JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Investigations on the Promoting Effect of Ammonium Hydrogencarbonate on the Formation of Acrylamide in Model Systems

THOMAS M. AMREIN,* LUCA ANDRES, GIUSEPPE G. G. MANZARDO, AND RENATO AMADÒ

Institute of Food Science and Nutrition, Swiss Federal Institute of Technology (ETH), ETH Zurich, CH-8092 Zurich, Switzerland

NH_4HCO_3 is known to promote acrylamide formation in sweet bakery products. This effect was investigated with respect to sugar fragmentation and formation of acrylamide from asparagine and sugar fragments in model systems under mild conditions. The presence of NH_4HCO_3 led to increases in acrylamide and α-dicarbonyls from glucose and fructose, respectively. As compared to glucose or fructose, sugar fragments such as glyoxal, hydroxyethanal, and glyceraldehyde formed much higher amounts of acrylamide in reaction with asparagine. The enhancing effect of NH_4HCO_3 is explained by (1) the action of NH_3 as base in the retro-aldol reactions leading to sugar fragments, (2) facilitated retro-aldol-type reactions of imines in their protonated forms leading to sugar fragments, and (3) oxidation of the enaminols whereby glyoxal and other reactive sugar fragments are formed. These α-dicarbonyl and α-hydroxy carbonyl compounds may play a key role in acrylamide formation, especially under mild conditions.

KEYWORDS: Acrylamide; ammonium hydrogencarbonate; Maillard reaction; retro-aldol; retro-aldol type; reactive sugar fragments; glyoxal; α-dicarbonyls; α-hydroxycarbonyls.

INTRODUCTION

The presence of acrylamide in a broad range of heated foods (*1*) is considered as a potential health concern because of the carcinogenic properties of acrylamide (*2*). Acrylamide is generated in the Maillard reaction via reaction of reducing sugars or other carbonyls and free asparagine, the amino acid forming the backbone of the acrylamide molecule (*3*, *4*). Recently, 3-aminopropionamide (3-APA) was shown to be a very potent precursor for acrylamide (*5*). 3-APA can be thermally formed by reaction of asparagine with carbonyls and may be an important transient intermediate (*6*, *7*).

In general, fried potato products contain more acrylamide as compared to bakery products (*8*). This may be attributed to the high temperatures during processing and the presence of large amounts of precursors in the raw potato (*9*). However, some sweet bakery products such as gingerbread are known to contain surprisingly high amounts of acrylamide, sometimes exceeding 1000 μg/kg (*8*, *10*, *11*). This is in contrast to the rather low concentration of free asparagine in the dough (*10*, *12*). Several studies have shown that the baking agent NH_4HCO_3 promotes acrylamide formation (*10*, *13*, *14*) and that replacement of this baking agent by $NaHCO_3$ substantially reduces the acrylamide content of the product (*10*, *15*−*17*). In this context it was shown that the N atom from NH_4HCO_3 was not incorporated into the acrylamide molecule during baking of gingerbread. This excludes formation of acrylamide via amidation of acrylic acid (*10*). Interestingly, the promoting effect of NH_4HCO_3 was found to depend on the presence of reducing sugars. If the main sources of reducing sugars (i.e., honey, inverted sugar syrup) were replaced by sucrose, a large decrease in the acrylamide content was observed in several products despite the presence of NH_4HCO_3 (*10*, *15*, *16*). Thus, the promoting effect of NH_4HCO_3 on acrylamide formation is indirect and requires the presence of reducing sugars. This leads to the following hypothesis: Ammonia from the baking agent reacts with the reducing sugars whereby reactive sugar fragments such as glyceraldehyde, glyoxal, methylglyoxal, etc., are formed. These fragments in turn react with free asparagine, delivering more acrylamide as compared to the reaction between asparagine and glucose or fructose. These reactions should take place under mild conditions since acrylamide was found in the crumb of gingerbread where temperature stayed below 120 °C during baking (*10*).

The aim of the present study was to check this hypothesis and deduce mechanistic explanations for the promoting effect of NH_4HCO_3. In a first experiment formation of acrylamide from asparagine with sugars or sugar fragments was explored. Then formation of such fragments from sugars in the presence of baking agent was investigated. As a simplified approach, only the α-dicarbonyls glyoxal, methylglyoxal, and diacetyl were monitored. The influence of temperature, time, and type of

* To whom correspondence should be addressed. Phone: +41 44 632 69 41. Fax: +41 44 632 11 23. E-mail: thomas.amrein@ilw.agrl.ethz.ch.

10.1021/jf0625860 CCC: $33.50
Published on Web 12/08/2006

buffer on formation of acrylamide and α-dicarbonyls was investigated as well.

MATERIALS AND METHODS

Chemicals and Buffers. Glucose, fructose, maltose, sucrose, asparagine, ammonium hydrogencarbonate, sodium hydrogencarbonate, and citric acid were all from Fluka (Buchs, Switzerland). Glyoxal, methylglyoxal, diacetyl, 2,3-pentanedione, hydroxyethanal, glyceraldehyde, hydroxyacetone, and erythrose were also from Fluka. The concentrations of asparagine, sugars, and sugar fragments in the model systems were all 0.025 M. NH_4HCO_3 and $NaHCO_3$ were added at 1.00% (w/v) and 1.07% (w/v, giving the same molarity of HCO_3 anions), respectively, or at one-half of these concentrations. Citric acid was added at 0.8% (w/v).

Heating experiments were carried out in phosphate ($NaHPO_4$, K_2HPO_4) or 3-morpholinopropanesulfonic acid (MOPS) buffers (both from Fluka). The buffers were prepared using the equation of Henderson−Hasselbalch. The concentration of buffer salts was 50 or 100 mM and the pH set to 7.0. A 3 mL amount of the solutions was filled into glass ampoules which were sealed and immersed into a preheated oil bath equipped with a thermostat. Heating conditions comprised temperatures from 80 to 140 °C and heating times from 3 to 30 min. The standard procedure was 120 °C for 30 min. After heating, the ampoules were immediately immersed in an ice−water bath to stop further reactions.

Analysis of α-Dicarbonyls. Glyoxal, methylglyoxal, and diacetyl were determined by GC-MS as their corresponding quinoxalines after derivatization with *o*-phenylenediamine (OPD) following the method described by Nedvidek et al. (*18*). A 100 μL amount of 2,3-pentanedione (internal standard, 0.02 M in methanol, Fluka) and 0.5 mL of OPD solution (0.025 M in phosphate buffer pH 7.0, Fluka) was added to 2 mL of the solution after heating. The reaction tubes were wrapped with aluminum foil, and the solutions were stirred in a water bath at 45 °C for 40 min. The quinoxalines were extracted twice with 0.5 mL of dichloromethane (Fluka). The combined organic extracts were dried over anhydrous magnesium sulfate (Fluka), and 2 μL of the solution was injected. Determination by GC-MS was carried out on a 2000 series "TRACE GC" gas chromatograph with split injector (Thermo Quest CE Instruments, Milan, Italy) coupled to a TSQ quadrupole mass spectrometer (Finnigan Mat, San Jose, CA). The separation column was a ZB-50 capillary (BGB Analytik, Böckten, Switzerland) with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm. The GC was programmed as follows: 120 °C for 5 min; rate of 10 °C/min to 240 °C; 240 °C for 5 min. An amount of 1−2 μL was injected in split mode (split ratio 1:10) with an injector temperature of 280 °C. The MS was operated in the SIM and full scan modes (*m*/*z* 40−240, scan time 0.5 s). In the SIM mode the following ions were monitored for the different quinoxalines (ions used for quantification are given in bold): Quinoxaline (*m*/*z* 76, 77, 103, **130**), methylquinoxaline (*m*/*z* 76, 77, 117, **144**), dimethylquinoxaline (*m*/*z* 76, 77, 117, **158**), methyl-ethylquinoxaline (103, 117, 130, 144, 171, **172**) with a scan time of 0.02 s each. Identification was done by comparing retention times and mass spectra to reference substances and the mix used for the determination of response factors. For every experiment a mix of glyoxal, methylglyoxal, diacetyl, and 2,3-pentanedione (0.01 M in methanol, Fluka) was derivatized with OPD, extracted, and injected to determine response factors ($n = 2$). Quinoxaline and methylquinoxaline were available from Fluka; dimethylquinoxaline and methyl-ethylquinoxaline were prepared by derivatization of diacetyl and 2,3-pentandione with OPD, respectively (*18*).

Analysis of Acrylamide. Determination of acrylamide was based on the GC-MS method published by Biedermann et al. (*19*) with the following modifications: $^{13}C_3$-acrylamide (CIL, Andover, MA) and methacrylamide (Fluka), both dissolved in methanol (Fluka), were used as internal standards with a concentration of 500 μg/mL. A 1.0 μL amount of internal standard was added to 2 mL of heated solution, and the sample was then mixed with 10 mL of 1-propanol. The water−propanol mixture was evaporated, and the residue was taken up in 2 mL of acetonitrile (Fluka). This extract was twice defatted with hexane (Fluka), centrifuged (1000*g*), and then injected on column. GC-MS involved a 2000 series "TRACE GC" gas chromatograph with on-column injector (Thermo Quest CE Instruments) coupled to a TSQ quadrupole mass spectrometer (Finnigan Mat). The precolumn (TSP deactivated, i.d. 0.53 mm) and separation column (BGB Wax, 12 m, i.d. 0.25 mm) were both from BGB Analytik.

Table 1. Acrylamide Formation in Different Model Systems (120 °C, 10 min; 50 mM Phosphate Buffer; Concentration of Reactant was 25 mM except for Baking Agents and Citric Acid; pH = 7.0; $n = 2$; n.d. not detectable)

reactants	acrylamide [μg/g Asn]
glucose + asparagines	5.5
fructose + asparagines	7.8
glucose + 0.5% NH_4HCO_3 + asparagines	202
glucose + 1% NH_4HCO_3 + asparagines	260
fructose + 1% NH_4HCO_3 + asparagines	539
glucose + 0.5% $NaHCO_3$ + asparagines	12.9
glucose + 1% $NaHCO_3$ + asparagine	13.4
glucose + 0.5% $NaHCO_3$ + citric acid + asparagine	1.6
glyoxal + asparagine	2001
methylglyoxal + asparagine	19
diacetyl + asparagine	10
glyceraldehyde + asparagine	100
hydroxyacetone + asparagine	n.d.
erythrose + asparagine	268
hydroxyethanal + asparagine	707

RESULTS AND DISCUSSION

Formation of Acrylamide. Acrylamide formation was determined in different model systems to check the influence of the different compounds. All model systems contained asparagine, glucose, or fructose with or without baking agents. In addition, acrylamide formation from asparagine and sugar fragments was investigated (**Table 1**). Mild heating conditions were chosen (120 °C, 10 min) in order to be comparable to the situation within gingerbread where acrylamide is also formed in the crumb (*10*). Phosphate buffer was chosen because it is a suitable and commonly used buffer for Maillard reaction model systems (*18*, *20*, *21*).

Table 1 shows that acrylamide was formed in substantial amounts despite the mild conditions. The reproducibility was done with four replicates; the mixture glucose + NH_4HCO_3 + asparagine gave an RSD of 6%, and the mixture fructose + NH_4HCO_3 + asparagine gave an RSD of 10%. Fructose formed more acrylamide in reaction with asparagine as compared to glucose, which is in accordance with previous publications reporting fructose being about twice as effective in acrylamide formation as glucose (*14*, *22*). The presence of NH_4HCO_3 dramatically increased acrylamide formation by a factor of 37 (glucose) and 69 (fructose). This demonstrates that the promoting effect of NH_4HCO_3 is also observed in the present experiments as it was reported for sweet bakery products (*10*, *13*, *14*). A higher concentration of NH_4HCO_3 led to more acrylamide, while the concentration of $NaHCO_3$ had virtually no effect on acrylamide. A similar effect was also observed in gingerbread (*10*). The presence of $NaHCO_3$ led only to a small increase of acrylamide in the glucose−asparagine system (approximately factor of 2) which could be due to a slightly higher pH (*23*). Thus, the presence of $NaHCO_3$ increased acrylamide contents by far less as compared to NH_4HCO_3. This was also previously observed in flour model systems (*13*, *14*) and bakery products (*10*, *15*, *16*, *24*). In the presence of citric acid almost no acrylamide was found after heating the model system containing glucose, asparagine, and $NaHCO_3$. This effect may at least be partially attributed to the decreased pH in this sample (pH = 6.3) and was also observed in bakery (*10*, *15*, *24*) and a similar model system (*23*).

Table 2. Acrylamide Formation in Model Systems with Different Buffers (120 °C, 30 min; concentration of sugars and asparagine was 25 mM, buffer concentration was 100 mM; $n = 2$)

	MOPS buffer	phosphate buffer
reactants	acrylamide [μg/g asparagine]	acrylamide [μg/g asparagine]
glucose + asparagine	2.6	161
fructose + asparagine	2.1	139
glucose + NH_4HCO_3 + asparagine	1063	2689
glucose + $NaHCO_3$ + asparagine	112	520
fructose + NH_4HCO_3 + asparagine	2302	4915
fructose + $NaHCO_3$ + asparagine	137	795
maltose + NH_4HCO_3 + asparagine	122	a
sucrose + NH_4HCO_3 + asparagine	1.1	a

[a] Experiment not performed.

Among the sugar fragments, glyoxal was clearly most effective in acrylamide formation, followed by hydroxyethanal, erythrose, glyceraldehyde, and methylglyoxal. Thus, sugar fragments form much higher amounts of acrylamide as compared to intact hexoses under the conditions used. In the presence of glyoxal about 0.2% of the asparagine was detected as acrylamide, which is considerable taking the mild conditions (low temperature, short time, and high water activity) into account. In the case of glyoxal, about 250−350 times more acrylamide was formed as compared to the model systems with the two hexoses, which points out the high reactivity of this sugar fragment. Therefore, it is concluded that glyoxal is the most important α-dicarbonyl among the investigated sugar fragments and may play a key role in acrylamide formation. Glyoxal, hydroxyethanal, glyceraldehyde, and methylglyoxal are all prominent sugar fragments (*25*−*27*), and the higher acrylamide yields from asparagine as compared to the hexoses reported here are in agreement with Zyzak et al. (*4*). In experiments with homogenized potato (*28*), acrylamide formation was doubled if glyceraldehyde was added, which pointed out the activity of this compound in a food matrix. The higher amounts of acrylamide formed in reaction with the sugar fragments is also in line with their much higher browning as compared to glucose and fructose (*29*). Diacetyl formed somewhat more acrylamide as compared to the two hexoses. Mottram et al. found more acrylamide from asparagine heated with glucose compared to diacetyl, which may be due to the more severe heat treatment (185 °C, 20 min) in their model system (*21*). Surprisingly, in the case of hydroxyacetone no quantifiable acrylamide signal was found in our system, which is in contrast to other reports where hydroxyacetone and asparagine formed rather large amounts of acrylamide in model systems (*7*, *22*, *30*). Different conditions used (open vs closed, dry vs aqueous, temperature, time) may have caused this discrepancy.

Phosphate buffers are known to enhance the Maillard reaction as compared to other buffers (*31*−*33*). Therefore, acrylamide formation was also investigated in model systems using MOPS buffer (**Table 2**). The heating conditions were set to 120 °C and 30 min to enhance effects and be comparable with the systems where α-dicarbonyls were monitored (see below). The data in **Table 2** are very comparable with the data in **Table 1**, but larger amounts of acrylamide were determined because the heating time was extended by 20 min. In the absence of baking agents much less acrylamide was formed as also observed with the shorter heating period (see **Table 1**). Fructose was more effective than glucose in the presence of baking agents, and NH_4HCO_3 had a much stronger effect than $NaHCO_3$. In the maltose−asparagine system about 10 times less acrylamide was found as compared to glucose, which is largely explained by the lower reactivity of a disaccharide (*25*). Sucrose and asparagine formed only traces of acrylamide because sucrose as a non-reducing disaccharide only undergoes Maillard reaction after hydrolysis. Phosphate buffer led to elevated levels of acrylamide in all experiments, and the effect was particularly large in the glucose−$NaHCO_3$ and fructose−$NaHCO_3$ systems as well as in the systems without baking agents. Thus, the type of buffer has a distinct effect which may be explained by its influence on the generation of α-dicarbonyls (see Results and Discussion below).

Formation of α-Dicarbonyls. Generation of α-dicarbonyl sugar fragments was monitored in model systems using phosphate and MOPS buffers, respectively. Reproducibility was checked with four replicates of fructose and NH_4HCO_3 in MOPS buffer heated at 120 °C for 30 min. The RSD for glyoxal concentration was 4.8%, and for methylglyoxal and diacetyl the RSD was 6%. As observed with acrylamide formation (see **Table 2**), phosphate buffer was found to enhance formation of α-dicarbonyls, while in MOPS buffer less α-dicarbonyls was formed in the absence of baking agents (**Table 3**). In phosphate buffer (no baking agent present) glucose formed about 6 times more glyoxal, 18 times more methylglyoxal, and 1.4 times more diacetyl as compared to MOPS buffer. In phosphate buffer Maillard reaction in terms of browning and loss of glycine and glucose is reported to be enhanced (*31*, *33*). This effect is explained by the function of phosphate as proton donor and acceptor in formation of the glycosylamine (*31*). Furthermore, Thornalley et al. found an influence of the buffer type and concentration on reaction rates in formation of α-oxoaldehydes from glucose and sugar fragments, respectively (*34*, *35*). In analogy to ref *31* retro-aldol reactions, which are key reactions during sugar fragmentation, may be facilitated by phosphate acting as proton donor and acceptor. As a consequence of the marked effect of phosphate buffer, further experiments were all performed in MOPS buffer.

As already observed with acrylamide formation, the presence of NH_4HCO_3 had a strong effect on formation of α-dicarbonyls. In the case of glucose and NH_4HCO_3 (MOPS buffer) about 30 times more glyoxal was determined compared to no baking agent and about 4.8 times more glyoxal compared to $NaHCO_3$. In the phosphate buffer 1.9 times more glyoxal was formed from glucose with NH_4HCO_3 compared to $NaHCO_3$. The patterns for glyoxal formation from fructose were similar: NH_4HCO_3 increased the yield of glyoxal by a factor of about 2 as compared to $NaHCO_3$ in both buffers. The yield of glyoxal in the presence of NH_4HCO_3 was similar for both sugars. Similar results were reported by Weenen in a Maillard model system with alanine (*36*).

Methylglyoxal was quantitatively the major α-dicarbonyl determined. Its yield was less influenced by the type of buffer and baking agent as compared to glyoxal. Mostly, more methylglyoxal was found in phosphate buffer as compared to MOPS buffer, which is mainly explicable by the effect of the phosphate buffer itself. The effect of phosphate almost covered up the effect of NH_4HCO_3. In general, more methylglyoxal was found in the fructose system as compared to glucose. The strongest difference was found for reaction with NH_4HCO_3 in MOPS buffer: Fructose formed 2.5 times more methylglyoxal than glucose. In other experiments this factor ranged from 1.2 to 1.6 only. The explanation for the higher methylglyoxal yields from fructose as compared to glucose lies in the ability of fructose to directly form both the 1- and 3-glucosone via the

Table 3. Influence of Buffer Type, Sugar, and Baking Agent on Formation of α-Dicarbonyls in Model Systems (120 °C, 30 min, $n = 2$)[a]

	phosphate buffer			MOPS buffer		
reactants	GO [mg/kg sugar]	mGO [mg/kg sugar]	DA [mg/kg sugar]	GO [mg/kg sugar]	mGO [mg/kg sugar]	DA [mg/kg sugar]
glucose	69	430	203	5	24	146
glucose + NH_4HCO_3	136	449	447	163	335	191
glucose + $NaHCO_3$	72	548	266	34	386	202
fructose + NH_4HCO_3	163	711	543	163	850	315
fructose + $NaHCO_3$	80	648	575	75	621	248

[a] GO, glyoxal; mGO, methylglyoxal; DA, diacetyl.

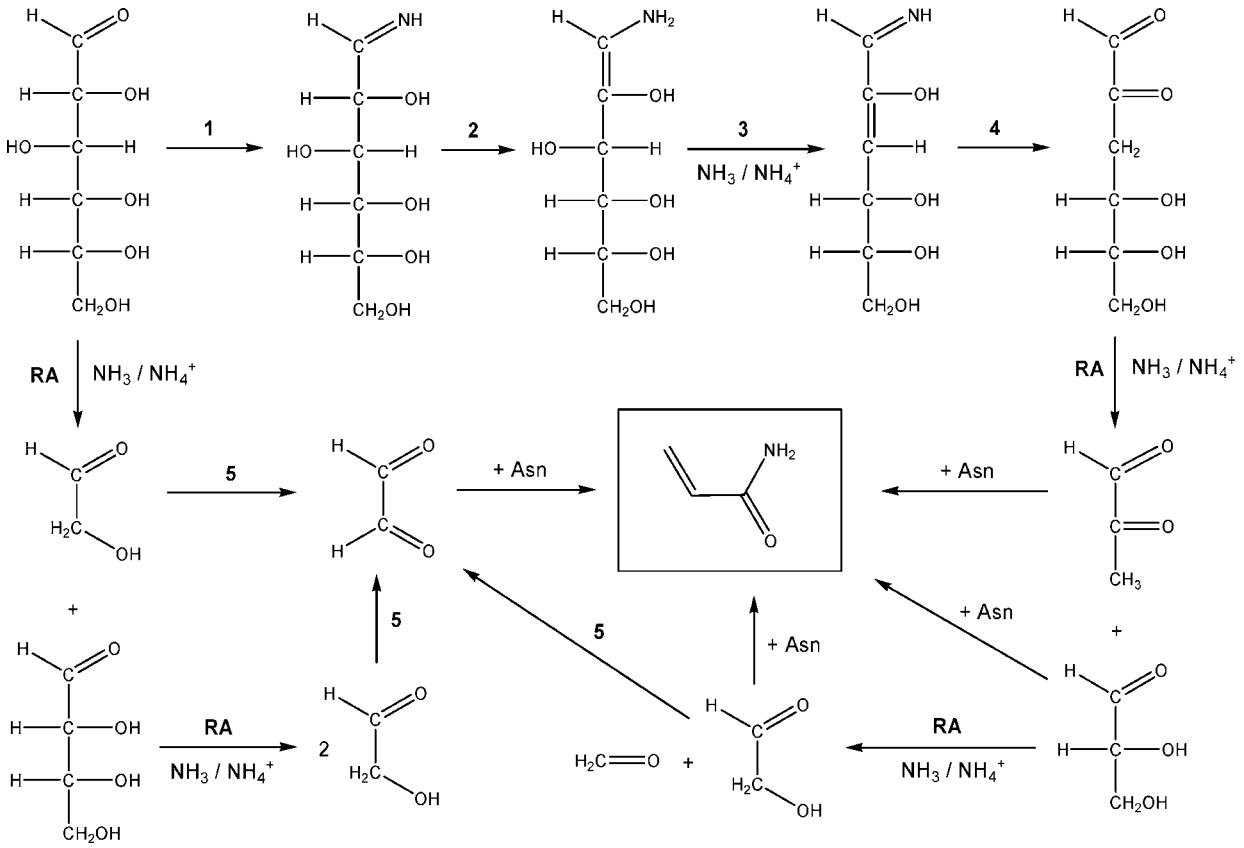

Figure 1. Formation of sugar fragments from glucose considering the role of NH_3 as a base (1, imine formation; 2, imin–enamine tautomerism; 3, dehydratation; 4, hydrolysis, only the unprotonated form of the imine shown; 5, oxidation; RA, retro-aldol reaction).

2,3-enediol (*37*, *38*) or the 2,3-enaminol, whereby the number of possible reaction pathways leading to methylglyoxal is virtually doubled. In principle, glucose can also form these desoxysones, but more steps are needed (*37*−*39*). Diacetyl yields were mainly influenced by the type of buffer (higher amounts in phosphate buffer) and type of sugar (more from fructose).

In addition to the experiments shown in **Table 3**, formation of glyoxal from erythrose and hydroxyethanal with and without NH_4HCO_3 was investigated (MOPS buffer, 120 °C, 30 min, $n = 2$). The amount of glyoxal from erythrose increased by a factor of about 9 if NH_4HCO_3 was present (90 and 800 mg/kg). NH_4HCO_3 obviously facilitates retro-aldol fragmentation of erythrose. In the case of hydroxyethanal, large amounts of glyoxal were determined for both cases as expected (about 1000 mg/kg). Thus, these two sugar fragments contribute to formation of glyoxal as shown in **Figures 1** and **2**.

The pH of the solution may also be an important factor. The difference of the pH between samples containing NH_4HCO_3 or $NaHCO_3$ was less than 0.1 pH units for both buffers ($c = 100$ mM). Although the pH of Maillard model systems may change during heating (*23*), only the pH before heating was considered because the pH during and after heating is not controllable. The pH of the NH_4HCO_3 model systems ranged from 7.2 to 7.4 for both buffers (data in **Table 3**). After heating the pH was increased by 0.5−0.8 pH units. The difference between the NH_4HCO_3 and NH_4HCO_3 systems was at most 0.3 pH units. To check if pH differences of about 0.3 pH units might have affected the results, experiments were repeated with solutions where the pH was adjusted to 7.0 before heating. In MOPS buffer the concentration of glyoxal and methylglyoxal decreased by 10−30% in the different model experiments. This is in line with the results from Nedvidek et al., who reported a decrease of these α-dicarbonyls of about 5−30% if the pH decreased from 7 to 5 (*18*). Hayashi and Namiki reported that formation of C2 and C3 fragments from hexoses was negligible at pH 3.5, substantial at pH 6.4, and large at pH 9.3 (*29*). However, the influence of the baking agent and the type of sugar remained the same in our model systems. This means that at pH = 7.0 NH_4HCO_3 led to higher amounts of glyoxal and methylglyoxal than $NaHCO_3$ and fructose was more efficient than glucose

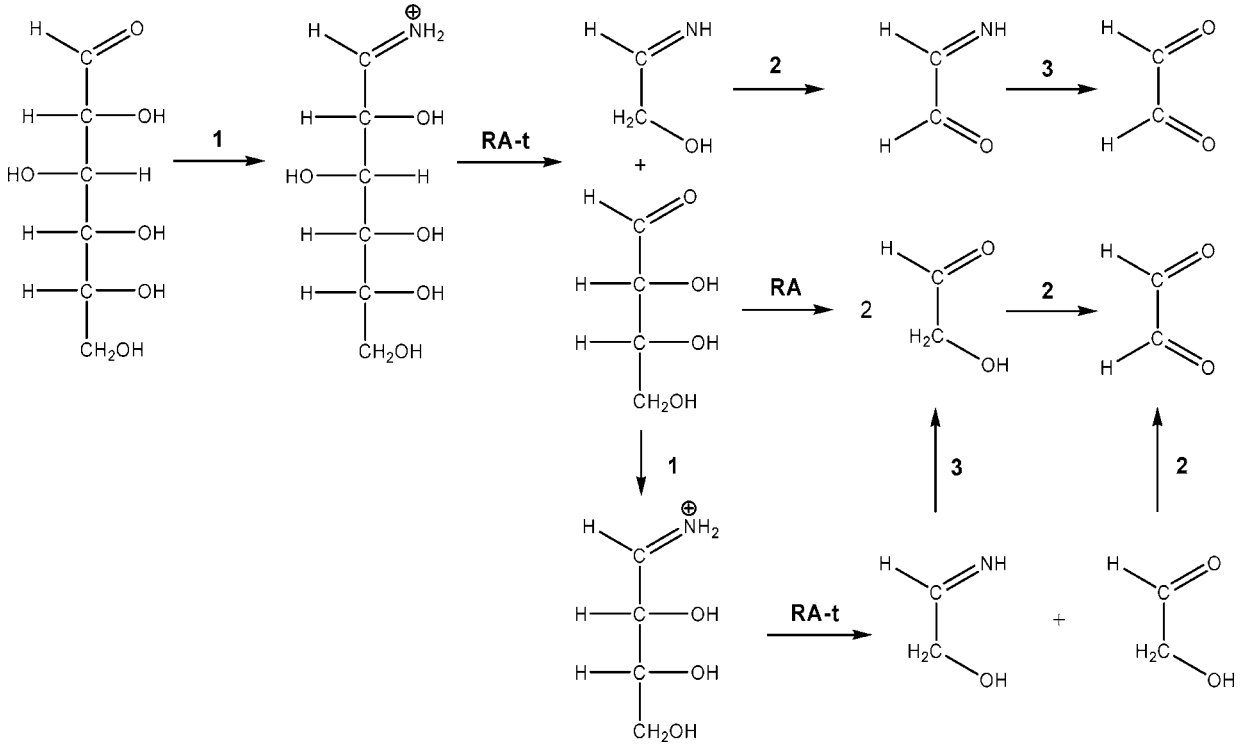

Figure 2. Reactions of various imines derived from glucose leading to glyoxal and 2-iminoethanal (1, imine formation and protonation; 2, oxidation; 3, hydrolysis, only the unprotonated forms of the imines shown; RA, retro-aldol reaction; RA-t, retro-aldol-type reaction).

(results not shown). Therefore, pH alone cannot explain the increased formation of α-dicarbonyls in the presence of NH_4HCO_3 although it has some impact.

If asparagine was present in the model systems (as it was in the experiments shown in **Tables 1** and **2**), similar results were obtained. The largest differences were observed with $NaHCO_3$ where significantly more α-dicarbonyls were detected. This may be explained by the increased number of nucleophilic groups (α-NH_2 group of asparagine) available for the first step of the Maillard reaction. This effect was not seen in the experiments with NH_4HCO_3 where the nucleophilic ammonia was present and probably outnumbered the effect of the α-NH_2 group of asparagine.

Altogether, it can be concluded that the presence of NH_4HCO_3 leads to higher amounts of α-dicarbonyls as compared to $NaHCO_3$, and this is not explained by pH effects around pH 7. This is in line with the results shown in **Table 1**. Glyoxal generation is strongly influenced by the type of baking agents and type of sugar. Together with its high efficacy of acrylamide generation with asparagine, glyoxal may be regarded as a key sugar fragment for acrylamide formation under the conditions used.

The effect of NH_4HCO_3 can be explained in the following ways (**Figure 1** and **2**). The pathways are all shown starting from glucose (fructose reacts in similar ways, see text below). NH_3 is a nucleophile and reacts with the carbonyl groups of glucose and fructose. The imines formed allow generation of glucosones, which can undergo retro-aldol reactions leading to reactive fragments such as methylglyoxal or glyceraldehyde. NH_3 may generally facilitate retro-aldol reactions of hexoses, 1-and 3-desoxysones, erythrose, and glyceraldehyde by acting as a base. NH_3 ($pK_b = 4.8$) is a stronger base compared to HPO_4^{2-} ($pK_b = 6.8$), HCO_3^- ($pK_b = 7.5$), and $H_2PO_4^-$ ($pK_b = 11.9$), which may explain the enhanced sugar fragmentation in the presence of NH_4HCO_3. Glyoxal is not formed from 1- and 3-glucosone (*36*) but via retro-aldol reactions of glucose, erythrose, and glyceraldehyde (and possibly of the corresponding imines) leading to hydroxyethanal (glycolaldehyde, hydroxyacetaldehyde), which will be oxidized by oxygen to glyoxal (*34*, *36*, *40*). Glyoxal, hydroxyethanal, erythrose, glyceraldehyde, and methylglyoxal formed more acrylamide in reaction with free asparagine as shown in **Table 1**. The reaction between glyoxal and asparagine can be formulated via the 3-aminopropionamide pathway (*6*, *7*).

In addition to the activity of NH_3 as a base, the imines formed have to be considered as well with respect to the promoting effect of NH_4HCO_3 on acrylamide and α-dicarbonyl formation (**Figure 2**). The protonated form of the glucosylimine undergoes retro-aldol reactions more easily due to the better electron-acceptor properties of the iminium ion compared to the carbonyl group. Likewise, hydrolysis of iminium ions is facilitated compared to imines (*41*). Both facts also apply to the imines of the glucosones and imines of glyceraldehyde and erythrose. Thereby, fragmentation via retro-aldol-type reaction is enhanced and 2-iminoethanol is formed, which is either oxidized by oxygen to 2-iminoethanal (which then can be hydrolyzed to glyoxal) or hydrolyzed to hydroxyethanal which then is oxidized to glyoxal (*34*, *35*).

Iminoethanal (glycolaldehyde imine, acetaldehyde imine) may facilitate decarboxylation of asparagine by the electron-withdrawing iminium group (**Figure 3**). From the decarboxylated intermediate a tautomer can be formulated which after hydrolysis produces 3-aminopropionamide. This amide was shown to form acrylamide very efficiently upon heating (*6*, *7*). Namiki and Hayashi postulated similar pathways for the reaction of sugars and amines: They also assumed a retro-aldol-type reaction of the imine formed leading to iminoethanol and its oxidized form iminoethanal (*42*).

Hofmann et al. (*27*) proposed another pathway for glyoxal formation (**Figure 4**): The enaminol tautomer of the imine of glucose is easily oxidized (*43*−*45*) by oxygen to an imino ketone, which procures a 1,2-dicarbonyl intermediate after

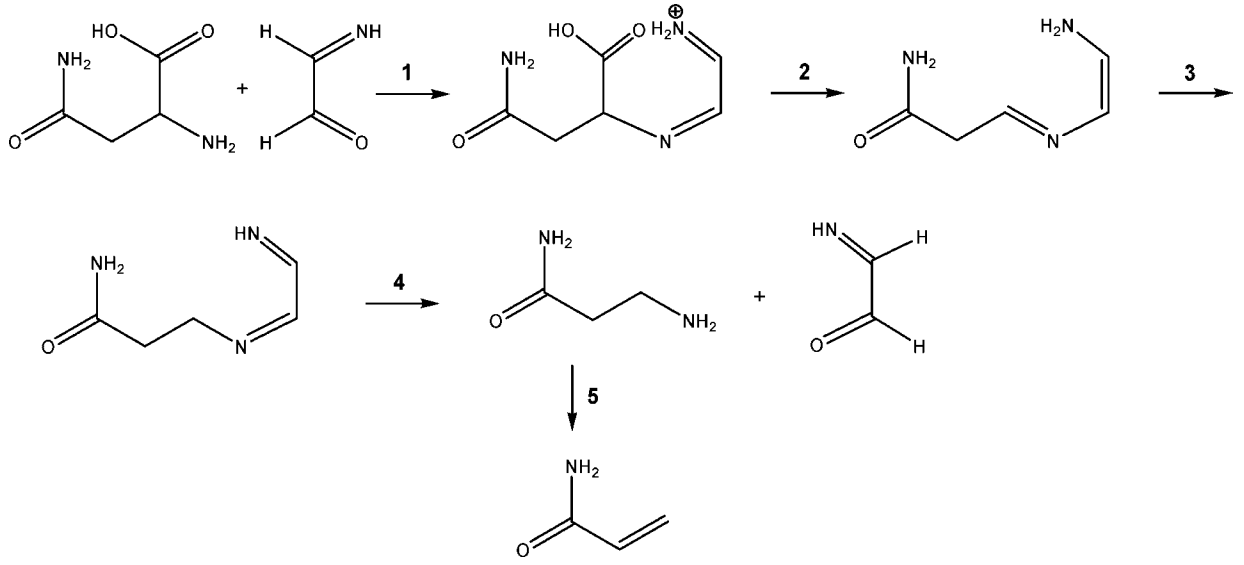

Figure 3. Formation of acrylamide from asparagine and 2-iminoethanal (1, imine formation and protonation; 2, decarboxylation; 3, enamine–imine tautomerism, 4, hydrolysis; 5, elimination of NH_3).

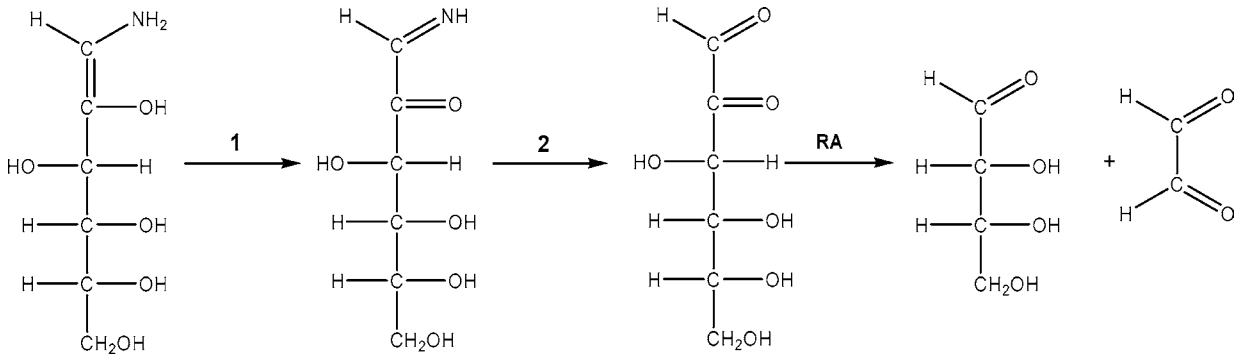

Figure 4. Formation of glyoxal from the 1,2-enaminol of glucose, adapted from Hofmann et al. (*27*) (1, oxidation; 2, hydrolysis; RA, retro-aldol reaction).

hydrolysis. Retro-aldol cleavage of this intermediate leads to glyoxal and erythrose (*27*). In a glucose−alanine model glyoxal formation preceded the one of hydroxyethanal, which indicates that glyoxal is instead reduced to hydroxyethanal, presumably by reductones (*27*). This explains the rather low amounts of glyoxal which are determined in our model systems.

NH_3 may also be important for acrylamide formation in potatoes, which contain large amounts of asparagine, glutamine, aspartic acid, and glutamic acid. Glutamine and other amino acids are known to release NH_3 already at 110 °C (*46*). Additionally, glutamine was shown to be degraded quickly during heating of grated potato at 120 °C (*9*).

Several publications on the effect of NH_4HCO_3 on acrylamide formation in a bakery exist in the literature. Weisshaar speculated that the Amadori product formed from glucose and NH_3 reacts with asparagine to an imine, which procures acrylamide via a β-elimination (*13*). However, the reaction pathway shown is somewhat doubtful. Biedermann and Grob (*14*) attributed the promoting effect of NH_4HCO_3 to an activation of fructose without any mechanistic explanations. However, they stated that NH_4HCO_3 acts merely as a catalyst and that it accelerates acrylamide formation, particularly at relatively low temperatures. In a dough model containing 39% fructose, 1% NH_4HCO_3, and dark flour the authors determined 990 μg/kg acrylamide even after storage at room temperature for 6 weeks. They also showed that other ammonium salts such as NH_4Cl, NH_4Br, and $(NH_4)_2SO_4$ were much less active (*14*). This indicates that ammonium cations need to be deprotonated before such effects become relevant. Levine and Smith attributed the lower acrylamide contents in crackers prepared with $NaHCO_3$ to an increased elimination of acrylamide and stated the same for NH_4HCO_3 (*47*). However, only very small amounts of reducing sugars (originating from the flour only) were present in the dough of their study. Therefore, these results cannot be compared to data from experiments with significant amounts of reducing sugars.

The amounts of α-dicarbonyls measured in the present model systems are not particularly large. However, it has to be stated that these compounds are highly reactive and prone to undergo further reactions, i.e., reduction (*27*) or formation of melanoidins (*29*, *39*, *40*). Thus, the amount measured is certainly smaller than the amount which was actually formed. If the trapping agent OPD is added to the reaction mixture before heating, lower amounts of dicarbonyls are lost via further reactions and higher results are obtained (no results shown). However, it is known that OPD, being a nucleophile itself, enhances formation of glucosones (*20*) and sugar fragments (*25*). Hollnagel and Kroh (*25*) determined about 700 mg of glyoxal per kilogram of fructose after heating a mixture of fructose−glycine−OPD at 100 °C for 60 min. The higher amount of α-dicarbonyls determined in their study may be attributed to the presence of OPD during heating and differences in the experimental set up (dry vs dissolved, temperature, time).

The influence of the type of sugar was further investigated, and results are shown in **Figure 5**. Glucose and fructose formed clearly more glyoxal and methylglyoxal as compared to maltose.

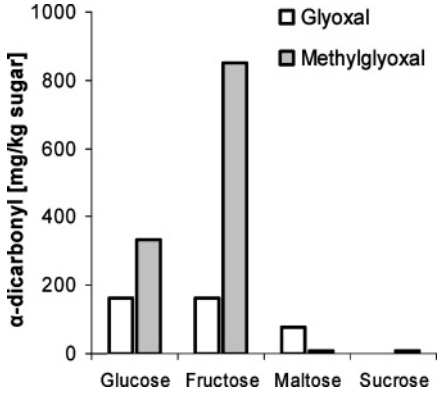

Figure 5. Generation of glyoxal and methylglyoxal from different sugars with 1% NH_4HCO_3 in MOPS buffer (120 °C, 30 min, $n = 2$).

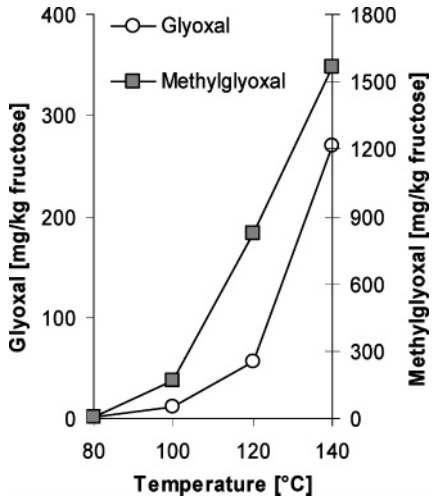

Figure 6. Influence of temperature on formation of glyoxal and methylglyoxal in the model system with fructose and NH_4HCO_3 in MOPS buffer. Note the different scale on the *y* axis (heating time 30 min; $n = 2$).

This pattern resembles the data for acrylamide formation in the model systems as shown in **Table 2**. This indicates that formation of α-dicarbonyls and acrylamide is interrelated. A similar effect for maltose was also reported in another Maillard model system (*25*). Sucrose, being a nonreducing disaccharide, formed no detectable amounts of glyoxal and hardly any methylglyoxal. The absence of glyoxal in the case of sucrose would be another explanation why bakery products prepared with sucrose and NH_4HCO_3 contain low amounts of acrylamide (*10*).

Influence of Temperature and Time. The influence of temperature and time on formation of α-dicarbonyls was studied with fructose and NH_4HCO_3 in MOPS buffer (**Figure 6** and **7**). Data points for diacetyl were omitted for a better readability of the figures. After heating at 80 °C only trace amounts of the two α-dicarbonyls were detected, while at 100 °C significant amounts were determined already. The amounts increased with temperature, in the case of glyoxal exponentially (**Figure 6**). Formation of methylglyoxal was faster as compared to glyoxal. This might be explained by their formation pathways from both glucose and fructose. Methylglyoxal can be directly derived from the 1- and 3-desoxysone via a retro-aldol reaction. On the other hand, formation of glyoxal is explained via an additional step, (a) oxidation of hydroxyethanal (*34*) which originates from retro-aldol reactions of glucose, 1- or 4-desoxysone, erythrose, and glyceraldehyde, or (b) oxidation of the enaminol or enediol as shown in **Figure 4**.

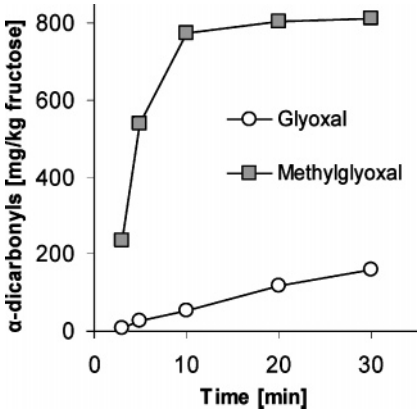

Figure 7. Influence of heating time on the generation of glyoxal and methylglyoxal from fructose and NH_4HCO_3 in MOPS buffer (120 °C; $n = 2$).

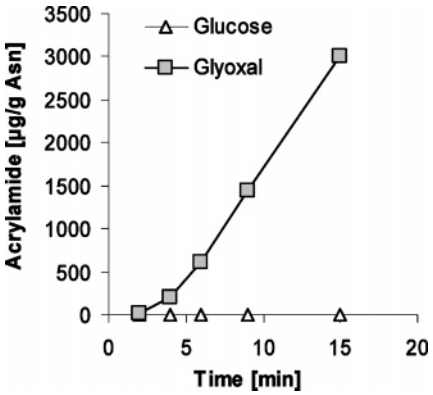

Figure 8. Formation of acrylamide from asparagine in reaction with glucose and glyoxal (MOPS buffer; 120 °C, $n = 1$).

Figure 7 shows that methylglyoxal was formed earlier and faster than glyoxal and that the concentration of methylglyoxal increased very little after 10 min. Generation of glyoxal was retarded and the amounts increased steadily with heating time. Both α-dicarbonyls were already formed at 100 °C (**Figure 6**), which may indicate that formation of such sugar fragments precedes acrylamide formation and that their generation may even be a prerequisite for acrylamide formation (see also **Figure 8** below). The fast consumption of sugars upon heating of food supports this assumption. During heating of grated potato at 120 °C glucose was consumed by about 50% in the first 10 min while almost no acrylamide was found after this time (*9*). During roasting of almonds at 165 °C one-third of glucose was already consumed after 2.5 min but no acrylamide was detectable yet (*48*). Thus, acrylamide may have been formed from asparagine and sugar fragments rather from glucose or fructose. It was also reported that sugar fragmentation precedes formation of glucosones and that sugar fragments significantly contribute to browning under mild and neutral conditions (*27*, *29*, *40*).

A similar course of formation of glyoxal and methylglyoxal in a glucose−glycine model system was shown by Hollnagel and Kroh under different conditions (buffer, temperature, concentration): The amount of methylglyoxal leveled at longer times and always exceeded that of glyoxal, which in turn increased more steadily over time (*25*).

Glyoxal was shown to form the highest amounts of acrylamide in reaction with asparagine (**Table 1**). Therefore, the influence of heating time on acrylamide formation was checked for glyoxal and glucose (**Figure 8**). MOPS buffer was chosen to minimize effects related to the type of buffer. Acrylamide

was already detected in substantial amounts in the glyoxal−asparagine system after 2 min (13 μg/g asparagine), and the contents steadily increased to very large amounts over time. In contrast, acrylamide formation in the case of glucose was several orders of magnitude lower and detected only after 9 min (0.6 μg/g asparagine). Thus, formation of sugar fragments such as glyoxal is likely to be a prerequisite for acrylamide formation under mild conditions.

Formation of α-dicarbonyls from reducing sugars is enhanced in the presence of NH_4HCO_3, which largely explains the promoting effect of this baking agent on acrylamide formation. In general, sugar fragmentation may play a key role for acrylamide formation in aqueous systems at moderate temperatures. Sugar fragments can be formed already under mild conditions via numerous pathways. These fragments form higher amounts of acrylamide when heated with asparagine as compared to glucose and fructose. The imines formed from NH_3 and sugars or sugar fragments may play an important role by facilitating various reactions including formation of 3-APA. The proposed pathway via the sugar imines formed in the presence of NH_4HCO_3 explains why replacement of either this baking agent or the reducing sugars is effective for acrylamide mitigation. Consideration of the new aspect of sugar fragments in acrylamide formation may also open further approaches for mitigation. Less acrylamide may be formed if sugar fragmentation is limited or if sugar fragments react further before they react with asparagine. The latter may be achieved by addition of other amino acids such as glycine, which has be shown to decrease the acrylamide content in bakery products (*10*, *49*).

LITERATURE CITED

(1) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.

(2) IARC Acrylamide. In *Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Industrial Chemicals*; International Agency for Research on Cancer, Ed.; IARC: Lyon, 1994; pp 389−433.

(3) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M. C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.

(4) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782−4787.

(5) Granvogl, M.; Jezussek, M.; Koehler, P.; Schieberle, P. Quantitation of 3-aminopropionamide in potatoes−A minor but potent precursor in acrylamide formation. *J. Agric. Food Chem.* **2004**, *52*, 4751−4757.

(6) Granvogl, M.; Schieberle, P. Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *J. Agric. Food Chem.* **2006**, *54*, 5933−5938.

(7) Schieberle, P.; Köhler, P.; Granvogl, M. New aspects on the formation and analysis of acrylamide. In *Chemistry and safety of acrylamide in food*; Friedman, M., Mottram, D. S., Eds.; Springer: New York, 2005; pp 205−222.

(8) Boon, P. E.; de Mul, A.; van der Voet, H.; van Donkersgoed, G.; Brette, M.; van Klaveren, J. D. Calculations of dietary exposure to acrylamide. *Mutat. Res.* **2005**, *580*, 143−155.

(9) Amrein, T. M.; Schönbächler, B.; Rohner, F.; Lukac, H.; Schneider, H.; Keiser, A.; Escher, F.; Amadò, R. Potential for acrylamide formation in potatoes: data from the 2003 harvest. *Eur. Food Res. Technol.* **2004**, *219*, 572−578.

(10) Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R. Acrylamide in gingerbread: Critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **2004**, *52*, 4282−4288.

(11) Konings, E. J. M.; Baars, A. J.; van Klaveren, J. D.; Spanjer, M. C.; Rensen, P. M.; Hiemstra, M.; van Kooij, J. A.; Peters, P. W. J. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem. Toxicol.* **2003**, *41*, 1569−1579.

(12) Springer, M.; Fischer, T.; Lehrack, A.; Freund, W. Acrylamidbildung in Backwaren (in German). *Getreide Mehl Brot* **2003**, *57*, 274−278.

(13) Weisshaar, R. Acrylamid in Backwaren-Ergebnisse von Modellversuchen (in German). *Dtsch. Lebensm.-Rundsch.* **2004**, *100*, 92−97.

(14) Biedermann, M.; Grob, K. Model studies on acrylamide formation in potato, wheat flour and corn starch; ways to reduce acrylamide contents in bakery ware. *Mitt. Lebensm. Hyg.* **2003**, *94*, 406−422.

(15) Graf, M.; Amrein, T. M.; Graf, S.; Szalay, R.; Escher, F.; Amadò, R. Reducing the acrylamide content of a semi-finished biscuit on industrial scale. *Food Sci. Technol.* **2006**, *39*, 724−728.

(16) Vass, M.; Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R. Ways to reduce the acrylamide formation in cracker products. *Czech J. Food Sci.* **2004**, *22*, 19−21.

(17) Grothe, K.; Unbehend, G.; Haase, N. U.; Ludewig, H.-G.; Matthäus, B.; Vosmann, K. Einfluss von Backtriebmitteln auf die Acrylamidgehalte von braunen Lebkuchen und Mürbekeksen (in German). *Getreidetechnol.* **2005**, *59*, 163−167.

(18) Nedvidek, W.; Ledl, F.; Fischer, P. Detection of 5-hydroxymethyl-2-methyl-3(2H)-furanone and of alpha-dicarbonyl compounds in reaction mixtures of hexoses and pentoses with different amines. *Eur. Food Res. Technol.* **1992**, *194*, 222−228.

(19) Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Grob, K.; Egli, P.; Mändli, H. Two GC-MS methods for the analysis of acrylamide in foods. *Mitt. Lebensm. Hyg.* **2002**, *93*, 638−652.

(20) Glomb, M. A.; Tschirnich, R. Detection of alpha-dicarbonyl compounds in Maillard reaction systems and *in vivo*. *J. Agric. Food Chem.* **2001**, *49*, 5543−5550.

(21) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.

(22) Biedermann, M.; Noti, A.; Biedermann-Brem, S.; Mozetti, V.; Grob, K. Experiments on acrylamide formation and possibilities to decrease the potential of acrylamide formation in potatoes. *Mitt. Lebensm. Hyg.* **2002**, *93*, 668−687.

(23) De Vleeschouwer, K.; Van der Plancken, I.; Van Loey, A.; Hendrickx, M. E. Impact of pH on the kinetics of acrylamide formation/elimination reactions in model systems. *J. Agric. Food Chem.* **2006**, *54*, 7847−7855.

(24) Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R. Factors influencing acrylamide formation in gingerbread. In *Chemistry and safety of acrylamide in food*; Friedman, M., Mottram, D. S., Eds.; Springer: New York, 2005; pp 431−446.

(25) Hollnagel, A.; Kroh, L. W. Formation of alpha-dicarbonyl fragments from mono- and disaccharides under caramelization and Maillard reaction conditions. *Eur. Food Res. Technol.* **1998**, *207*, 50−54.

(26) Severin, T.; Hiebl, J.; Popp-Ginsbach, H. Unterschuchungen zur Maillard Reaktion. XX: Nachweis von Glycerinaldehyd, Dihydroxyaceton und anderen hydrophilen Zuckerabbauprodukten in Caramellisierungsgemischen (in German). *Z. Lebensm. Unters. Forsch.* **1984**, *178*, 284.287.

(27) Hofmann, T.; Bors, W.; Stettmaier, K. Studies on radical intermediates in the early stage of the nonenzymatic browning reaction of carbohydrates and amino acids. *J. Agric. Food Chem.* **1999**, *47*, 379−390.

(28) Rydberg, P.; Eriksson, S.; Tareke, E.; Karlsson, P.; Ehrenberg, L.; Törnqvist, M. Investigations of factors that influence the acrylamide content of heated foodstuffs. *J. Agric. Food Chem.* **2003**, *51*, 7012−7018.

(29) Hayashi, T.; Namiki, M. Role of sugar fragmentation in an early stage browning of amino-carbonyl reaction of sugar with amino acid. *Agric. Biol. Chem.* **1986**, *50*, 1965−1970.

(30) Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550−5558.

(31) Bell, L. N. Maillard reaction as influenced by buffer type and concentration. *Food Chem.* **1997**, *59*, 143−147.

(32) Burton, H. S.; McWeeny, D. J. Non-enzymatic browning reactions: Consideration of sugar stability. *Nature* **1963**, *197*, 266−268.

(33) Potman, R. P.; Van Wijk, T. A. Mechanistic studies of the Maillard reaction with emphasis on phosphate-mediated catalysis. In *Thermal generation of aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 1989; pp 182−195.

(34) Thornalley, P. J.; Wolff, S.; Crabbe, J.; Stern, A. The autoxidation of glyceraldehyde and other simple monosaccharides under physiological conditions catalysed by buffer ions. *Biochim. Biophys. Acta.* **1984**, *797*, 276−287.

(35) Thornalley, P. J.; Langborg, A.; Minhas, H. S. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* **1999**, *344*, 109−116.

(36) Weenen, H. Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chem.* **1998**, *62*, 393−401.

(37) Anet, E. F. L. J. 3-Deoxyglycosuloses (3-deoxyglycosones) and the degradation of carbohydrates. *Adv. Carbohydr. Chem.* **1964**, *19*, 181−218.

(38) Belitz, H.-D.; Grosch, W.; Schieberle, P. *Food Chemistry*; Springer: Berlin, 2004.

(39) Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 565−594.

(40) Hayashi, T.; Namiki, M. Formation of 2-carbon sugar fragment at an early stage of the browning reaction of sugar with amine. *Agric. Biol. Chem.* **1980**, *44*, 2575−2580.

(41) Hauser, C. R.; Lednicer, D. Addition reactions of the methiodide of benzophenonemethylimine and its 4-methyl analog with nucleophilic reagents. *J. Org. Chem.* **1959**, *24*, 46−49.

(42) Namiki, M.; Hayashi, N. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In *The Maillard reaction in foods and nutrition*; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 21−46.

(43) Cocker, W.; Schwarz, P. Some anilo-compounds related to reductone. *Chem. Ind. (London)* **1953**, 1126−1126.

(44) Kurata, T.; Otsuka, Y. Amino-reductones, formation mechanisms and structural characteristics. In *Process-induced chemical changes in food.*; Shahidi, F., Ho, C. T., van Chuyen, N., Eds.; Plenum Press: New York, 1998; pp 269−276.

(45) Hofmann, T.; Schieberle, P. New and convenient syntheses of the important roasty, popcorn-like smelling food aroma compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine from their corresponding cyclic alpha-amino acids. *J. Agric. Food Chem.* **1998**, *46*, 616−619.

(46) Sohn, M.; Ho, C. T. Ammonia generation during thermal degradation of amino acids. *J. Agric. Food Chem.* **1995**, *43*, 3001−3003.

(47) Levine, R. A.; Smith, R. E. Sources of variability of acrylamide levels in a cracker model. *J. Agric. Food Chem.* **2005**, *53*, 4410−4416.

(48) Amrein, T. M.; Lukac, H.; Andres, L.; Perren, R.; Escher, F.; Amadò, R. Acrylamide in roasted almonds and hazelnuts. *J. Agric. Food Chem.* **2005**, *53*, 7819−7825.

(49) Bråthen, E. B.; Kita, A.; Knutsen, S. H.; Wicklund, T. Addition of glycine reduces the content of acrylamide in cereal and potato products. *J. Agric. Food Chem.* **2005**, *53*, 3259−3264.

Received for review September 8, 2006. Revised manuscript received October 27, 2006. Accepted November 5, 2006. Financial support was provided by the Swiss Federal Office of Public Health (BAG), the Federation of Swiss Food Industries (FIAL), Cooperative Migros, and COOP Switzerland.

JF0625860