MECHANISMS OF ACRYLAMIDE FORMATION
Maillard-induced transformation of asparagine

Nestlé Research Center, 1000 Lausanne 26, Switzerland; Product Technology Center Orbe, CH-1350 Orbe, Switzerland; e-mail: imre.blank@rdls.nestle.com

Abstract: The formation of acrylamide (AA) from L-asparagine was studied in Maillard model systems under pyrolysis conditions. While the early Maillard intermediate N-glucosylasparagine generated ~2.4 mmol/mol AA, the Amadori compound was a less efficient precursor (0.1 mmol/mol). Reaction with α-dicarbonyls resulted in relatively low AA amounts (0.2-0.5 mmol/mol), suggesting that the Strecker aldehyde pathway is of limited relevance. Similarly, the Strecker alcohol 3-hydroxypropanamide generated low amounts of AA (0.2 mmol/mol). On the other hand, hydroxyacetone afforded more than 4 mmol/mol AA, indicating that α-hydroxycarbonyls are more efficient than α-dicarbonyls in transforming asparagine into AA. The experimental results are consistent with the reaction mechanism proposed, i.e. (i) Strecker-type degradation of the Schiff base leading to azomethine ylides, followed by (ii) β-elimination of the decarboxylated Amadori compound to release AA. The functional group in β-position on both sides of the nitrogen atom is crucial. Rearrangement of the azomethine ylide to the decarboxylated Amadori compound is the key step, which is favored if the carbonyl moiety contains a hydroxyl group in β-position to the N-atom. The β-elimination step in the amino acid moiety was demonstrated by reacting under pyrolysis conditions decarboxylated model Amadori compounds obtained by synthesis.

Key words: Maillard reaction; acrylamide; asparagine; carbonyls; β-elimination; Strecker degradation; pyrolysis; low moisture conditions.

1. INTRODUCTION

The recent discovery of relatively high amounts of acrylamide (AA) in carbohydrate-rich foods obtained by thermal processing (review by Friedmann, 2003) has led to numerous studies to help understand how AA is
formed. Maillard-type reactions have been shown as one major reaction pathway, in particular in the presence of asparagine, which directly provides the backbone of the acrylamide molecule (Mottram et al., 2002; Stadler et al., 2002; Weisshaar and Gutsche, 2002; Biedermann et al., 2002; Becalski et al., 2003; Zyzak et al., 2003). However, other reaction pathways and precursors have also been suggested, such as acrolein formed by oxidative lipid degradation leading to acrylic acid, which can react with ammonia to give AA (Gertz and Klostermann, 2002; Yasuhara et al., 2003). Acrylic acid can also be formed from aspartic acid, in analogy to the formation of AA from asparagine (Stadler et al., 2003).

There are basically two major hypotheses published thus far pertaining to the formation of AA from asparagine in foods by Maillard-type reactions. Mottram et al. (2003) have suggested that α-dicarbonyls are necessary co-reactants in the Strecker degradation reaction affording the Strecker aldehyde as precursor of AA. Glycoconjugates, such as N-glycosides and related compounds formed in the early phase of the Maillard reaction, have been proposed as key intermediates leading to AA (Stadler et al., 2002). This hypothesis is supported by the work recently published by Yaylayan et al. (2003) and Zyzak et al. (2003). Both groups have shown some evidence for the importance of the Schiff base of asparagine, which corresponds to the dehydrated N-glucosyl compound. The key mechanistic step is decarboxylation of the Schiff base leading to Maillard intermediates that can directly release AA. However, as the key intermediates were not or only partially characterized, the chemical reactions leading to AA remained largely hypothetical.

The objective of this study was to further clarify the mechanism of AA formation from asparagine and to study the role of reaction conditions such as temperature, time, moisture, and pH.

2. EXPERIMENTAL SECTION

2.1 Materials

L-Asparagine, D-fructose, D-glucose, butane-2,3-dione, hydroxyacetone, methylglyoxal, glyoxal, glyoxalic acid, acrylamide (AA), 1-butanal, 1,2-dihydroxybutane, tetrabutylammonium chloride, potassium carbonate, 2,2,6,6-tetramethylpiperidine 1-oxyl (free radical, TEMPO), and N-chlorosuccinimide were from Fluka/Aldrich (Buchs, Switzerland). Methanol, formic acid, dichloromethane, water for HPLC, and Silica gel 60 were from Merck (Darmstadt, Germany). 3-Hydroxypropanamide was custom synthesized by Toronto Research Chemicals (Toronto, Canada). 2,3,3-\textsuperscript{2}H\textsubscript{3}-
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Acrylamide (isotopic purity 98%) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). All other reagents were of analytical grade and were used without further purification.

**Caution:** Acrylamide (CAS 79-06-1) is classified as toxic and may cause cancer. Wear suitable protective clothing, gloves and eye/face protection when handling this chemical.

### 2.2 Synthesis

The synthesis of several Maillard intermediates used in this study, such as potassium N-(D-glucos-1-yl)-L-asparaginate (PGA), N-(1-deoxy-D-fructos-1-yl)-L-asparagine (DFA), N-(1-deoxy-D-fructos-1-yl)-benzylamine (DFB), and N-(1-deoxy-D-fructos-1-yl)-2-phenylethylamine (DFP), has been reported elsewhere (Stadler et al., 2004).

2-Hydroxy-1-butanal was prepared as follows. 1,2-Dihydroxybutane (5 g, 0.056 mol), N-chlorosuccinimide (8.16 g, 0.061 mol), and tetrabutylammonium chloride (1.55 g) were added to dichloromethane (110 mL) in a round flask (0.5 L) at 0°C. Then, an aqueous buffer solution (K₂CO₃/NaHCO₃, pH 8.6, 110 mL) and TEMPO (0.87 g) were added to the mixture, which was stirred with a mechanical system for 16 h at 0°C. The organic layer was extracted and the aqueous phase washed with dichloromethane (3 x 100 mL). The organic layers were collected and dried above magnesium sulfate. The solvent was finally filtered and evaporated under vacuum. Distillation of the crude product (55 °C, 0.02 mbar) led to the target compound composed of 2-hydroxy-1-butanal and the corresponding hydroxyketone in a 3:2 ratio. The two tautomers were characterized by mass spectrometry (MS/EI, m/z, rel-%): 2-hydroxy-1-butanal: 88 (M⁺), 59 (100), 31 (55); 1-hydroxy-2-butanone: 88 (M⁺), 57 (100), 31 (55).

It should be noted that 2-hydroxy-1-butanal is very unstable and easily undergoes a time-dependent tautomerization to its hydroxyketone form under acidic condition (e.g. silica gel), as observed by NMR (data not shown). GC-MS clearly indicated the complete conversion of hydroxyaldehyde to hydroxyketone observed during separation of the 2 tautomers by column chromatography.

### 2.3 Pyrolysis procedure

The chemicals of interest (0.2 mmol each, with 20 µL water added) were heated in a temperature controlled heating module (Brouwer) at 180°C in closed 6 mL Pyrex vacuum hydrolysis tubes (16 cm x 0.9 mm) that were immersed in silicone oil. After a defined heating period, the tubes were cooled in ice. For quantification of acrylamide, the pyrolysates were spiked
with \(^2\)H\(_3\)-acrylamide (250 ng, 125 μL of 5 μg/mL), suspended in water (2.325 mL), vortexed (1 min), and sonicated in an ultrasonic water bath (5 min). After suspension, the extracts were centrifuged (3.5 min, 9000 rpm). SPE extraction cartridges (Isolute Multimode, 3 mL, 500 mg, IST Hengoed Mid Glamorgan, UK) were preconditioned with one-bead volume of methanol and two-bead volumes of water. A portion of the clear supernatant (1 mL) was loaded onto the SPE cartridge and water (1 mL) was added, both fraction being collected. The aqueous extract (2 mL) was then reduced to 500 μL under N\(_2\) at 60°C and filtered (0.2 μm syringe filters) prior to analysis of an aliquot (0.06 mL) by LC-MS/MS, as described below.

Samples for on-line measurement of headspace volatiles obtained in pyrolysis experiments were analyzed by proton transfer reaction mass spectrometry (PTR-MS) as previously described (Pollien et al., 2003). The precursors (each 0.35 mol) were ground, mixed, and heated from room temperature to 190°C at a 5°C/min heating rate. Acrylamide (m/z 72) was monitored in the scan mode (m/z 21-200, 0.2 s/mass).

2.4 Quantification of acrylamide

This was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) as recently described (Stadler et al., 2004; Riediker and Stadler, 2003) using some modifications as follows. Analytical separation was achieved by using a Shodex RSpack DE-413L HPLC column (polymethacrylate gel, 250 x 6 mm i.d., Showa Denko K.K., Japan). The elution mode was a gradient, beginning with 0.01% aqueous formic acid/methanol 90:10 (v/v) and ramping linearly to 0.01% aqueous formic acid/methanol 60:40 (v/v) within 12 min. The initial flow rate was set at 0.6 mL/min, and reduced by post-column splitting after the LC column to 0.3 mL/min.

The different fragment ion transitions at t\(_R\) = 7.5 min were m/z 72→55, m/z 72→54, and m/z 72→27 for acrylamide as well as m/z 75→58 and 75→29 for the internal standard. The MS settings were 3.2 kV capillary voltage and for acrylamide 22 V for the cone voltage. The collision energy was set at -20 eV for all acrylamide transition reactions, except the fragmentation transitions m/z 72→55 and 72→58 that were set at -11 eV. External calibration curves (6 points) were established in the concentration range from 15 pg/μL to 20000 pg/μL acrylamide, containing a defined amount of the internal standard (250 pg/μL). Good linearity was obtained for all standard curves.
3. RESULTS AND DISCUSSION

3.1 The Strecker aldehyde route

To clarify the role of the Strecker aldehyde route compared to that employing glycoconjugates, the formation of AA from the Strecker alcohol of asparagine (3-hydroxypropanamide) was studied under pyrolytic conditions. This Strecker alcohol is the direct precursor of AA as it can be formed by a one-step dehydration process. As shown in Figure 1, the lower amounts of AA were generated from the Strecker alcohol compared to the binary mixture of glucose/asparagine, in particular at milder temperatures.

![Figure 1](image-url)

*Figure 1. Formation of acrylamide from equimolar fructose/asparagine mixtures (■) and the Strecker alcohol 3-hydroxypropanamide (◇) upon pyrolysis for 5 min. (Adapted from Stadler et al., 2004).*

Considering the fact that the Strecker aldehyde first needs to be generated through a cascade of reactions and then reduced to the alcohol, it seems unlikely that the Strecker aldehyde route plays a major role in AA formation. Furthermore, it is questionable if the reducing potential of Maillard systems (Ledl and Schleicher, 1990) is sufficiently strong to reduce the Strecker aldehyde to the corresponding alcohol.

All attempts thus far failed to identify substantial amounts of the Strecker aldehyde 3-oxopropanamide. As shown in Figure 2, even on-line measuring tools based on PTR-MS indicated only traces of a compound with m/z 88 (C₃H₅NO₂, protonated). Possible fragments pointing to the Strecker aldehyde could neither be found, i.e. [M+1-NH₃]⁺ (m/z 71) and [M+1-H₂O]⁺ (m/z 70). However, the signal representing acrylamide (m/z 72) formed under the same reaction conditions could easily be monitored.
Figure 2. (A) Formation of acrylamide (m/z 72) and putative 3-oxopropanamide (m/z 88) from binary mixtures of fructose and asparagine monohydrate monitored by PTR-MS. (B) Traces of potential fragments of putative 3-oxopropanamide.

These data suggest that acrylamide is preferably generated compared to the Strecker aldehyde. Interestingly, no published data could be found on 3-oxopropanamide as Strecker aldehyde of asparagine using SciFinder®. In general, it has only been mentioned in a few papers, mainly dealing with computational chemistry related to intramolecular N-H⋯O resonance-assisted hydrogen bonding in β-enaminones (Gilli et al., 2000; Rios and Rodriguez, 1991). In fact, 3-oxopropanamide may preferably occur as 3-amino-3-hydroxy-2-propanenal stabilized by intramolecular N-H⋯O bonding.

In agreement with this suggestion, only relatively low amounts of AA were formed in the presence of α-dicarbonyls, which are known to promote
the formation of Strecker aldehydes from amino acids (Schönberg and Moubacher, 1952; Yaylayan, 2003). As shown in Table 1, the typical amounts of AA generated from asparagine in the presence of α-dicarbonyls were 0.2-0.5 mmol/mol (samples A-C). However, the α-dicarbonyl moiety is not a prerequisite for generating AA, as shown for glyoxylic acid (sample D) and 1-butanal (sample E). Surprisingly, hydroxyacetone gave rise to ca. 4 mmol/mol AA (sample G), ~10 times higher than in the samples containing α-dicarbonyls or the Strecker alcohol (sample F). However, it should be noted that the reproducibility of data obtained by pyrolysis is limited. As an example, values of 0.2-0.7 mmol/mol were obtained with methylglyoxal in the course of this study. The data are therefore only indicative, but show the tendency in reactivity.

Table 1. Acrylamide generated from asparagine in the presence of carbonyls upon pyrolysis (180 °C, 5 min, 20 μL water).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbonyl reactant</th>
<th>Acrylamide b</th>
<th>CV (%) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Butane-2,3-dione (diacetyl)</td>
<td>0.26</td>
<td>6.1</td>
</tr>
<tr>
<td>B</td>
<td>2-Oxopropanal (methylglyoxal)</td>
<td>0.52</td>
<td>5.7</td>
</tr>
<tr>
<td>C</td>
<td>Glyoxal</td>
<td>0.38</td>
<td>20.2</td>
</tr>
<tr>
<td>D</td>
<td>Glyoxylic acid</td>
<td>0.08</td>
<td>3.2</td>
</tr>
<tr>
<td>E</td>
<td>1-Butanal</td>
<td>0.01</td>
<td>2.9</td>
</tr>
<tr>
<td>F</td>
<td>3-Hydroxypropanamide</td>
<td>0.24 d</td>
<td>&lt;20</td>
</tr>
<tr>
<td>G</td>
<td>Hydroxyacetone (acetol)</td>
<td>3.97</td>
<td>23.4</td>
</tr>
<tr>
<td>H</td>
<td>2-Hydroxy-1-butanal e</td>
<td>15.8</td>
<td>13.3</td>
</tr>
<tr>
<td>I</td>
<td>Glucose</td>
<td>2.22</td>
<td>11.8</td>
</tr>
</tbody>
</table>

* Sample heated at 180 °C for 5 min. b Concentration in mmol/mol asparagine. c Coefficient of variation. d Taken from Stadler et al., 2004. e Mixture of both tautomers (see text).

The structural features of the carbonyl reactants were further studied, in particular the role of the functional group in α-position to the carbonyl group, by reacting asparagine with various functionalized carbonyls. 2-Hydroxy-1-propanal was commercially not available to compare it with hydroxyacetone. The higher homologue, 2-hydroxy-1-butanal, was synthesized and turned out to be very unstable, easily tautomerizing to the corresponding hydroxyketone as shown in Figure 3.

![Figure 3. Tautomerization of 2-hydroxy-1-aldehydes to 1-hydroxy-2-ketones.](image-url)
Thus, the sample synthesized was composed of both tautomers, with the equilibrium favoring the hydroxyketone. Consequently, a direct comparison with acetol was not possible. 2-Hydroxy-1-butanal (Table 1, sample H) was much more efficient in forming AA (15.8 mmol/mol) than was glucose (sample I, 2.2 mmol/mol) or any of the dicarbonyls. These observations demonstrate the importance of the α-hydroxyl group of the carbonyl for acrylamide formation.

3.2 The glycoconjugate route

The type of early Maillard intermediate was studied to identify the key mechanistic step transforming asparagine into AA. In dry model systems, N-glucosyl asparagine was found to be a more efficient precursor (2.4 mmol/mol) than the binary mixture of glucose and asparagine (0.7 mmol/mol). Moreover, the corresponding Amadori compound N-(1-deoxy-D-fructos-1-yl)-L-asparagine (DFA) formed only traces of AA (0.1 mmol/mol), suggesting the key precursor to be a Maillard intermediate prior to the Amadori rearrangement. As shown in Figure 4, AA can be formed from N-glucosyl asparagine under mild reaction conditions.

![Graph of acrylamide formation over time](image)

*Figure 4. Formation of acrylamide from potassium N-(D-glucos-1-yl)-L-asparaginate over time at 100°C. (Adapted from Stadler et al., 2004).*

The Schiff base, which is relatively stable under low moisture conditions (Zyzak et al., 2003), and the decarboxylated Amadori compound (Yaylayan et al., 2003) have been suggested as a key intermediates. We have prepared model decarboxylated Amadori compounds, i.e. N-(1-deoxy-D-fructos-1-yl)-
benzylamine (DFB) and \(N\)-(1-deoxy-D-fructos-1-yl)-2-phenylethylamine (DFP) (Stadler et al., 2004), to study the validity of the \(\beta\)-elimination step suggested by Yaylayan et al. (2003). Only the latter Maillard intermediate shows a \(\beta\)-proton susceptible to a Hofmann-type \(\beta\)-elimination reaction. Indeed, GC-MS analysis of the reaction products indicated that styrene was only generated from DFP, while its lower homologue DFB (no \(\beta\)-proton) resulted in the Strecker degradation product benzaldehyde (Figure 5). This observation supports the hypothesis that AA formation may proceed via \(\beta\)-elimination of early Maillard intermediates based on asparagine. Consequently, the Strecker aldehyde route is likely to be a minor source of acrylamide compared to asparagine glycoconjugates.

\[\text{Figure 5. Formation of (A) benzaldehyde and (B) styrene from model Amadori compounds. (Adapted from Stadler et al., 2004).}\]

### 3.3 Mechanism of acrylamide formation from asparagine

The experimental data shown above confirm the role of early Maillard intermediates as suggested earlier, \(i.e.\) \(N\)-glycosyl asparagine (Stadler et al., 2002) and the corresponding decarboxylated Schiff base (Zyzak et al., 2003) and decarboxylated Amadori compound (Yaylayan et al., 2003). The role of the \(\beta\)-elimination step was substantiated to release AA from the Maillard intermediate. Furthermore, some critical features of the carbonyl reactant were found, \(i.e.\) \(\alpha\)-hydroxycarbonyls (\(e.g.\) acetol) are more efficient than \(\alpha\)-dicarbonyls (\(e.g.\) 2,3-butandione). On the basis of these observations, the following mechanistic scheme is proposed, which is in line with the experimental data obtained (Figure 6).
The first critical step is the amino-carbonyl reaction of asparagine and a carbonyl compound, preferably an α-hydroxycarbonyl, resulting in the corresponding conjugate, which under elevated temperatures dehydrates forming the Schiff base. Under low moisture conditions, both the N-glycosyl compound and Schiff base are relatively stable. However, in aqueous systems, the Schiff base may hydrolyze to the precursors or rearrange to the Amadori compound (pathway I), not an efficient precursor in AA formation. Even under low moisture conditions, this reaction is the main pathway initiating the early Maillard reaction cascade that leads to 1- and 3-deoxyosones. The deoxosones then further decompose to generate color and flavor (Ledl and Schleicher, 1990). This is in agreement with the relatively low transformation yield of asparagine to AA, typically below 1 mol%.

Alternatively, the Schiff base may decarboxylate to the intermediary azomethine ylide (pathway II), which after tautomerization leads to the decarboxylated Amadori compound (pathway III). The prerequisite for this reaction is the presence of an OH-group in β-position to the N-atom. As α-hydroxycarbonyls are proper precursors to yield such azomethine ylides, in contrast to α-dicarbonyls, reactants such as 1-hydroxy-2-ketones (e.g. fructose, acetol) and 2-hydroxyaldehydes (glucose, 2-hydroxy-1-butanal) generate more AA than do α-dicarbonyls (2,3-butanedione, methylglyoxal).

Decarboxylation of the Schiff base to the azomethine ylide may proceed via the zwitterionic form (pathway IIa), claimed as more probable (Grigg et al., 1988, Grigg and Thianpatanagul, 1984) compared to the classical Strecker degradation mechanism (Schönberg and Moubacher, 1952). Alternatively, the Schiff base may undergo an intramolecular cyclization to the oxazolidine-5-one derivative (pathway IIb). Such compounds have been reported to easily decarboxylate (Manini et al., 2001), thus giving rise to stable azomethine ylides, which after tautomerization lead to the decarboxylated Amadori compound (pathway III).

Acrylamide is then released, along with the corresponding aminoketone, via a β-elimination reaction and cleavage of the carbon-nitrogen covalent bond. Our model decarboxylated Amadori compounds confirmed the β-elimination step. This mechanistic pathway is supported by the fact that co-pyrolysis of a reducing sugar with aspartic acid, glutamine, and phenylalanine also leads to the corresponding vinylogous compounds, i.e. acrylic acid (Stadler et al., 2003), 3-butenamide (Weisshaar and Gutsche, 2002), and styrene (Keyhani and Yaylayan, 1996), respectively.
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Figure 6. Formation of acrylamide from asparagine in the presence of α-hydroxycarbonyls. R represents the rest of the carbonyl moiety. (Adapted from Stadler et al., 2004).

Zyzak and coworkers (2003) have reported some evidence for the decarboxylated Schiff base of asparagine in model systems containing an excess of reducing sugar by MS(ESI+) measurement of m/z 251, which however may also represent the decarboxylated Amadori compound. They suggested AA to be formed directly from the azomethine ylide (pathway IV)
and claimed that the decarboxylation of the Schiff base to be the limiting step. In the oxazolidine-5-one pathway (Yaylayan et al., 2003), it is not the decarboxylation step that is thought to be the limiting step, but rather the cleavage of the strong carbon-nitrogen covalent bond in the β-elimination reaction.

The formation of AA in Maillard reaction systems containing reducing sugars, i.e. polyhydroxy carbonyls, may alternatively proceed as shown in Figure 7. This scheme employs the classical Strecker degradation mechanism leading to a concomitant release of CO₂ and water. H-transfer in the intermediary imine gives rise to a secondary amine, which undergoes the β-elimination reaction to afford AA. The driving force for this reaction is the carbonyl group of the sugar moiety in γ-position to the nitrogen atom. However, none of the intermediates were thus far isolated to substantiate this hypothesis.

Results obtained with binary mixtures composed of carbonyls and asparagine (Table 1) clearly indicate that the α-dicarbonyl function is not a prerequisite for AA formation. Also Becalski et al. (2003) found low AA levels in dry systems containing diacetyl (0.2 mmol/mol). Mottram et al. (2002) reported 0.5 mmol/mol. However this concentration was almost 10-times lower compared to the glucose system. On the other hand, we found α-hydroxycarbonyls to be very efficient precursors for AA formation.

Figure 7. Hypothesis of the formation of acrylamide from asparagine in the presence of reducing sugars.

This observation can be explained by the type of azomethine ylide preferably formed upon decarboxylation of the Schiff base. As shown in
Figure 8, the Schiff base leads after decarboxylation to the azomethine ylide with a 1,3-dipole structure shown in two resonance-stabilized forms. In general, the final location of the proton in the neutral imines depends on the kinetically controlled proton transfer to the site of the dipole with the greatest electron density (Grigg and Thianpataanagul, 1984). However, as the negative charge in 1b can delocalize at the carbonyl group in β-position to the nitrogen atom, the 1,2-prototropic H-shift may preferably lead to imine 2b, which upon hydrolysis furnishes the Strecker aldehyde.

Alternatively, the azomethine ylide 1a may react to imine 2a, which hydrolyzes to the decarboxylated amino acid, *i.e.* 3-aminopropionamide from asparagine. This compound has been reported to release acrylamide (Zyzak et al., 2003, Granvogl et al., 2004). Thus, the relatively low amounts of acrylamide reported in Table 1 (samples A-C) is most likely due to preferred formation of imine 2b, resulting in a Strecker aldehyde that does not release high amounts of AA.

In the presence of α-hydroxycarbonyls, imines 4a and 4b can be formed (Figure 9). Due to the higher electron density in the azomethine ylide 3b (hydroxy group in β-position to the N-atom), the 1,2-prototropic H-shift preferably leads to imine 4b, which upon hydrolysis furnishes the Strecker aldehyde. However, imine 4a formed in a side reaction via the azomethine ylide 3a has the required structural features to rearrange to the decarboxylated Amadori compound, which can then undergo the β-elimination reaction, directly affording the vinyllogous compound, *i.e.* acrylamide from asparagine. This reaction is less probable with imine 2a obtained in the presence of α-dicarbonyls (Figure 8).
Figure 8. Formation of acrylamide ($R' = \text{CONH}_2$), 3-aminopropanamide (primary amine), and the 3-oxopropanamide (Strecker aldehyde) from asparagine ($R' = \text{CONH}_2$) in the presence of an $\alpha$-dicarbonyl.
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Thus, the relatively low transformation yield (< 1 mol%) of asparagine to AA might be explained as described below:

- The major Maillard reaction flux follows 1,2- and 2,3-enolization pathways (Amadori pathway I in Figure 6) forming deoxyosones;
- Imines 2b and 4b are preferably formed, leading to Strecker aldehydes with low efficiency for acrylamide release;
- Imines 2a and 4a are minor reaction products, of which only 4a shows proper structural features needed to rearrange to the decarboxylated Amadori compound, which can then release AA;
- Reaction conditions such as temperature, moisture, pH, and time may markedly influence the various steps of the reaction cascade until AA is released.

3.4 Effect of reaction parameters on the formation of acrylamide from asparagine

It is known that AA formation is favored under low moisture conditions. The water content may affect both the chemical reaction and the physical state of the reaction system, which is linked to reaction temperature and heat transfer. However, the role of water in AA formation has not been studied in great detail. As shown in Figure 10, the amounts of AA do indeed depend on the water content. In the reaction system based on fructose, the AA increases with increasing water content. They accumulate in the 200 μL water sample and decline at higher water content. The glucose system showed a broad maximum in the 20-200 μL water samples. The physical state of the reaction systems changes from solid to suspension (50-200 μL water) with increasing solubility to become a solution (500 μL water).
Low pH has been reported as a means to control AA amounts in certain food products (Jung et al., 2003). This idea refers to the fact that the initial amino-carbonyl reaction is hampered due to protonation of the amino group at low pH. Our data indicate only a relatively weak influence of the pH on AA formation from asparagine (Figure 11). However, the fructose reaction series formed significantly less AA at pH 3 (2.2 mmol/mol), while pH 8 represented optimum reaction conditions forming more AA (3.3 mmol/mol).

Also Rydberg et al. (2003) reported optimal AA formation at around pH 8. Interestingly, all samples containing fructose (Figure 10) gave rise to higher amounts of AA compared to glucose. Even in the sample with no water, fructose was more efficient, despite the fact that glucose, as an aldohexose, should be chemically more reactive, since the aldehyde group is not hydrated. Therefore, other parameters than chemical reactivity may play a role to explain this phenomenon, which is subject of ongoing studies dealing with the physical state and its effect on AA formation (Robert et al., 2004).
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Figure 11. Formation of acrylamide in binary mixtures of asparagine and glucose (black bars) or fructose (white bars) as function of pH (180°C, 5 min, 20 μL water).

Figure 12. Formation of acrylamide by heating a binary equimolar mixture of asparagine and glucose for 5 (▲) and 60 (●) minutes.

In another series of experiments, the role of reaction time and temperature (heat load) was investigated. As shown in Figure 12, the interplay of these parameters strongly influenced AA formation. Pyrolysis of glucose and asparagine at different temperatures for either 5 min or 60 min resulted in different curves, indicating high amounts of AA formed already at 120°C at long reaction times, whereas 160°C was required to obtain
highest amounts of AA at short pyrolysis times. These two parameters are covariant and represent a means for controlling AA formation under food processing conditions. The decline of the curves is most likely due to polymerization as recently shown (Stadler et al., 2004).

3.5 Conclusions

Our studies have confirmed that glycoconjugates of asparagine are the major source of AA in foods under low moisture conditions at elevated temperatures in the presence of reducing sugars or a suitable carbonyl source. Moisture content and heat load (reaction time versus temperature) may be suitable parameters to minimize AA formation. Occurrence and chemical reactivity of the precursors are also important factors to consider. However, the physical state of the reaction system may be equally essential to achieve reduced AA amounts, while keeping desirable product attributes such as flavor and color generated by similar Maillard reaction pathways. This aspect is subject of further studies and will be published elsewhere.

REFERENCES

Mechanisms of Acrylamide Formation


