 ± 103	3135 ± 181	3454 ± 285
	tagatose	3.9 ± 1.1	155 ± 18	708 ± 27	1088 ± 57	1487 ± 37	1841 ± 71	2333 ± 205	2625 ± 223
	glucose	47.8 ± 3.1	368 ± 21	982 ± 14	1430 ± 54	1745 ± 54	2123 ± 172	2366 ± 119	2678 ± 40

[a] Analysis performed in triplicate; nd, not detected.

which is in agreement with the findings of Amrein et al. (*8*). Similar results have been found in fried potato model systems heated at 180 °C for 2 min (*30*). The amounts of pyrazines in all low-moisture WMS systems of this study were greater than the amounts of acrylamide. In a study in aqueous glucose−asparagine solutions heated at 140 °C in sealed tubes, the relative pyrazine/acrylamide yields were much lower, with acrylamide being preferentially formed, but there was also a strong correlation between the two (*15*). The formation of pyrazines is inhibited by the presence of water, whereas it increases with temperature of the heat treatment (*31*); therefore, differences in methodologies employed could contribute significantly to the relative yields of Maillard reaction products.

Correlation of pyrazines with acrylamide formation in our model systems with added sugars revealed a linear relationship ($R^2 = 0.937$) only for the first 20 min of the heating regimen, while the average ratio was approximately 4:1 (**Figure 6**). Low et al. (*13*) determined the formation of pyrazines and acrylamide in potato cakes and found a total pyrazine to acrylamide molar ratio of approximately 14:1 at both 15 and 30 min (maximum acrylamide yield) of heating at 180 °C while asparagine represented 32% of the total amino acids. In low final moisture

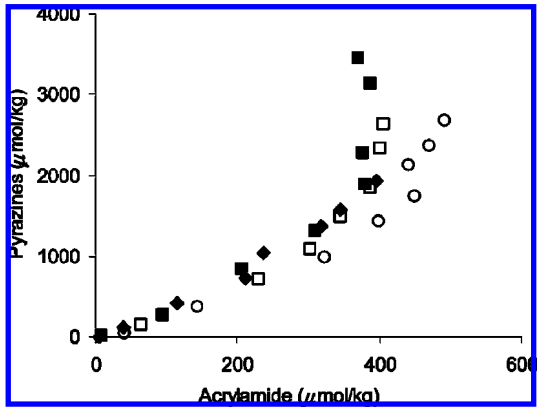

Figure 6. Correlation of acrylamide formation with pyrazines in asparagine−sugar WMS model systems (50 µmol/g, equimolar) heated at 160 °C for 2.5−60 min in sealed vials: glucose (○), fructose (■), maltose (◆), tagatose (□).

food systems the relative yields of pyrazines to acrylamide are expected to be much higher because other amino acids will contribute both to the formation of reactive intermediates and pyrazines and possibly to Michael addition reactions with acrylamide. Acrylamide polymerization and/or Michael addition reactions with other components at prolonged heating times could lead to loss of acrylamide (*19*), whereas pyrazines could continue to be formed from residual carbonyls and ammonia (*24*). This is especially true in sealed model systems in which both volatile reactive sugar fragmentation products and ammonia are contained within the headspace of the sealed vials.

A more detailed examination of the relationship between pyrazines and acrylamide in our model systems revealed some significant differences between the individual sugars studied. In particular, the relative pyrazine/acrylamide molar ratios for the first 20 min of the reaction were 3.5:1 and 4.5:1 for glucose and fructose, respectively, with tagatose and maltose being in between. These differences are in agreement with our observations on the relative yields of glyoxal from the parent monosaccharides as well as the relative reactivities of glyoxal and methylglyoxal toward acrylamide and pyrazine formation. The tautomerization of the decarboxylated asparagine−carbonyl Schiff base is a key step in determining the relative pyrazine/acrylamide yields, and that was shown to be greatly influenced by the chemical identity of the carbonyl.

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Received for review December 21, 2007. Revised manuscript received April 3, 2008. Accepted May 18, 2008. Financial support was provided by the U.K. Food Standards Agency (C03048).

JF703744K