

Toward a Kinetic Model for Acrylamide Formation in a Glucose–Asparagine Reaction System

JEROEN J. KNOL,^{†,§} WIL A. M. VAN LOON,^{†,§} JOZEF P. H. LINSSEN,^{*,†,‡}
 ANNE-LAURE RUCK,[‡] MARTINUS A. J. S. VAN BOEKEL,[†] AND
 ALPHONS G. J. VORAGEN[‡]

Product Design and Quality Management Group and Laboratory of Food Chemistry,
 Department of Agrotechnology and Food Science, Wageningen University,
 Post Office Box 8129, 6700 EV Wageningen, The Netherlands

A kinetic model for the formation of acrylamide in a glucose–asparagine reaction system is proposed. Equimolar solutions (0.2 M) of glucose and asparagine were heated at different temperatures (120–200 °C) at pH 6.8. Besides the reactants, acrylamide, fructose, and melanoidins were quantified after predetermined heating times (0–45 min). Multiresponse modeling by use of nonlinear regression with the determinant criterion was used to estimate model parameters. The proposed model resulted in a reasonable estimation for the formation of acrylamide in an aqueous model system, although the behavior of glucose, fructose, and asparagine was slightly underestimated. The formation of acrylamide reached its maximum when the concentration of sugars was reduced to about 0. This supported previous research, showing that a carbonyl source is needed for the formation of acrylamide from asparagine. Furthermore, it is observed that acrylamide is an intermediate of the Maillard reaction rather than an end product, which implies that it is also subject to a degradation reaction.

KEYWORDS: Acrylamide; multiresponse kinetic modeling; Maillard reaction

INTRODUCTION

In April 2002, Tareke et al. (1) reported that several heat-processed foods contain relatively high amounts of acrylamide. These results were confirmed by other research groups: fried and baked potato products were found to contain the highest amounts of acrylamide, followed by cereal products, whereas only low amounts were detected in meat products (2–4). A high amount in foods is of major concern as acrylamide is known to be a neurotoxic, genotoxic, and carcinogenic compound in animals (5), which is classified by the IARC (6) as a probable human carcinogen.

About half a year later the suggestion was published that acrylamide is formed in the Maillard reaction and that asparagine is the precursor (7–9). Further elucidation of the pathway by use of ¹³C-labeled isotopes showed that asparagine needs a carbonyl source to form acrylamide (10, 11). According to Friedman (5), the proposed mechanisms predict that acrylamide may result from the general reaction of asparagine with any aldehyde or ketone. Simultaneously, many researchers focused on optimization of methods for determination of acrylamide. A

review about this subject was published by Wenzl et al. (12). Evaluation of the health risk resulted in the recommendation that intake levels of acrylamide should be reduced to protect health, although there is no direct risk on cancer with the current intake of about 35 μg day⁻¹ person⁻¹ (13, 14). Possible ways to reduce the formation of acrylamide include reducing the temperature, reducing precursors during processing, and influencing the pathway of formation by changing the pH (15–17). More information on research, analysis, formation, and control of acrylamide is available in an extensive review that was published recently (18).

The Maillard reaction is highly complex and cannot be described with simple reaction kinetics. To better understand mechanisms behind the formation of acrylamide, an advanced approach is necessary (19). Kinetic modeling, using multiresponse models, has proven to be a useful tool to enable further unraveling of reaction mechanisms in the Maillard reaction (20, 21). Knowledge of kinetics provides the tool to predict the effect of certain time–temperature combinations in a quantitative way.

Model systems of asparagine and a carbonyl such as glucose, both dry and in aqueous solutions, have been used mainly to study the pathway of acrylamide formation (3, 22). The yield of acrylamide was about 0.1% of asparagine, and a wide range of other compounds (e.g., melanoidins) was found. Furthermore, an effect of time and temperature on both formation and

* To whom correspondence should be addressed: telephone +31-317-483548; fax +31-317-483669; e-mail jozef.linszen@wur.nl.

[†] Product Design and Quality Management Group.

[‡] Laboratory of Food Chemistry.

[§] These authors contributed equally.

degradation of acrylamide has been found. Recently, a kinetic model for the formation of acrylamide in potato, wheat, and rye model systems was published by Wedzicha et al. (23). However, using food model systems to gain kinetic insights into reactions can cause disturbances by the presence of food matrix components. Therefore, the aim of this study was to attempt to model the formation of acrylamide in a glucose–asparagine reaction system at different time–temperature combinations. Once information about the kinetics of formation of acrylamide is obtained, one can move on to more realistic systems to describe the effect of complicating factors as present in real foods.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: D-glucose, D-fructose, Na_2HPO_4 , and KH_2PO_4 (Merck, Darmstadt, Germany) and L-asparagine (Fluka, Buchs, Switzerland). All chemicals used in this study were of analytical grade.

Preparation of Reaction Mixtures. Equimolar solutions of glucose and asparagine (0.2 M) were prepared in phosphate buffer (0.1 M, pH 6.8). Samples (10 mL) were heated in hermetically closed screw-capped glass tubes (Schott, 16 × 160 mm) at 120, 140, 160, 180, and 200 °C in an oil bath. The tubes were immersed in the oil up to the cap. At predetermined heating times (0, 1, 2, 4, 9, 15, 30, and 45 min), samples were taken and immediately cooled in ice and stored at –20 °C prior to analysis. Experiments were carried out in triplicate.

Analysis of Acrylamide. The analysis of acrylamide was based on the HPLC method described by Barber et al. (24). Prior to analysis, samples were diluted in Millipore water (1:10) and centrifuged for 5 min at 16000g. The HPLC system used for the analysis consisted of a P4000 pump, an AS3000 autosampler, and a UV3000 detector (Thermo Separation Products, San Jose, CA). A Synergi 4 μm hydro reversed-phase C18 column (80 Å, 250 × 2.00 mm) with AJO-4286 guard column (Phenomenex, Aschaffenburg, Germany) was used for the analysis (25). Samples (20 μL) were eluted isocratically at 20 °C with a solution containing 1% methanol and 99% 5 mM heptanesulfonic acid in Millipore water at a flow rate of 0.2 mL/min. Acrylamide ($t_r = 6.2$ min) was detected by its absorbance at 200 nm with a UV detector and quantified by the external standard procedure with a calibration curve. The limit of detection obtained ($10 \mu\text{g kg}^{-1}$) was similar to what Barber et al. (24) found.

Analysis of Sugars. The diluted samples (1:10) were analyzed for sugars by HPLC (see Analysis of Acrylamide) by use of the method of Martins et al. (26). An ion-exchange column (ION-300, Interaction Chromatography Inc., San Jose, CA) with guard column was used for the analysis at 85 °C. Sulfuric acid (2.5 mM) in Millipore water was used as the eluent with a flow rate of 0.4 mL/min. Sugars were detected by monitoring the refractive index and quantified by the external standard procedure with a calibration curve. The formation of sugar isomers in the Maillard reaction has been confirmed by HPLC and electrochemical detection recently (27, 28).

Analysis of Asparagine. Samples were diluted 1:1000 with Millipore water and the EZ:faast amino acid analysis kit (Phenomenex, Aschaffenburg, Germany) was used for determination of asparagine (29). Norvaline was added as an internal standard (20 nmol) before sample preparation. After sample preparation, samples were analyzed on a Carlo Erba GC5300 system (Interscience BV, Breda, The Netherlands) on a Zebtron amino acid column (10 m × 0.25 mm) that was included in the kit. Samples (2.5 μL) were injected onto the column at 250 °C (split ratio 1:15). The oven temperature started at 110 °C and was programmed to heat to 250 °C at a rate of 20 °C/min, followed by a 1 min isothermal hold. The external standard procedure was used for quantification.

Analysis of Melanoidins. Quantification of melanoidins was performed by measuring the absorbance at 470 nm on a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). When necessary, the samples were diluted with Millipore water. The concentration of melanoidins was calculated from the Lambert–Beer equation with an

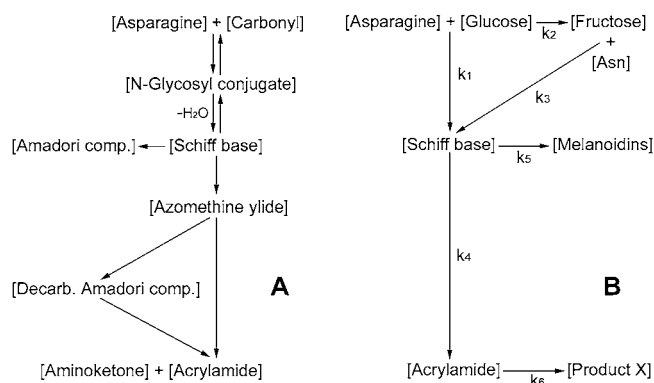


Figure 1. (A) Reaction network for the formation of acrylamide from asparagine through early Maillard reaction adapted from Stadler et al. (26). (B) Simplified reaction network for the formation of acrylamide from asparagine and glucose/fructose through early Maillard reaction. Asn, asparagine; Decarb. Amadori comp., decarboxylated Amadori compound; Product X, assumed product(s) formed from acrylamide.

extinction coefficient of 282 L/mol·cm (30), a value derived for melanoidins formed from glucose and asparagine. The concentration of melanoidins is thus expressed as moles of sugar incorporated in the brown polymers. Although melanoidins are of course complex mixtures of various molecules, several studies have shown that they can be quantified in this way (20, 31, 32).

Kinetic Modeling. A kinetic model was derived from the proposed reaction network taken from Stadler et al. (33) (shown in Figure 1). Our arguments for using the reaction network published by Stadler et al. (33) and the steps taken to derive a kinetic model will be discussed further on. For each reaction step, a differential equation was set up, and these were translated into a mathematical model. The differential equations were solved by numerical integration. For both numerical integration and parameter estimation, the software package Athena Visual Studio v. 10.0 (34) was used. The parameters of the model, that is, the rate constants and activation energies, were estimated by nonlinear regression by use of the determinant criterion (20). For modeling purposes, the individually measured concentrations were used (measured in triplicate). For the visualization of the experimental results (Figure 2), the average values with their standard deviation (represented by the error bars) were used.

RESULTS AND DISCUSSION

Identification and Quantification of Reactants and Main Products. During heating of the glucose–asparagine reaction mixtures, the concentrations of the reactants decreased over time. As expected, the rate of degradation of glucose and asparagine increased with temperature. At 180 and 200 °C, a complete loss of glucose was observed after 9 and 5 min, respectively (Figure 2A). The loss of asparagine (Figure 2B) was slower compared to the loss of glucose. A complete loss of asparagine was measured at 180 and 200 °C after 30 and 9 min, respectively. The loss of sugar being faster than the loss of amino acid is in line with what has been found in a glucose–glycine reaction system by Martins and Van Boekel (20). The slower loss can possibly be explained by the regeneration of asparagine from the initial condensation products such as the Amadori rearrangement product and the possible formation of diglucosylamine (20).

As fructose and mannose are known to be formed from glucose by isomerization (26), some formation of these compounds was expected. Analysis showed no formation of mannose, but fructose was indeed formed (Figure 2C). This corresponds with findings of Brands and Van Boekel (35) and Martins and Van Boekel (20), where only fructose was found as an isomerization product of glucose. There was a clear

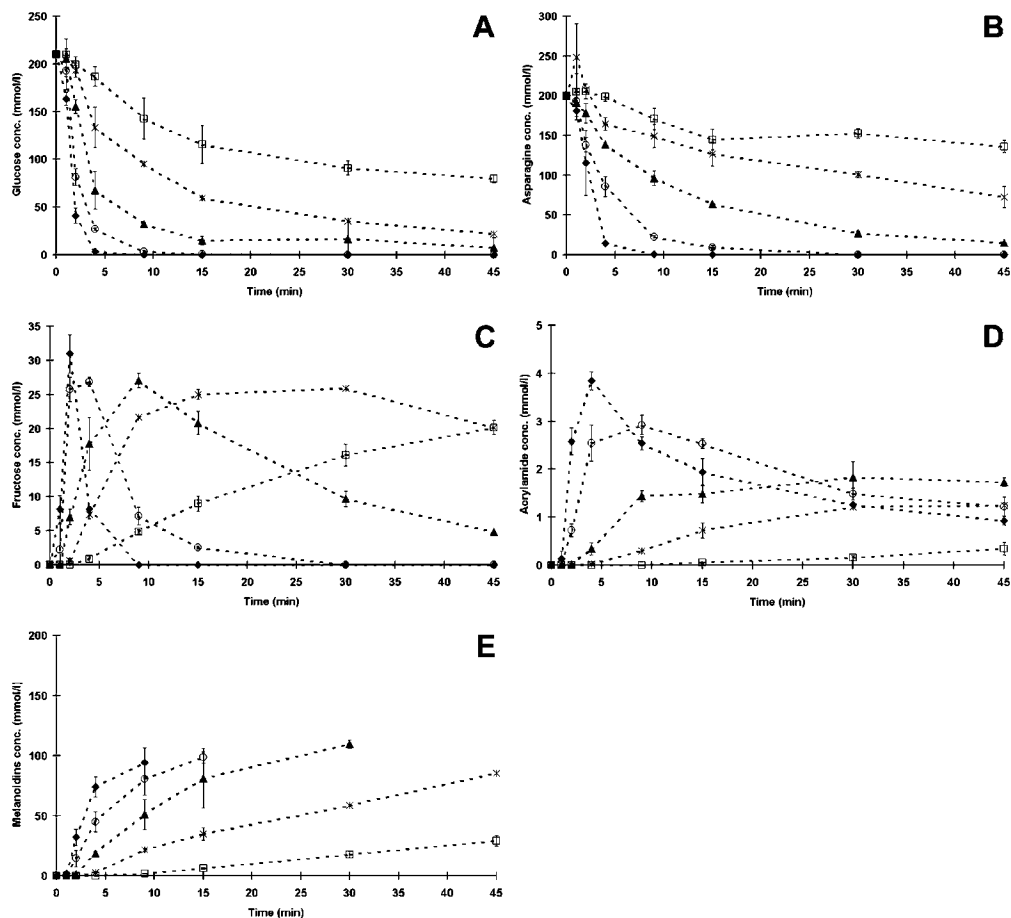


Figure 2. Experimental data of glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120 (□), 140 (*), 160 (▲), 180 (○), and 200 °C (◆). (A) Loss of glucose; (B) loss of asparagine; (C) formation of fructose; (D) formation of acrylamide; (E) formation of melanoidins.

maximum at 160, 180, and 200 °C in the formation of fructose at 9, 4, and 2 min, respectively. For the reaction mixtures heated at 180 and 200 °C, these maximum values were followed by a complete loss after 30 and 9 min, respectively. The decrease in fructose concentration can be explained by the reversible isomerization reaction between glucose and fructose so that fructose re-forms back into glucose, which is then consumed. However, it is also quite likely that fructose participates in the formation of acrylamide (36–38).

The initial rate of formation of acrylamide increased with temperature (Figure 2D). At 140 and 160 °C, the increase in acrylamide concentration was followed by a steady state in which the formation of acrylamide was probably in equilibrium with its degradation. At 180 and 200 °C, the increase was followed by a fast decrease. The maximal acrylamide formation at 180 and 200 °C corresponded with the time at which the concentration of glucose and fructose reached zero (Figure 2A,C). This is in line with the findings of Yaylayan et al. (10), who showed that asparagine needs a carbonyl source to form acrylamide. The decrease in acrylamide can be explained by its reactive nature. The increase and subsequent decrease of the acrylamide concentration at 180 and 200 °C is a typical behavior for an intermediate (20). Acrylamide may have polymerized or reacted further in Michael-type addition reactions (33). Wedzicha et al. (23) attributed the loss of acrylamide after prolonged heating at 180 °C to the reactivity of acrylamide with food components. These pathways have not been fully elucidated, and further research in this area is necessary to expand the reaction network of the Maillard reaction between asparagine and glucose.

Melanoidins are known as the main end products of the Maillard reaction. The structure of these brown nitrogenous polymers and copolymers is largely unknown, which makes quantification difficult. Previous studies have shown that it is possible to relate the absorbance to the number of sugar molecules incorporated in the melanoidins in aqueous model systems (20, 30, 32). At 160, 180, and 200 °C, we observed after some time the formation of insoluble particles, which are probably high molecular weight melanoidins. The absorbance measurements were hindered by the scattering effect of these particles. Moreover, the values for absorbance decreased during formation of these particles, causing an underestimation of the melanoidins concentration. Because of this, we excluded the results for the reaction mixtures where these solid compounds were found. This was the case for the reaction mixtures heated for 45 min at 160, 180, and 200 °C, for 30 min at 180 and 200 °C, and for 15 min at 200 °C. Considerable browning was observed in samples during heating. The induction time, during which no browning was detected, decreased with increasing temperature (Figure 2E). Furthermore, the formation of acrylamide could not be associated directly with the degree of coloring of the reaction mixtures at 160, 180, and 200 °C after 15, 9, and 4 min, respectively. This is due to their difference in status: acrylamide is an intermediate, whereas melanoidins are the main end products of the Maillard reaction. As our proposed kinetic model applies only to an aqueous model system, this relationship cannot be used yet directly in food systems. The relationship between acrylamide and color formation in foods, for instance the one found in yeast-leavened wheat bread by Surdyk et al. (37), is induced by effects such as concentration

gradients, diffusion and evaporation, and the presence of other compounds such as other amino acids and sugars and buffering agents. Therefore, more research is needed with model systems that can take these effects into consideration.

Proposal of a Kinetic Model Based on a Reaction Network. By analyzing the reactant degradation and the formation of the main products in the Maillard reaction between glucose and asparagine, a kinetic model could be proposed. The mathematical model derived from the kinetic model was then confronted with the experimental data to test the hypothesized model. Several reaction networks for the formation of acrylamide in foods have been proposed. These publications have revealed the Maillard reaction as the major reaction pathway involved (3, 7–9). The hypothesis proposed by Mottram and co-workers (7, 23) emphasizes the pathway of Strecker degradation. The hypothesis proposed by Stadler et al. (8), however, pointed toward glycoconjugates such as N-glycosides formed in the early Maillard reaction as key intermediates. A recent publication by Stadler et al. (33) supports their hypothesis as well as the work published by Zyzak et al. (11) and Yaylayan et al. (10). Furthermore, Stadler et al. (33) suggest that the mechanism proposed by Mottram et al. (7) is not a main one. We did not pursue the identification and quantification of all these suggested intermediates in this study and, therefore, we are not able to shed more light on this matter. However, we chose the reaction network proposed by Stadler et al. (8), shown in **Figure 1A**, as initial basis for the proposal of a kinetic model, as their proposed reaction network has been more elaborately described in recent literature (33). To fit the model to the experimental data, this reaction network was first adapted to reduce the number of parameters. Because the intermediates (e.g., N-glycosyl conjugate, azomethine ylide) were not analyzed and quantified in our experiments, the number of parameters was relatively high in comparison to the number of measured responses. Second, the new reaction network proposed in **Figure 1B** also takes the measured responses for fructose and melanoidins and the observed loss of acrylamide into account. The formation of fructose was suggested by including the reversible isomerization reaction between glucose and fructose. Furthermore, the reaction between asparagine and fructose was added, since fructose is also a reactant in the formation of acrylamide (36–38). The formation of melanoidins was suggested to result from further reaction of Schiff base, and the loss of acrylamide was accounted for by the putative formation of hitherto unknown product(s).

Test of the Hypothesized Mechanism. The proposed reaction model presented in **Figure 1B** was translated into a mathematical model. For each reaction step, a differential equation was set up by use of the law of mass action, and the obtained differential equations were solved by numerical integration. The temperature dependence, which plays a major role in the Maillard reaction, was also taken into account by including an Arrhenius relationship between the rate constants (k) of the different reactions. The Arrhenius equation

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \quad (1)$$

where k_0 is the so-called frequency factor, R is the gas constant [8.314 J/(mol·K)], E_a is the activation energy (joules/mole), and T is the absolute temperature (kelvins), was reparametrized to avoid statistical problems in estimation (39) as follows:

$$k = X \exp(-YE_a) \quad (2)$$

where

$$X = k_0 \exp\left(\frac{-E_a}{RT_{av}}\right) \quad (3)$$

$$Y = \frac{1}{R}\left(\frac{1}{T} - \frac{1}{T_{av}}\right) \quad (4)$$

$$T_{av} = \frac{\sum T}{n} \quad (5)$$

The model was fitted to the data obtained at 120, 140, 160, 180, and 200 °C at pH 6.8 simultaneously by substituting eq 2 for the rate constants.

At the lower reaction temperatures (120 and 140 °C), quite a good fit was observed for all compounds (**Figures 3A–E**). For the acrylamide and melanoidins concentrations, the model fits the data well at all temperatures (**Figure 3D,E**). However, the good fit for the melanoidins concentration may have been partially caused by the lack of data for the higher temperatures. For the estimation of the acrylamide concentration, the model was not restrained by experimental data for the products formed in the degradation reaction from acrylamide, and therefore, the model was able to fit the loss of acrylamide (k_6) to the experimental observations for acrylamide. More knowledge about the reaction products from acrylamide would validate the estimated rate constants for the formation and loss of acrylamide.

The observed lack of fit for the individual responses was rather high, especially for fructose and at the higher temperatures. The model underestimated the decrease in glucose concentration, especially during the first 10 min (**Figure 3A**). The increase in fructose was also underestimated, but the model predicted the tendencies as found in the experimental observations (**Figure 3C**). The same goes for asparagine, where there was an underestimation for the decrease (**Figure 3B**). These underestimations are undoubtedly caused by the limitations of the present model. Reactions of glucose and fructose are limited to the isomerization reaction and the reaction with asparagine to form the Schiff base. The model does not take into account the degradation of sugars to carbohydrate fragments and the formation of compounds via the Strecker degradation route. Therefore, this model is currently under further investigation, but nevertheless the results already show some remarkable phenomena that are worth discussing.

The estimated parameters, that is, rate constants and activation energies, are shown in **Table 1**. The rate constants for the different reaction temperatures have been derived from the estimated values found for X . The estimated values of X are the same as the derived values for the rate constants at 160 °C, as T_{av} in this case is 160 °C. The temperature dependence is consistent for all six reactions, but the precision of estimation for the parameters is low. The average 95% highest posterior density interval is about $\pm 27\%$ for the rate constants and $\pm 11\%$ for the activation energies. The activation energy for the formation of the Schiff base from fructose and asparagine is about 2 times higher than the activation energy for the same formation reaction with glucose and asparagine as reactants, suggesting that the contribution from fructose becomes more important at higher temperatures. The rate of the Schiff base formation reaction from fructose and asparagine (k_3) increases, therefore, much faster with increasing temperature than the rate constant of reaction 1 (k_1). At 120 °C, k_3 is 35% lower than k_1 , but at 180 °C k_3 is 2–3 times higher than k_1 . This is consistent with the observations by Robert et al. (36) that fructose is more efficient in the formation of acrylamide than glucose by a factor

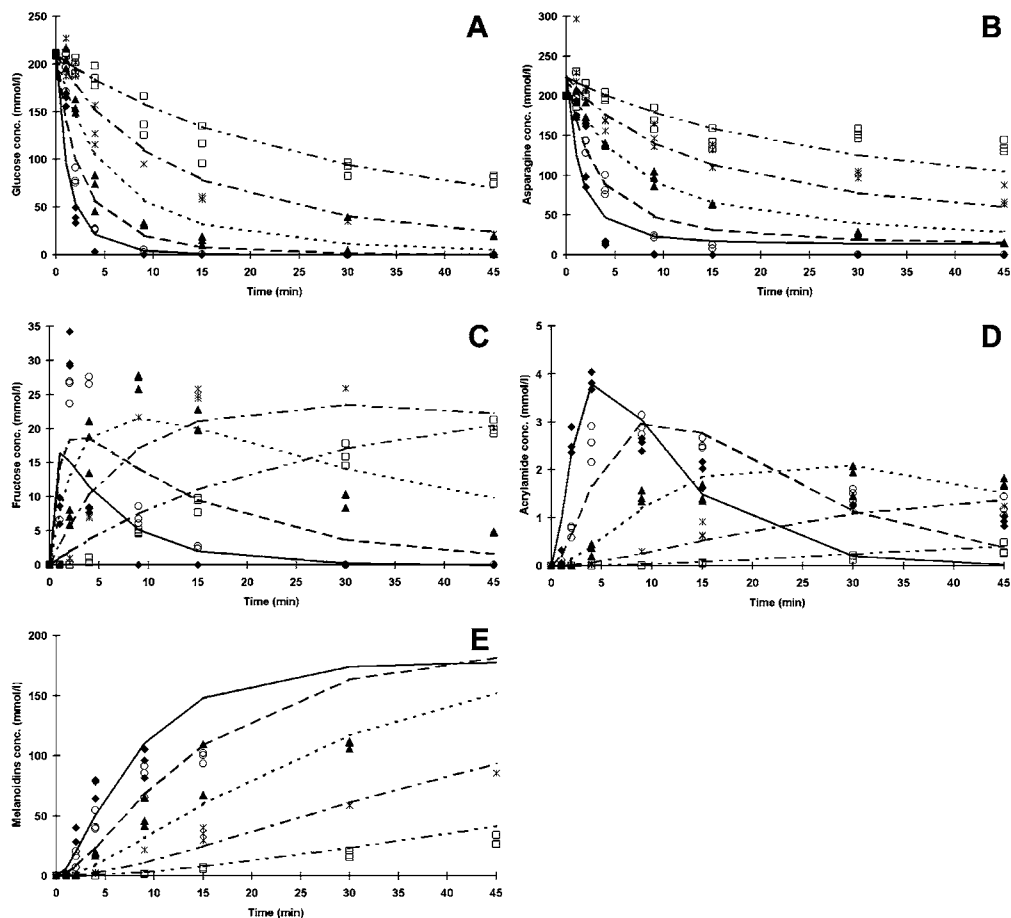


Figure 3. Model fit (lines) to experimental data (symbols) of glucose/asparagine (0.2 mol/L, pH 6.8) aqueous reaction system heated at 120 (□; - · - · -), 140 (*; - · - · -), 160 (▲; · · ·), 180 (○; - - -) and 200 °C (◆; -). (A) Loss of glucose; (B) loss of asparagine; (C) formation of fructose; (D) formation of acrylamide; (E) formation of melanoidins.

Table 1. Estimates of Rate Constants^a and Activation Energies^a as Found by Kinetic Modeling for the Proposed Kinetic Model

$k (\times 10^{-3} \text{ min}^{-1})$	120 °C	140 °C	160 °C	180 °C	200 °C	E_a (kJ/mol)
k_1	0.131 ± 0.025	0.308 ± 0.049	0.668 ± 0.13	1.35 ± 0.36	2.58 ± 0.89	57.6 ± 8.0
k_2	4.98 ± 1.2	16.7 ± 3.0	50.1 ± 9.6	136 ± 35	341 ± 114	81.7 ± 8.6
k_3	0.0819 ± 0.045	0.369 ± 0.14	1.45 ± 0.44	5.04 ± 1.6	15.8 ± 6.4	102 ± 14
k_4	0.176 ± 0.081	0.712 ± 0.23	2.53 ± 0.51	8.05 ± 1.2	23.2 ± 4.3	94.4 ± 11
k_5	15.7 ± 3.5	28.4 ± 4.6	48.7 ± 5.9	79.6 ± 8.9	125 ± 16	40.1 ± 5.0
k_6	7.96 ± 5.1	28.1 ± 13	88.1 ± 25	250 ± 45	650 ± 136	85.1 ± 14

^a ±95% highest posterior density (HPD) interval.

of about 3 at 180 °C. The higher reactivity of fructose with asparagine in the formation of the Schiff base could make the reversible isomerization of fructose to glucose insignificant. Therefore, model discrimination was performed for our proposed model with or without the reversible isomerization reaction from fructose to glucose. Model discrimination can be used to provide information about the most plausible model (20). In this case we used the Akaike criterion (the model with the lowest value is preferred) and the posterior probability (the model with the highest posterior probability is preferred) to assess the most plausible model (40, 41). The results from our test, shown in **Table 2**, support the model without the reversible isomerization reaction.

The aim of this study was to work toward a kinetic model for the formation of acrylamide in the Maillard reaction of glucose and asparagine at an initial pH of 6.8. Despite the current underestimation for the behavior of sugars and asparagine, the proposed model gives a reasonable estimation for the formation

Table 2. Model Discrimination Results for Our Proposed Model with (A) or without (B) the Reversible Isomerization Reaction from Fructose to Glucose^a

model	p	SS	n	AIC _c	ΔAIC _c	PPB	PPS
A	16	1.32×10^5	600	6505.2	27.3	-89.83	0.234
B	14	1.29×10^5	600	6477.9	0.0	-89.33	0.757

^a p, number of parameters; SS, residual sum of squares; n, number of data points including the replicates; AIC_c, Akaike criterion; ΔAIC_c, (AIC_c difference with the smallest value taken as reference); PPB, log₁₀ of posterior probability; PPS, normalized posterior probability share.

of acrylamide in an aqueous model system. The kinetic model supports the observations (11) that for the formation of acrylamide from asparagine a carbonyl source is needed, and our results suggest an important role for fructose, especially at the higher temperatures. Fructose is invariably formed from glucose isomerization. Furthermore, the experimental observa-

tions and the kinetic modeling suggested that acrylamide is not an end product in the Maillard reaction. The behavior of acrylamide suggests that it is an intermediate. The multiresponse model derived in this study is a first step into the realization of a tool that can be used to predict how acrylamide reduction in foods containing asparagine and reducing sugars can be accomplished. Further research is on the way to determine intermediate reaction products in order to extend the kinetic model. In addition, the formation of compounds via the Strecker degradation route and the degradation of sugars into carbohydrate fragments will be investigated to improve the current kinetic model in estimating the behavior of sugars and asparagine.

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) Ahn, J. S.; Castle, L.; Clarke, D. B.; Lloyd, A. S.; Philo, M. R.; Speck, D. R. Verification of the findings of acrylamide in heated foods. *Food Addit. Contam.* **2002**, *19*, 1116–1124.
- (3) Becalski, A.; Lau, B. P.-Y.; Lewis, D.; Seaman, S. W. Acrylamide in foods: Occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802–808.
- (4) 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.
- (5) Friedman, M. Chemistry, biochemistry, and safety of acrylamide. A review. *J. Agric. Food Chem.* **2003**, *51*, 4504–4526.
- (6) IARC. Acrylamide. In *IARC Monographs on the evaluation of carcinogen risk to humans: some industrial chemicals*; International Agency for Research on Cancer: Lyon, France, 1994; Vol. 60, pp 389–433.
- (7) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448–449.
- (8) 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.
- (9) Weisshaar, R.; Gutsche, B. Formation of acrylamide in heated potato products. Model experiments pointing to asparagine as precursor. *Deutsch. Lebensm.-Rundsch.* **2002**, *98*, 397–400.
- (10) Yaylayan, V. A.; Wnorowski, A.; Perez Locas, C. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753–1757.
- (11) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Loye Eberhart, B.; 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.
- (12) Wenzl, T.; Beatriz de la Calle, M.; Anklam, E. Analytical methods for the determination of acrylamide in food products: a review. *Food Addit. Contam.* **2003**, *20*, 885–902.
- (13) Svensson, K.; Abramsson, L.; Becker, W.; Glynn, A.; Hellenäs, K.-E.; Lind, Y.; Rosén, J. Dietary intake of acrylamide in Sweden. *Food Chem. Toxicol.* **2003**, *41*, 1581–1586.
- (14) Mucci, L. A.; Dickman, P. W.; Steineck, G.; Adami, H.-O.; Augustsson, K. Dietary acrylamide and cancer of the large bowel, kidney, and bladder: Absence of an association in a population-based study in Sweden. *Br. J. Cancer* **2003**, *88*, 84–89.
- (15) Jung, M. Y.; Choi, D. S.; Ju, J. W. A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *J. Food Sci.* **2003**, *68*, 1287–1290.
- (16) Grob, K.; Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Imhof, D.; Amrein, T.; Pfefferle, A.; Bazzocco, D. French fries with less than 100 $\mu\text{g}/\text{kg}$ acrylamide. A collaboration between cooks and analysts. *Eur. Food Res. Technol.* **2003**, *217*, 185–194.
- (17) Biedermann-Brem, S.; Noti, A.; Grob, K.; Imhof, D.; Bazzocco, D.; Pfefferle, A. How much reducing sugar may potatoes contain to avoid excessive acrylamide formation during roasting and baking? *Eur. Food Res. Technol.* **2003**, *217*, 369–373.
- (18) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R. H.; Gondé, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Müller, D.; Tallmadge, D.; O'Brien, J.; Thompson, S.; Silvani, D.; Whitmore, T. A review of acrylamide: an industry perspective on research, analysis, formation, and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323–347.
- (19) Van Boekel, M. A. J. S. Kinetic aspects of the Maillard reaction: a critical review. *Nahrung* **2001**, *45*, 150–159.
- (20) Martins, S. I. F. S.; Van Boekel, M. A. J. S. A kinetic model for the glucose/glycine Maillard reaction pathways. *Food Chem.* **2005**, *90*, 257–269.
- (21) Mundt, S.; Wedzicha, B. L. A kinetic model for the glucose–fructose–glycine browning reaction. *J. Agric. Food Chem.* **2003**, *51*, 3651–3655.
- (22) Yasuhara, A.; Tanaka, Y.; Hengel, M.; Shibamoto, T. Gas chromatographic investigation of acrylamide formation in browning model systems. *J. Agric. Food Chem.* **2003**, *51*, 3999–4003.
- (23) Wedzicha, B. L.; Mottram, D. S.; Elmore, J. S.; Koutsidis, G.; Dodson, A. T. Kinetic models as a route to control acrylamide formation in food. In *Chemistry and Safety of Acrylamide in Food*; M. Friedman and D. S. Mottram, Eds.; Springer: New York, 2005; pp 235–253.
- (24) Barber, D. S.; Hunt, J.; LoPachin, R. M.; Ehrich, M. Determination of acrylamide and glycidamide in rat plasma by reversed-phase high performance liquid chromatography. *J. Chromatogr. B* **2001**, *758*, 289–293.
- (25) Peng, L.; Farkas, T.; Loo, L.; Dixon, A.; Teuscher, J.; Kallury, K. Rapid and reproducible extraction of acrylamide in French fries using a single solid-phase sorbent. *Am. Lab. News.* **2003**, *10*–14.
- (26) Martins, S. I. F. S.; Marcelis, A. T. M.; Van Boekel, M. A. J. S. Kinetic modelling of Amadori *N*-(1-deoxy-D-fructos-1-yl)glycine degradation pathways. Part I—Reaction mechanism. *Carbohydr. Res.* **2003**, *338*, 1651–1663.
- (27) Davidek, T.; Clety, N.; Aubin, S.; Blank, I. Degradation of the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)glycine in aqueous model systems. *J. Agric. Food Chem.* **2002**, *50*, 5472–5479.
- (28) Davidek, T.; Clety, N.; Devaud, S.; Robert, F.; Blank, I. Simultaneous quantitative analysis of Maillard reaction precursors and products by High-Performance Anion Exchange Chromatography. *J. Agric. Food Chem.* **2003**, *51*, 7259–7265.
- (29) Husek, P. Method for preparing sample for amino acid analysis and kit for analyzing the same. Phenomenex Inc., EP 1,033,576, March 1999.
- (30) Leong, L. P. Modelling of the Maillard reaction involving more than one amino acid. Ph.D. Dissertation, University of Leeds, Leeds, U.K., 1999.
- (31) Leong, L. P.; Wedzicha, B. L. A critical appraisal of the kinetic model for the Maillard browning of glucose with glycine. *Food Chem.* **2000**, *68*, 21–28.
- (32) Brands, C. M. J.; Wedzicha, B. L.; Van Boekel, M. A. J. S. Quantification of melanoidin concentration in sugar–casein systems. *J. Agric. Food Chem.* **2002**, *50*, 1178–1183.
- (33) 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 vinyllogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550–5558.
- (34) <http://www.athenavision.com>
- (35) Brands, C. M. J.; Van Boekel, M. A. J. S. Reactions of monosaccharides during heating of sugar–casein systems: building of a reaction network model. *J. Agric. Food Chem.* **2001**, *49*, 4667–4675.

- (36) Robert, F.; Vuataz, G.; Pollien, P.; Saucy, F.; Alonso, M.-I.; Bauwens, I.; Blank, I. Acrylamide formation from asparagine under low-moisture Maillard reaction conditions. 1. Physical and chemical aspects in crystalline model systems. *J. Agric. Food Chem.* **2004**, *52*, 6837–6842.
- (37) Surdyk, N.; Rosén, J.; Andersson, R.; Åman, P. Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. *J. Agric. Food Chem.* **2004**, *52*, 2047–2051.
- (38) Stadler, R. H.; Verzeznassi, L.; Varga, N.; Grigorov, M.; Studer, A.; Riediker, S.; Schilter, B. Formation of vinylogous compounds in model Maillard reaction systems. *Chem. Res. Toxicol.* **2003**, *16*, 1242–1250.
- (39) Van Boekel, M. A. J. S. Statistical aspects of kinetic modeling for food science problems. *J. Food Sci.* **1996**, *61*, 477–485, 489.
- (40) Burham, K. P.; Anderson, D. R. *Model selection and inference. A practical information and theoretic approach*; Springer-Verlag: New York, 1998.
- (41) Stewart, W. E.; Shon, Y.; Box, G. E. P. Discrimination and goodness of fit of multiresponse mechanistic models. *AIChE J.* **1998**, *44*, 1404–1412.

Received for review March 7, 2005. Revised manuscript received May 20, 2005. Accepted May 21, 2005. This work was carried out partly with support from a joint program of the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, and the Ministry of Housing, Spatial and Environment of The Netherlands (EETK20038) and with support from the European Commission, Priority 5 on Food Quality and Safety (Contract FOOD-CT-2003-506820 Specific Targeted Project), Heat-generated food toxicants—identification, characterization and risk minimization. This publication reflects the author's views and not necessarily those of the Dutch government or the EC. The information in this document is provided as is and no guarantee or warranty is given that the information is fit for any particular purpose. The user thereof uses the information at its sole risk and liability.

JF050504M