RESEARCH ARTICLE

Conversion of 3-aminopropionamide and 3-alkylaminopropionamides into acrylamide in model systems

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Carbonyl compounds have been shown to play a major role in the conversion of asparagine into acrylamide. However, it is unclear at this point if its role is only restricted to the decarboxylation of the amino acid or if carbonyl compounds also play a role in the deamination reaction of the decarboxylated intermediates 3-aminopropionamide and 3-(alkylamino)propionamides. This study describes the deamination reaction of 3-aminopropionamide and 3-(alkylamino)propionamides (benzyl, phenylethyl, butyl, and octyl) in model systems and in the presence, or not, of different carbonyl compounds (alkadienals, alkenals, and alkanals). All these reactions were mainly produced at almost neutral or basic pH values. In addition, the reaction yields and the activation energies not only depended on the type of aminopropionamide involved but also on the water activity (a_w) and in the presence, or not, of carbonyl compounds. However, there was not a clear correlation among the activation energies calculated for the different deamination reactions and the yields of acrylamide obtained; therefore, suggesting the existence of diverse pathways by which 3-aminopropionamide and 3-(alkylamino)propionamides are converted into acrylamide. In addition, these reactions are also competing with other carbonyl–amine reactions when carbonyl compounds are present. All these results suggest that the type of the intermediate aminopropionamide involved is going to play a major role in both the amount of acrylamide produced and the conditions required for its formation. On the other hand, the role of carbonyl compounds in the acrylamide produced, but not in the activation energy of the reactions implicated, seems to be more limited than either the type of amine or the a_w. A detailed analysis of the type of the intermediate aminopropionamide formed in foods may help to define strategies for mitigating the formation of this food toxicant.

Keywords:
3-(Alkylamino)propionamide / 3-Aminopropionamide / Acrylamide / Carbonyl–amine reactions / Maillard reaction

1 Introduction

Acrylamide is produced by degradation of asparagine in the presence of reducing sugars as a consequence of the Maillard reaction [1–5]. The general features for the mechanisms involved in this reaction are now known. Thus, different studies have pointed out to the decarboxylated Schiff base [*N*-(D-glucos-1-yl)-3′-aminopropionamide] and the corresponding Amadori product [*N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide] as possible direct precursors, in addition to 3-aminopropionamide [6–10].

Analogously to reducing sugars, other carbonyl compounds, like some produced in the lipid oxidation pathway, are also able to degrade amino acids, including asparagine, to their corresponding vinylogous derivatives [11–12]. Although the mechanisms involved in these reactions are not fully elucidated yet, the decarboxylation of the

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Abbreviations: a_w, water activity; E_a, activation energy

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amino acid and the later deamination of the biogenic amines produced seem to be key steps.

All these results point out to the deamination reaction of 3-aminopropionamide, or its *N*-alkyl derivative, as the final step in the formation of acrylamide. However, the role of carbonyl compounds in the deamination reaction of 3-aminopropionamide (or its *N*-alkyl derivative) is not yet clarified. In fact, Granvogl *et al.*, found that 3-aminopropionamide produced less acrylamide in the presence of a reducing sugar than in its absence [13]. On the other hand, Perez Locas and Yaylayan found that acrylamide formation from 3-aminopropionamide in the presence of excess sugar was enhanced sixfold relative to the system with no sugar, and this increase was even more enhanced in a high moisture system [10].

In an attempt to understand the reaction pathways by which carbonyl compounds are able to convert asparagine into acrylamide, this study analyzes in detail the deamination reaction of both 3-aminopropionamide and 3-(alkylamino)propionamides in the presence, or not, of carbonyl compounds. As model carbonyl compounds, alkanals, alkenals, and alkadienals, were selected in order to compare the influence of the aldehyde structure. In addition, 2,4-decadienal has been shown to convert asparagine into acrylamide to a high extent in model systems [12].

2 Materials and methods

2.1 Materials

All chemicals were purchased from Aldrich (Milwakee, WI, USA), Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany), and were analytical grade. 3-Aminopropionamide was obtained from TCI Europe (Zwijndrecht, Belgium). Labeled [1,2,3-$^{13}C_3$]acrylamide was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). 2,4-Decadienal (93%) was obtained from Aldrich. It was further purified by column chromatography on silica gel 60 using hexane–acetone (9:0.25) as solvent. The aldehyde recovered from the column was chromatographically pure as determined by GC.

3-(Alkylamino)propionamides were prepared by reaction of acrylamide with alkylamines (benzylamine, phenylethylamine, butylamine, and octylamine). Briefly, acrylamide (14.5 mmol) was dissolved in methanol (29 mL) and treated with the alkylamine (14.5 mmol). The obtained solution was heated at 60°C for 24 h and, then, the solvent was evaporated. The residue was treated with toluene–hexane (1:1) in order to crystallize the 3-(alkylamino)propionamide (except for 3-(phenylethylamino)propionamide, which was crystallized from *m*-xylene). The obtained compounds were recrystallized using the same solvent employed for the crystallization. Chemical structures of the prepared compounds were confirmed by ^{1}H and ^{13}C NMR and MS.

3-(Benzylamino)propionamide: ^{1}H NMR (CD_3OD): δ 2.44 *t* (2H, $J = 6.9$ Hz, $C\underline{H}_2CONH_2$), 2.84 *t* (2H, $J = 6.9$ Hz, $CH_2C\underline{H}_2NH$), 3.76 *s* (2H, $PhC\underline{H}_2NH$), and 7.34 *m* (5H, Ph); ^{13}C NMR (CD_3OD): δ 35.64 ($\underline{C}H_2CONH_2$), 45.85 ($CH_2\underline{C}H_2NH$), 54.26 ($Ph\underline{C}H_2NH$), 128.82 (C4 of Ph), 129.49 (C2, C3, C5, and C6 of Ph), 140.46 (C1 of Ph), and 177.36 ($CONH_2$); MS (relative intensity, ion structure): *m/z* 178 (0.4, M^+), 120 (6, $M^+ - CH_2CONH_2$), 118 (17), 106 (73, $M^+ - CH_2CH_2CONH_2$), 91 (100, $PhCH_2$), 87 (31, $NHCH_2CH_2CONH_2$), 65 (15), and 44 (21).

3-(Phenylethylamino)propionamide: ^{1}H NMR (CD_3OD): δ 2.41 *t* (2H, $J = 6.9$ Hz, $C\underline{H}_2CONH_2$), 2.86 *t* (2H, $J = 6.9$ Hz, $CH_2C\underline{H}_2NH$), 2.82 *m* (4H, $PhC\underline{H}_2C\underline{H}_2NH$), and 7.23 *m* (5H, Ph); ^{13}C NMR (CD_3OD): δ 35.64 ($\underline{C}H_2CONH_2$), 36.71 ($Ph\underline{C}H_2CH_2NH$), 46.32 ($CH_2\underline{C}H_2NH$), 51.93 ($PhCH_2\underline{C}H_2NH$), 127.29 (C4 of Ph), 129.56 and 129.69 (C2, C3, C5, and C6 of Ph), 140.93 (C1 of Ph), and 177.26 ($CONH_2$); MS (relative intensity, ion structure): *m/z* 193 (0.1, $M^+ + 1$), 134 (2, $M^+ - CH_2CONH_2$), 105 (14, $PhCH_2CH_2$), 101 (100, $M^+ - PhCH_2$), 91 (100, $PhCH_2$), 84 (75, $M^+ - PhCH_2 - NH_3$), 44 (12), and 42 (55).

3-(Butylamino)propionamide: ^{1}H NMR (CD_3OD): δ 0.95 *t* (3H, $J = 7.3$ Hz, $C\underline{H}_3$), 1.37 *sx* (2H, $J = 7.3$ Hz, $CH_3C\underline{H}_2$), 1.50 *qu* (2H, $J = 7.3$ Hz, $CH_3CH_2C\underline{H}_2$), 2.43 *t* (2H, $J = 6.9$ Hz, $C\underline{H}_2CONH_2$), 2.61 *t* (2H, $J = 7.3$ Hz, $CH_3CH_2CH_2C\underline{H}_2$), and 2.84 *t* (2H, $J = 6.9$ Hz, $CH_2C\underline{H}_2NH$); ^{13}C NMR (CD_3OD): δ 14.28 (CH_3), 21.46 ($CH_3\underline{C}H_2$), 32.44 ($CH_3CH_2\underline{C}H_2$), 35.33 ($\underline{C}H_2CONH_2$), 46.38 ($CH_2\underline{C}H_2NH$), 50.06 ($CH_3CH_2CH_2\underline{C}H_2$), and 177.22 ($CONH_2$); MS (relative intensity, ion structure): *m/z* 144 (1, M^+), 101 (55, $M^+ - CH_3CH_2CH_2$), 84 (65, $M^+ - CH_3CH_2CH_2 - NH_3$), 56 (22, $M^+ - CH_3CH_2CH_2 - NH_3 - CO$), 44 (100), and 42 (77).

3-(Octylamino)propionamide: ^{1}H NMR (CD_3OD): δ 0.90 *t* (3H, $J = 6.7$ Hz, $C\underline{H}_3$), 1.32 *m* (10H, 5 $C\underline{H}_2$), 1.51 *m* (2H, $CH_2C\underline{H}_2CH_2NH$), 2.42 *t* (2H, $J = 6.9$ Hz, $C\underline{H}_2CONH_2$), 2.59 *t* (2H, $J = 7.3$ Hz, $CH_2CH_2C\underline{H}_2NH$), and 2.83 *t* (2H, $J = 6.9$ Hz, $CH_2C\underline{H}_2NH$); ^{13}C NMR (CD_3OD): δ 14.44 (CH_3), 23.74 ($CH_3\underline{C}H_2$), 28.40 ($\underline{C}H_2CH_2CH_2NH$), 30.36 ($CH_2\underline{C}H_2CH_2NH$), 30.41 and 30.63 (C4 and C5 of octyl chain), 33.01 ($CH_3CH_2\underline{C}H_2$), 35.41 ($\underline{C}H_2CONH_2$), 46.41 ($CH_2\underline{C}H_2NH$), 50.40 ($CH_2CH_2\underline{C}H_2NH$), and 177.29 ($CONH_2$); MS (relative intensity, ion structure): *m/z* 200 (1, M^+), 171 (1, $M^+ - CH_3CH_2$), 157 (1, $M^+ - CH_3CH_2CH_2$), 142 (3), 128 (13), 101 (100, M^+–heptyl), 84 (57, M^+ – heptyl – NH_3), 56 (9, M^+ – heptyl–NH_3–CO), 44 (27), and 42 (29).

2.2 Carbonyl–amine reactions

Model reactions were carried out analogously to Granvogl and Schieberle [9], with the modifications described by Zamora and Hidalgo [12]. Briefly, mixtures of the amino compound (3.75 μmol) and the carbonyl compound (0–3.75 μmol) were singly homogenized with

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0.063–0.200 mm silica gel 60 (300 mg) (Macherey-Nagel, Düren, Germany), 30 μL of 0.3 M buffer (sodium citrate for pH 3–6 and sodium phosphate for pH 6–8) and 0–270 μL (0–45%) of water, and heated under nitrogen at 180°C in closed test tubes for 10 min, unless otherwise indicated. The amino compounds assayed were 3-aminopropionamide, 3-(benzylamino)propionamide, 3-(phenylethylamino)propionamide, 3-(butylamino)propionamide, and 3-(octylamino)propionamide. The carbonyl compounds assayed were 2,4-decadienal, 2,4-heptadienal, 2,4-hexadienal, 2-octenal, 2-pentenal, and hexanal. The water activity (a_w) of the samples was determined with a Pawkit Decagon analyzer (Pullman, WA, USA). The a_w determined when 0, 75, and 150 μL of water were added to the reaction mixtures were: 0.60, 0.73, and 0.95, respectively. Additions of more than 150 μL of water always resulted in a_w 1. The reaction pH was maintained upon heating.

After cooling (15 min at −20°C), 10 μL of internal standard solution (1 mg/mL of labeled [1,2,3-$^{13}C_3$]acrylamide in methanol) and 2 mL of 0.3 M sodium citrate buffer, pH 2.2, were added. Suspensions were stirred for 1 min, the supernatant was then filtered and its acrylamide content determined. In addition, other samples were cooled (15 min at −20°C) and extracted with methanol (2 × 2 mL). These extracts were studied directly by GC–MS.

2.3 Analysis of acrylamide

Acrylamide was analyzed as the stable 2-bromopropenamide by GC–MS using the method of Castle *et al.* [14] with the modifications of Andrawes *et al.* [15]. Briefly, 1 mL of the supernatant was treated with 0.3 g of potassium bromide and 400 μL of saturated bromine solution in water. After 1 h in the dark at 0°C, the excess of bromine was removed by addition of 1 M sodium thiosulfate until the solution became colorless, and the solution was extracted with 1 mL of ethyl acetate/hexane (4:1). The organic layer was finally dried with sodium sulfate, evaporated until a volume of ~50 μL, treated with 50 μL of triethylamine, and analyzed by GC–MS.

The ions monitored for the identification of the analyte, 2-bromopropenamide, were $[C_3H_4NO]^+ = 70$, $[C_3H_4\,^{79}BrNO]^+ = 149$, and $[C_3H_4\,^{81}BrNO]^+ = 151$, using *m/z* 149 for quantitation. The ions monitored for identification of the internal standard (2-bromo[$^{13}C_3$]propenamide) were $[^{13}C_2H_3\,^{81}Br]^+ = 110$ and $[^{13}C_3H_4\,^{81}BrNO]^+ = 154$, using *m/z* 154 for quantitation. Mass spectra of both compounds are collected, for example, by Pittet et *al.* [16]. The separation of acrylamide analyte after derivatization was performed on GC capillary columns of middle to high polarity. GC–MS analyses were conducted with a Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (Mass Selective Detector-Quadrupole type). In most experiments, a 30 m × 0.25 mm id × 0.25 μm HP5-MS capillary column was used. Working conditions were as follows: carrier gas helium (1 mL/min at constant flow); injector, 250°C; oven temperature: from 50 (1 min) to 240°C at 5°C/min and then to 325°C at 10°C/min; transfer line to MSD, 280°C; and ionization EI, 70 eV.

Quantification of acrylamide was carried out by preparing standard curves of this compound in the 300 mg of silica gel and following the whole procedure described in Section 2.2. For each curve, 15 different concentration levels of acrylamide (0–200 μg) were used. Acrylamide content was directly proportional to the acrylamide/internal standard area ratio ($r = 0.999$, $p < 0.0001$). The coefficients of variation at the different concentrations were lower than 10%.

2.4 GC–MS analyses

GC–MS analyses of heated 3-aminopropionamide/2,4-decadienal reaction mixtures were carried out in an attempt to identify other reaction products in addition to acrylamide. A Hewlett-Packard 6890 GC Plus coupled with an Agilent 5973 MSD (Mass Selective Detector-Quadrupole type) and a 30 m × 0.25 mm id × 0.25 μm HP5-MS capillary column was used in these experiments. Working conditions were as follows: carrier gas helium (1 mL/min at constant flow); injector, 250°C; oven temperature: from 40 (1 min) to 240°C at 5°C/min and then to 300°C at 10°C/min; transfer line to MSD, 280°C; and ionization EI, 70 eV.

3 Results

3.1 Effect of reaction conditions in the formation of acrylamide during the heating of 3-amino propionamide, 3-(benzylamino)propionamide, and their mixtures with 2,4-decadienal

The heating of either 3-aminopropionamide or 3-(benzylamino)propionamide in the presence or in the absence of 2,4-decadienal always produced acrylamide to an extent that depended on the reaction conditions, including the reaction pH and the a_w. Thus, the amount of acrylamide increased with pH until achieving a maximum at pH 6–8 (Fig. 1). When 3-aminopropionamide was heated alone, the acrylamide yield depended on the a_w (Fig. 1A). Thus, at a_w 0.6 the reaction yield after heating 10 min at pH 7 was 41%. However, this yield was lower (11%) for a_w 0.95.

A similar behavior was observed when 3-aminopropionamide was heated in the presence of an equimolecular amount 2,4-decadienal (Fig. 1B). When the aldehyde was present, the acrylamide yield was 42% at a_w 0.6 and pH 7, and 18% at a_w 0.95 and the same pH.

These yields increased, and significant differences were observed, when the formation of acrylamide from 3-(benzylamino)propionamide was studied (Fig. 1C). Thus, a significant acrylamide yield (20%) was observed at pH 2.15 and this yield increased with pH until achieving yields of about 50% at pH values higher than 5. In addition, very similar yields were obtained at the two a_w assayed.

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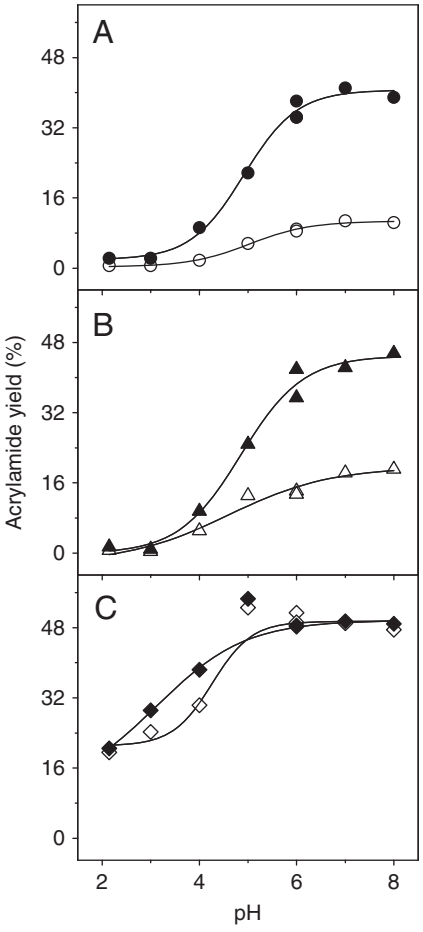

Figure 1. Effect of reaction pH in the formation of acrylamide from: (A) 3-aminopropionamide, (B) an equimolecular mixture of 3-aminopropionamide and 2,4-decadienal, and (C) 3-(benzylamino)propionamide, at 180°C for 10 min. Reaction mixtures were heated at two water activities: 0.60 (filled symbols) and 0.95 (open symbols).

This major role of a_w in the formation of acrylamide from 3-aminopropionamide, and not from 3-(benzylamino)propionamide, was confirmed when the effect of the water added to the reaction mixture was studied (Fig. 2). Thus, the acrylamide yield decreased linearly between a_w 0.6 and 0.95 (water addition between 0 and 150 μL, respectively) for 3-aminopropionamide heated alone ($r = -0.98$, $p < 0.0001$) or in the presence of 2,4-decadienal ($r = -0.99$, $p < 0.0001$). Further additions of water did not reduce the amount of acrylamide produced. In fact, the amount of acrylamide produced from 3-aminopropionamide heated alone seemed to increase when a higher amount of water was present. Differently to 3-aminopropionamide, 3-(benzylamino)propionamide always produced acrylamide to very similar extents when heated under different a_w. According to all these results, pH 7 and two a_w were employed in the rest of this study. The selected a_w were 0.6 (no water addition), which produced the highest acrylamide yields and still

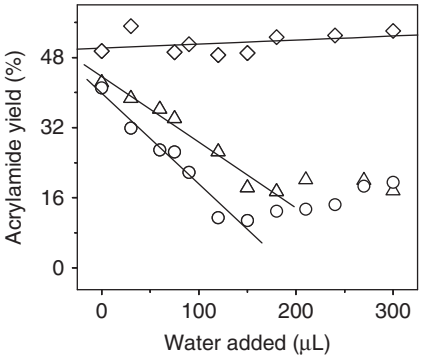

Figure 2. Effect of water content in the formation of acrylamide from: 3-aminopropionamide (○), an equimolecular mixture of 3-aminopropionamide and 2,4-decadienal (△), and 3-(benzylamino)propionamide (◇), at 180°C for 10 min.

maintained the pH, and 0.95 (addition of 150 μL of water), in which the highest effect of 2,4-decadienal was observed.

3.2 Effect of 2,4-decadienal in the formation of acrylamide during the heating of 3-amino propionamide or 3-(benzylamino) propionamide

As shown in Figs. 1 and 2, the presence of 2,4-decadienal seemed to have an effect on the acrylamide produced in the degradation of 3-aminopropionamide. This was confirmed when 3-aminopropionamide was heated in the presence of increasing amounts of 2,4-decadienal, but only at relatively high a_w (Fig. 3). As shown in the figure, acrylamide yield increased linearly ($r = 0.9999$, $p < 0.0001$) as a function of 2,4-decadienal concentration at a_w 0.95 but not at a_w 0.6. This increase was also observed at the intermediate a_w 0.73.

Differently to 3-aminopropionamide, the presence of 2,4-decadienal did not play a significant role in the amount of acrylamide produced by 3-(benzylamino)propionamide decomposition. As observed in the figure, the amount of acrylamide obtained was always approximately the same independently of the presence of 2,4-decadienal and the a_w. For this reason, only the decomposition of 3-(benzylamino)propionamide in the absence of 2,4-decadienal was analyzed in the rest of this study.

3.3 Determination of activation energies for the formation of acrylamide during the heating of 3-aminopropionamide, 3-(benzylamino) propionamide, and their mixtures with 2,4-decadienal

Acrylamide yield always increased linearly ($r > 0.972$, $p < 0.009$) as a function of the heating time and temperature. The formation of acrylamide from 3-aminopropionamide as a function of the heating time at the two a_w assayed is shown in

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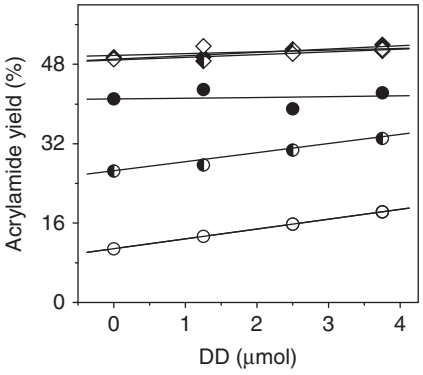

Figure 3. Effect of decadienal concentration in the formation of acrylamide from 3-aminopropionamide (○), and 3-(benzylamino)propionamide (◇), at 180°C for 10 min. Reaction mixtures were heated at three water activities: 0.60 (filled symbols), 0.73 (half-filled symbols), and 0.95 (open symbols).

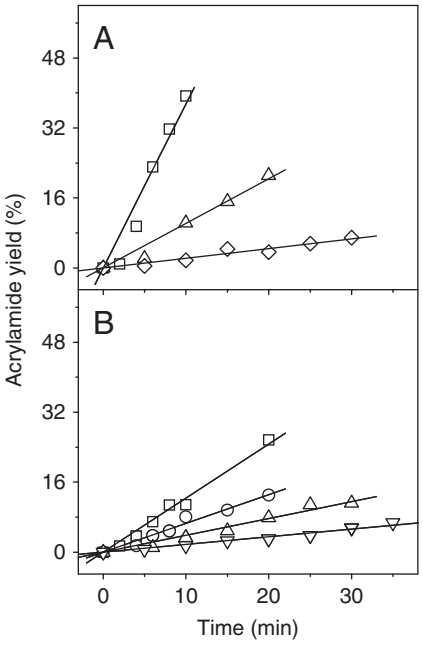

Figure 4. Effect of time and temperature in the formation of acrylamide from 3-aminopropionamide at two water activities: (A) 0.60 and (B) 0.95. The assayed temperatures were: 180 (□), 170 (○), 160 (△), 150 (▽), and 140 (◇) °C.

Fig. 4. Reaction rates at the different assayed temperatures were calculated by using the following equation:

$$[\text{acrylamide}] = [\text{acrylamide}]_0 + kt \qquad (1)$$

where $[\text{acrylamide}]_0$ represents the intercept, k is the rate constant, and t is the time. These rate constants were used in an Arrhenius plot (Fig. 5A) for the calculation of activation energy (E_a) of acrylamide formation from 3-aminopropionamide at the two a_w assayed. The obtained

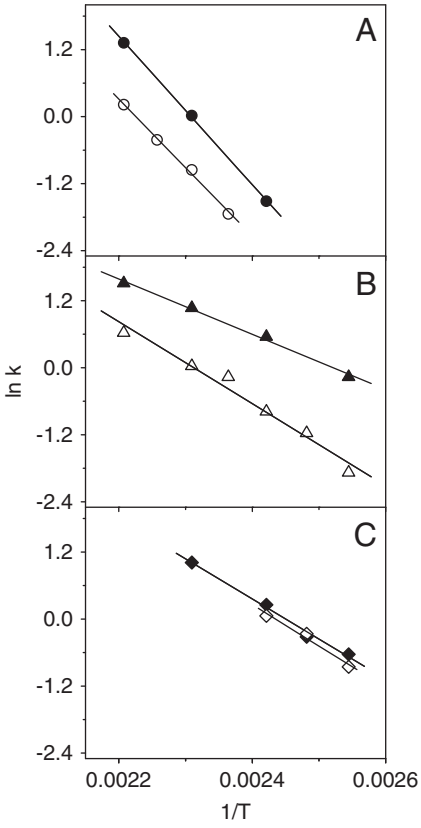

Figure 5. Arrhenius plot for acrylamide formation from: (A) 3-aminopropionamide, (B) an equimolecular mixture of 3-aminopropionamide and 2,4-decadienal, and (C) 3-(benzylamino)propionamide. Reaction mixtures were heated at two water activities: 0.60 (filled symbols) and 0.95 (open symbols).

E_a are listed in Table 1. Although a_w had a major role in the amount of acrylamide formed, it did not have major effects in the determined E_a. In fact, they were relatively similar among them and were higher than 100 kJ/mol.

Analogously to 3-aminopropionamide heated alone, when 3-aminopropionamide was heated in the presence of 2,4-decadienal, acrylamide yield also increased linearly as a function of the heating time and temperature at the two assayed a_w (Fig. 6). Reaction rates, calculated as described previously, were also used in an Arrhenius plot (Fig. 5B) for the calculation of E_a of acrylamide formation from 3-aminopropionamide in the presence of 2,4-decadienal at the two a_w assayed. The obtained E_a are listed in Table 1. The presence of 2,4-decadienal decreased the E_a of 3-aminopropionamide degradation and a_w had a major role in the obtained E_a. Thus, the E_a of the degradation of 3-aminopropionamide in the presence of 2,4-decadienal was 61 kJ/mol at a_w 0.95 and it decreased to 41 kJ/mol at a_w 0.6.

Acrylamide yield also increased linearly as a function of the heating time and temperature at the two assayed a_w when 3-(benzylamino)propionamide was heated (Fig. 7). As

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Table 1. Activation energies (E_a) of acrylamide formation from 3-aminopropionamide and 3-(benzylamino)propionamide

Amino compound	Carbonyl compound	a_w	E_a (kJ/mol)
3-Aminopropionamide	None	0.6	110
		0.95	102
3-Aminopropionamide	2,4-Decadienal	0.6	41
		0.95	61
3-(Benzylamino) propionamide	None	0.6	60
		0.95	62

Values were determined at pH 7.

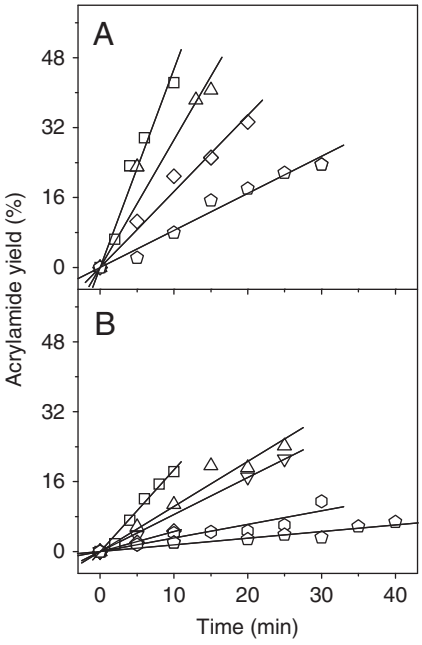

Figure 6. Effect of time and temperature in the formation of acrylamide from an equimolecular mixture of 3-aminopropionamide and 2,4-decadienal at two water activities: (A) 0.60 and (B) 0.95. The assayed temperatures were 180 (□), 160 (△), 150 (▽), 140 (◇), 130 (hexagon), and 120 (pentagon) °C.

expected, and differently to the heating of 3-aminopropionamide in the presence, or not, of 2,4-decadienal, the observed increases in acrylamide yield at the two a_w analyzed were similar among them. In fact, reaction rates, calculated according to Eq. (1), were very similar and the Arrhenius plot (Fig. 5C) exhibited two lines, which were very close to one another. E_a of acrylamide formation from 3-(benzylamino)-propionamide at the two a_w assayed were calculated from the slopes of these lines. The obtained results are summarized in Table 1. E_a for acrylamide formation from 3-(benzylamino)-propionamide at the two a_w assayed were almost identical among them. In addition, they had the same value as acrylamide formation from 3-aminopropionamide in the presence of 2,4-decadienal at a_w 0.95.

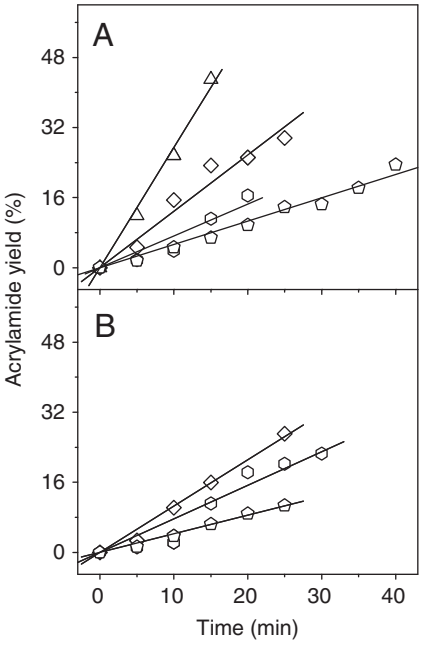

Figure 7. Effect of time and temperature in the formation of acrylamide from 3-(benzylamino)propionamide at two water activities: (A) 0.60 and (B) 0.95. The assayed temperatures were 160 (△), 140 (◇), 130 (hexagon), and 120 (pentagon) °C.

3.4 Effect of the type of aldehyde and the alkyl chain of 3-(alkylamino)propionamide in the formation of acrylamide

In an attempt to understand the role of the aldehyde structure, and the role of the alkyl chain in 3-(alkylamino)-propionamide, in the acrylamide yield, different aldehydes and 3-(alkylamino)propionamides were assayed. The assayed aldehydes were 2,4-decadienal, 2,4-heptadienal, 2,4-hexadienal, 2-octenal, 2-pentenal, and hexanal. All of them were tested by studying the degradation of 3-aminopropionamide at a_w 0.95. These conditions were selected because 2,4-decadienal showed its highest effect under these conditions. The obtained results (Table 2) showed that acrylamide yield was similar for the different alkadienals assayed. However, it decreased for alkenals, and no positive effect was observed for the tested saturated aldehyde.

Differently to the unsaturation of the alkyl chain in the carbonyl compound, when different alkyl chains were present in the amino group of the aminopropionamide, all assayed 3-(alkylamino)propionamides produced analogous acrylamine yields (Table 3).

3.5 Formation of lipid derivatives in 3-aminopropionamide/2,4-decadienal reaction mixtures

3-Aminopropionamide/2,4-decadienal reaction mixtures were also studied by GC–MS to determine the formation of

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Table 2. Effect of carbonyl compounds in the formation of acrylamide by degradation of 3-aminopropionamide

Carbonyl compound	Acrylamide (%)
2,4-Decadienal	18.3
2,4-Heptadienal	18.1
2,4-Hexadienal	18.5
2-Octenal	14.2
2-Pentenal	15.1
Hexanal	8.6
None	10.8

Equimolecular mixtures of the carbonyl compound and 3-aminopropionamide at pH 7 and a_w 0.95 were heated at 180°C for 10 min.

Table 3. Effect of the alkyl chain in the formation of acrylamide by degradation of 3-(alkylamino)propionamides

3-(Alkylamino)propionamide	Acrylamide (%)
3-(Benzylamino)propionamide	49.0
3-(Phenylethylamino)propionamide	49.6
3-(Butylamino)propionamide	48.7
3-(Octylamino)propionamide	50.7
3-Aminopropionamide	10.8

Amino compounds at pH 7 and a_w 0.95 were heated at 180°C for 10 min.

other compounds in addition to acrylamide. Among other compounds, the formation of 2-pentylpyridine was observed. This compound was identified on the basis of its retention index and mass spectrum as compared with those of a standard.

4 Discussion

The above results show that the conversion of 3-aminopropionamide and 3-(alkylamino)propionamides into acrylamide is a process that depends on reaction conditions as well as on the type of amine involved. Amine deamination is an energetically difficult process due to the basicity of the leaving group. For this reason, the E_a mainly depended on the amino group involved. Thus, the E_a was 100–110 kJ/mol for primary amino groups, such as in 3-aminopropionamide, when carbonyl groups were not present. The common method of deamination for these amines is through their conversion into quaternary ammonium salts followed by Hofmann elimination (Fig. 8). This reaction is favored at basic pH values and in the absence of water [17], which is in accordance to the results shown in Fig. 1.

When the amino group was alkylated, the E_a decreased considerably ($E_a \cong 60$ kJ/mol). This is likely a consequence of the changes produced in the mechanisms involved. Thus, in addition to ammonium salts, the 3-(alkylamino)propionamides can also be transformated into iminium ions, which can later suffer a milder elimination [18] to produce acrylamide (Fig. 8).

In the presence of carbonyl compounds, 3-(alkylamino)-propionamides are not expected to change significantly their deamination mechanism and no differences should be expected whether the carbonyl compound is present or not. However, this is not the case for 3-aminopropionamide. Carbonyl compounds can form easily imines with 3-aminopropionamide, which can later evolve into iminium ions as an intermediate step in the formation of acrylamide. Figure 8 shows the reaction mechanism proposed for the conversion of 3-aminopropionamide into acrylamide in the presence of alkadienals. In this case, the reaction is favored by the aromatization of the lipid, which produces 2-pentylpyridine. In fact, 2-pentylpyiridine has been found to be produced during the formation of acrylamide by 3-aminopropionamide degradation, and also by asparagine degradation, in the presence of 2,4-decadienal. The similarity between the elimination step in this mechanism and that proposed for 3-(alkylamino)propionamides is in agreement with the similarity of determined E_a for these degradations. Only the E_a determined for 3-aminopropionamide degradation in the presence of 2,4-decadienal at a_w 0.6 was lower, which may be related to the stabilities of Schiff bases at low a_w. In addition, this mechanism is also in agreement with the reactivities exhibited by the different carbonyl compounds. Thus, the formation of the pyridine ring should favor the elimination reaction and no significant differences should be expected among different alkadienals. In addition, the formation of the iminium ion should also be less favored for alkenals, which are not able to cycle into pyridines. Nevertheless, this iminium ion would be conjugated. Finally, alkanals should be the least reactive compounds for this reaction. Alternatively, 3-aminopropionamide can also undergo Michael addition with carbonyl compounds. In this case, the Michael adduct would also form exactly the same pyridine ring as when the reaction is initiated by the formation of the imine. Thus, Michael addition would distinguish alkadienals and alkenals from alkanals in catalyzing elimination, because alkanals can only undergo imine formation and are prone to be deactivated in the presence of water. On the other hand, alkenals and alkadienals can undergo both types of interactions (imine and Michael adduct formation) and are more stable due to conjugation.

Although E_a can be easily interpretated on the basis of the proposed reaction mechanisms, the acrylamide yields obtained did not clearly follow the order found for E_a. Thus, after heating for 10 min at 180°C and a_w 0.6, acrylamide yields followed the following order (Fig. 3): 3-(benzylamino)propionamide/2,4-decadienal $\cong$ 3-(benzylamino) propionamide $>$ 3-aminopropionamide/2,4-decadienal $\cong$ 3-aminopropionamide. This order changed at a_w 0.95: 3-(benzylamino)propionamide/2,4-decadienal $\cong$ 3-(benzylamino)propionamide $\gg$ 3-aminopropionamide/2,4-decadienal $>$ 3-aminopropionamide. Two main differences were

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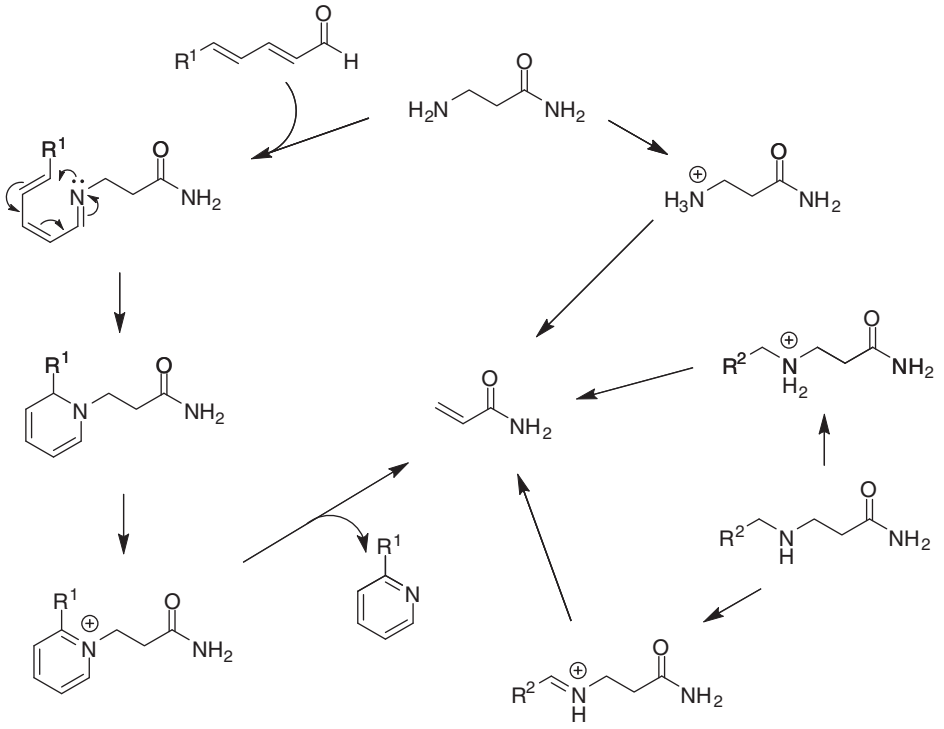

Figure 8. Proposed pathways for the degradation of 3-aminopropionamide and 3-(benzylamino)-propionamide in the presence, or not, of 2,4-decadienal.

observed between acrylamide yields and E_a. The first one was the effect of a_w. Water activity had not a major effect in the E_a of 3-aminopropionamide degradation, but it clearly determined the yield of acrylamide produced. On the contrary, the null effect of a_w observed in the E_a of 3-(benzylamino)propionamide degradation and the decrease of E_a when a_w decreased in 3-aminopropionamide/2,4-decadienal mixtures were clearly correlated to the obtained acrylamide yields. These apparently contradictory results might be related to a different role of a_w in the Hofmann elimination of 3-aminopropionamide and in the elimination reaction of the quaternary iminium ions formed with either 3-(benzylamino)propionamide or 3-aminopropionamide/2,4-decadienal. The second significant difference was the relatively low contribution of carbonyl compounds to acrylamide yields in spite of its significant role in lowering E_a. This is likely a consequence of the reactivity of these compounds, which can react with amino compounds in several other ways, in addition to contribute to its deamination. Thus, the formation of Strecker aldehydes and diverse heterocyclic compounds, among other reactions, has been described by carbonyl–amine reactions in amino acids/alkadienals reactions mixtures [19–21].

All these results suggest the existence of diverse competitive pathways by which 3-aminopropionamide and 3-(alkylamino)propionamides are converted into acrylamide. At low a_w and high temperatures, both types of amino compounds are converted rapidly into acrylamide to high extent (40–50%) and carbonyl compounds do not seem to play a significant role, at least in relation to the amount of acrylamide produced. When a_w increases, the conversion of 3-(alkylamino)propionamides into acrylamide does not seem to change significantly. However, the amount of acrylamide produced from 3-aminopropionamide decreases and carbonyl compounds have a positive effect in the amount of acrylamide formed. Therefore, in foods, the type of precursor involved (either 3-aminopropionamide or 3-(alkylamino)propionamides that are produced during the Maillard reaction or even 3-(alkylamino)propionamides that might be naturally present in those foods) is likely to play a major role in the amount of acrylamide produced and the conditions required for its formation. A detailed analysis of these types of intermediates in the different foods may help to define strategies for mitigating the formation of acrylamide.

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