Food Chemistry 111 (2008) 719–729

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The kinetics of acrylamide formation/elimination in asparagine–glucose systems at different initial reactant concentrations and ratios

Kristel De Vleeschouwer, Iesel Van der Plancken, Ann Van Loey *, Marc E. Hendrickx

Laboratory of Food Technology, Center for Microbial and Food Technology, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B-3001 Heverlee, Belgium

ARTICLE INFO

Article history:
Received 23 November 2007
Received in revised form 3 March 2008
Accepted 21 April 2008

Keywords:
Acrylamide
Kinetics
Reactant concentration
Reactant ratio
Thermal treatment
Food

ABSTRACT

The effect of initial reactant concentration and ratio on the kinetics of acrylamide formation and elimination was studied using diluted asparagine–glucose model systems, heated at temperatures between 120 and 200 °C. By means of single response modelling, acrylamide concentrations measured could be modelled using a simplified kinetic model, in which acrylamide formation and elimination reactions were included. Changing the reactant concentration in equimolar systems did not affect significantly the kinetic parameters (k_{Fref} and E_{aF}) for acrylamide formation, whereas the rate constant for acrylamide elimination increased with increasing reactant concentration, in the range from 0.01 M to 0.125 M. Modifying the reactant ratio, while keeping one of the reactants at a fixed concentration (0.125 M), did not affect the estimated parameters as compared to the equimolar system. In addition, multiresponse modelling was applied on three model systems with a different initial reactant ratio. Besides acrylamide, the concentration of the reactants and fructose and the level of browning were quantified. By means of deduction, a mechanistic model was proposed which was able to model all responses simultaneously in an adequate way. It appeared that caramelisation has a predominant role in browning, however, only in the system with an excess of sugar. The corresponding kinetic parameters estimated were in some cases significantly different, but in the same order of magnitude.

1. Introduction

In 2002 relatively high amounts of acrylamide, a potential carcinogen to humans (IARC, 1994), were detected in thermally processed carbohydrate-rich foods, for example, potatoes. Numerous studies have been performed to gain insight into acrylamide formation. The major pathway of formation seems to be the Maillard reaction, in particular in the presence of the amino acid asparagine, which directly delivers the backbone of the acrylamide molecule (Mottram, Wedzicha & Dodson, 2002; Stadler et al., 2002; Zyzak et al., 2003). Reducing sugars, in the case of potatoes mainly glucose and fructose, are required precursors for the conversion of asparagine into acrylamide (Yaylayan et al., 2003).

Various factors, such as pH, water activity, reactant concentrations and the ratio between reactants, temperature and time of processing, etc., influence the formation of acrylamide in foods (De Vleeschouwer, Van der Plancken, Van Loey & Hendrickx, 2006; Franke, Sell, & Reimerdes, 2005; Jung, Choi, & Ju, 2003; Rydberg et al., 2003). In order to predict and control acrylamide formation affected by these factors, knowledge of kinetic parameters, such as rate constant and activation energy, is necessary. By applying multiresponse modelling, taking into account more than one response, not only acrylamide formation can be predicted, but also other aspects related to the Maillard reaction, for example, browning.

It is well-known for potato products, which are the predominant source of acrylamide exposure, that they are particularly rich in free asparagine (Brierley, Bonner & Cobb, 1996). The chemical composition of the raw material is, however, influenced by different factors, such as the variety, the use of fertilisers, the climate, but most of all by the storage conditions applied (Blenkinsop, Copp, Yada & Marangoni, 2002; EFSA, 2003; Eppendorfer, 1996; Olsson et al., 2004; Silva and Simon, 2005). Storage below 8 °C leads to the accumulation of sugars ("low temperature sweetening"), while the level of asparagine does not vary significantly with storage temperature (Coffin, Yada, Parkin, Grodzinski & Stanley, 1987; Haase et al., 2004; Noti et al., 2003). Consequently, this leads to potatoes with a wide range of sugar concentrations and thus of potatoes with highly variable sugar–asparagine ratios (Amrein et al., 2003; Ehling and Shibamoto, 2005). So far, the effect of initial reactant concentrations and ratio on acrylamide formation has only been studied qualitatively for a single temperature–time combination. According to Leufvén and Lingnert (2003), an equimolar ratio of glucose to asparagine results in the highest yield of acrylamide, which was confirmed by Becalski, Lau, Lewis and Seaman

* Corresponding author. Tel.: +32 16 32 15 85; fax: +32 16 32 19 60.
E-mail address: ann.vanloey@biw.kuleuven.be (A. Van Loey).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.04.045

Nomenclature

List of symbols

AA	acrylamide
Asn	asparagine
Asp	aspartic acid
DPn	unidentified acrylamide degradation products
Frc	fructose
Glc	glucose
Int1	intermediary 1
Int2	intermediary 2
Mel	melanoidins
Cf	caramelisation from fructose
Cg	caramelisation from glucose
E	elimination
Exp	experimental
F	formation
I	isomerisation
INTf	formation of intermediate from fructose
INTg	formation of intermediate from glucose
M	Maillard
Pred	predicted
Ref	reference

List of subscripts

Asp	aspartic acid formation
B	browning

(2003) for another reaction temperature. The concentration of acrylamide measured decreases at both higher and lower molar ratio (Leufvén & Lingnert, 2003). A kinetic study concerning the Maillard reaction has shown that initial concentration and ratio of reactants does not have a significant effect on the estimated rate constants, on the assumption that the kinetic model proposed is consistent with the reaction mechanism (Martins & van Boekel, 2005a). The initial reactant ratio also seems to affect the extent of browning, which is used as an indicator of the Maillard reaction in food. Increasing the amino acid concentration shows a greater increase in browning than increasing the sugar content on a molar basis for diluted model systems at pH 7 (Davies & Labuza, 1997). However, a study by Warmbier et al. (1976) in systems of intermediate moisture content showed that the rate of browning increased with increasing sugar to amino acid ratio up to a plateau at a ratio of 3.

In this study, the impact of changing reactant concentration and ratio on the kinetic parameters describing acrylamide formation and elimination was evaluated. Knowledge on how changes in reactant concentration influence these kinetics is of great relevance, given the fluctuating concentration of the reactants in raw materials, especially potatoes, with different parameters, which are not always controllable. This study was conducted in diluted asparagine–glucose model systems, in order to be able to determine the exact effect of reactant concentration and ratio without the interference of complicating factors present in real food matrices. Acrylamide concentration measured in thermally treated equimolar model systems with various initial reactant concentrations and in thermally-treated model systems with an excess of glucose or asparagine was modelled using a simplified kinetic model. For a selected number of model systems, the kinetic model and corresponding kinetic parameters were validated using multiresponse modelling, in which changes in the concentration of all measured components (acrylamide, asparagine, glucose, fructose and melanoidins) are taken into account simultaneously. This approach can help to obtain better insight into the reaction mechanism.

2. Materials and methods

2.1. Chemicals

Acrylamide (99.9%) was obtained from Bio-Rad Laboratories (Hercules, CA), whereas methacrylamide (⩾99%) was supplied by Merck (Darmstadt, Germany) and butyramide (⩾98%) by Fluka (Buchs, Switzerland). L-Asparagine, L-aspartic acid, D-fructose, sucrose and lactose were also obtained from Fluka and D-glucose was from Sigma (Steinheim, Germany); all these chemicals were of HPLC grade. All chemicals used for the extraction of acrylamide were of GC-grade. Sodium hydroxide (JT Baker, 50%) for sugar analysis was purchased from Boom Laboratories (Meppel, The Netherlands). Reagent-grade water (Simplicity™ Water Purification System, Millipore, Molsheim, France) was used throughout the experiments.

2.2. Preparation of reaction mixtures

Equimolar solutions of glucose and asparagine of different initial concentration (0.005 M, 0.01 M, 0.025 M, and 0.125 M) were prepared in citrate buffer (0.05 M, pH 6.0). In order to evaluate the effect of varying reactant ratio, two additional asparagine–glucose mixtures were prepared in a comparable way, in which one of the reactants was added at a lower level (0.031 M), compared to the second reactant (0.125 M).

2.3. Heat treatment

Samples were heated in hermetically closed reactor tubes (inox, 8 mm × 100 mm, custom-made) in order to avoid, as much as possible, side phenomena during heating, such as water evaporation and absorption of oil, which affect acrylamide formation. Heat treatment was performed in a thermostatted oil bath (UH2D, Grant Instruments Ltd., Cambridge, UK) at 120, 140, 160, 180 and 200 °C. For the kinetic experiments, samples were taken at different heating times, which were chosen depending on the treatment temperature. After thermal treatment, samples were immediately cooled in ice water, to stop any further reaction, and stored at −40 °C prior to analysis. During the heating and subsequent cooling phase, temperature of the samples was registered within the closed reactor tubes at regular time intervals (4 s) using thermocouples (type T, Thermo Electric Benelux, Balen, Belgium) connected to a data logger (TM 9616, Ellab, Roedovre, Denmark).

2.4. Analysis of acrylamide

The analysis of acrylamide was performed by gas chromatography coupled to mass spectrometry with chemical ionization based on the method described by Biedermann, Biedermann-Brem, Noti, and Grob (2002), without prior derivatization of acrylamide. After acrylamide extraction and further clean-up, the samples were analysed using the 5973 inert GC–MS system (Agilent Technologies, Diegem, Belgium). One microlitre was injected at low temperature, i.e., 60 °C, on an HP-InnoWax column (30 m × 250 μm i.d., 0.25 μm film thickness, Agilent Technologies) with a 0.5 m × 530 μm i.d.

precolumn of deactivated fused silica. The chromatographic separation was carried out with a constant flow rate of 2 ml/min of helium as carrier gas, using the following temperature program: initially the oven temperature was set to 60 °C (0.2 min), from which the temperature was increased to 100 °C at a rate of 35 °C/min and further increased at a rate of 12 °C/min to 230 °C (3 min). The detection was performed with a quadrupole mass spectrometer operating in positive chemical ionization mode with 20% methane as ionization gas in 'selected ion monitoring' (SIM) at *m/z* 72 (acrylamide), *m/z* 86 (methacrylamide) and *m/z* 88 (butyramide). Acrylamide concentration was quantified using methacrylamide as an internal standard, which was added at the start of the sample preparation step. By comparing this internal standard with a second internal standard, i.e., butyramide, added to the samples prior to injection, losses during sample preparation and analysis could be accounted for.

2.5. Analysis of amino acids

The analysis of asparagine was performed using the EZ:faast amino acid analysis kit (Phenomenex, Torrance, CA). Thermally treated samples were diluted so that the total amino acid concentration was maximally 0.01 M. The procedure for sample preparation consists of a solid-phase extraction step, followed by a derivatisation step and a liquid/liquid extraction. Derivatised samples were analysed by gas chromatography coupled to mass spectrometry. Samples were injected (2.0 μl) at 250 °C in split mode (1:15) onto a Zebron ZB-AAA column (10 m × 0.25 mm i.d., Phenomenex). The oven temperature was set initially at 110 °C (1 min) and further increased to 320 °C, at a rate of 30 °C/min. Helium was used as carrier gas at a constant flow rate of 1.1 ml/min. The detection was carried out in electron ionization mode over a scan range from *m/z* 45 to *m/z* 450. Quantification for asparagine was performed based on the ratio of the peak area of the *m/z* 155 ion to the peak area of the *m/z* 158 ion for the internal standard, norvaline.

2.6. Analysis of sugars

Samples were diluted (1:100 to 1:1000, depending on the sugar concentration) and analysed by high-performance anion-exchange chromatography (HPAEC; Dionex Bio-LC system, Sunnyvale, CA) using a CarboPac PA1 column (4 × 250 mm) with corresponding guard column at 30 °C. An isocratic gradient of 150 mM sodium hydroxide was used as the eluent for the sample analysis, with a flow rate of 1 ml/min. After 10 min the eluent concentration was increased to 200 mM NaOH in order to regenerate the column. Sugars, i.e., glucose, fructose and sucrose, were detected using an ED50 electrochemical detector in the pulsed amperometric detection mode (PAD; Dionex) and quantified by use of an internal standard, lactose.

2.7. Analysis of browning

Browning was determined spectrophotometrically by measuring the absorbance at 470 nm. When necessary, samples were diluted with reagent-grade water. The corresponding melanoidin concentration, formed from asparagine and glucose, could be calculated from the absorbance measured by using the equation of Lambert–Beer with an extinction coefficient of 282 l/mol cm, as described by Knol et al. (2005).

2.8. Repeatability of the heat treatment and the subsequent analyses

For every temperature–time combination two reactor tubes were heated in the oil bath, which were combined after cooling in ice water. From the resulting sample, the concentration of the different responses was determined once. In order to assess the standard error of the heat treatment and the subsequent analyses for the different responses, five identical samples were heated independently of each other at 120 °C and 200 °C. For both the temperatures, the resulting standard error of the concentration of the different responses, measured in the five identical samples, was calculated and was found to be no greater than 10%.

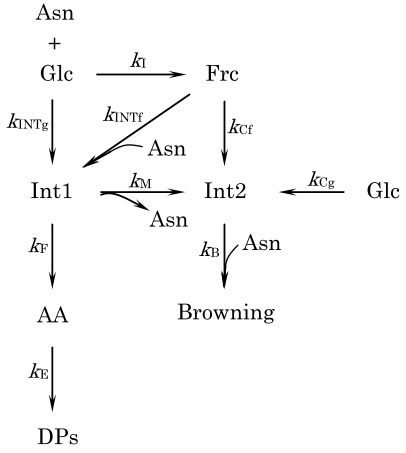
2.9. Kinetic modelling

A simplified kinetic model, taking into account simultaneous formation and elimination reactions (Scheme 1), has proven to be suitable to describe experimental acrylamide concentrations in diluted mixtures in previous studies (Claeys, De Vleeschouwer, & Hendrickx, 2005a; De Vleeschouwer, Van der Plancken, Van Loey, & Hendrickx, 2006).

$$\text{Asn} + \text{Glc} \xrightarrow{k_F} \text{AA} \xrightarrow{k_E} \text{DPs}$$

Scheme 1. Simplified kinetic model for the formation and elimination of acrylamide in asparagine–glucose mixtures.

In a second part, multiresponse modelling was performed to validate the estimated kinetic parameters obtained by simple single response modelling. In order to model the five measured responses (namely acrylamide (AA), asparagine (Asn), glucose (Glc), fructose (Frc) and browning, expressed as melanoidins (Mel)) simultaneously, a more complicated model was constructed, based on the comprehensive reaction mechanism, which is given in Scheme 2. This was built up starting from the simplified reaction schedule as presented in Scheme 1 and the kinetic model as proposed by Knol et al. (2005). This reaction network (Scheme 2) also includes caramelisation reactions and browning resulting from these reactions. The first intermediary presented, 'Int1' refers to a Schiff's base, whereas the second intermediary 'Int2' refers to the group of products, osuloses, that are intermediaries formed in both the Maillard reaction and caramelisation reactions and that can be linked by amino compounds to form the brown-coloured melanoidins (Davies & Labuza, 1997; Martins, 2003; van Boekel, 2001). If these second intermediaries are polymerized without intervention of an amino compound, the resulting products belong to the group of caramel colours.



Scheme 2. Proposed reaction network for the formation and elimination of acrylamide from asparagine and glucose through the Maillard reaction.

For each step in the proposed reaction network (Scheme 2), a differential equation was set up describing the reaction rate:

$$\frac{d[Asn]}{dt} = -k_{INTg}[Asn][Glc] - k_{INTf}[Asn][Frc] + k_M[Int1] - k_B[Int2][Asn] \quad (1)$$

$$\frac{d[Glc]}{dt} = -k_{INTg}[Asn][Glc] - (k_{Cg} + k_I)[Glc] \quad (2)$$

$$\frac{d[Frc]}{dt} = k_I[Glc] - k_{INTf}[Asn][Frc] - k_{Cf}[Frc] \quad (3)$$

$$\frac{d[Int1]}{dt} = k_{INTg}[Asn][Glc] + k_{INTf}[Asn][Frc] - (k_F + k_M)[Int1] \quad (4)$$

$$\frac{d[Int2]}{dt} = k_M[Int1] + k_{Cg}[Glc] + k_{Cf}[Frc] - k_B[Int2][Asn] \quad (5)$$

$$\frac{d[Mel]}{dt} = k_B[Int2][Asn] \quad (6)$$

$$\frac{d[AA]}{dt} = k_F[Int1] - k_E[AA] \quad (7)$$

$$\frac{d[DPn]}{dt} = k_E[AA] \quad (8)$$

where [compound] represents the concentration of the different compounds, k is the reaction rate constant and t is the reaction time. Initially, at time t = 0, the concentration of acrylamide, fructose, Int1, Int2, melanoidins and acrylamide degradation/elimination products are considered to be zero, whereas, for the reactants, the initial asparagine and glucose concentrations were considered.

The effect of temperature on each reaction was expressed by the Arrhenius equation, in which the temperature dependence of the rate constant k is quantified by the activation energy E_a (J/mol) according to

$$k = k_{ref} e\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad (9)$$

where R is the universal gas constant (8.3145 J/mol K), T the temperature concerned and k_{ref} the reaction rate constant at a reference temperature T_{ref} of 160 °C. Substitution of Eq. (9) into the differential Eqs. (1)–(8), renders the mathematical model to be solved using numerical integration of the registered temperature–time profile of each sample. The corresponding kinetic parameters, the reaction rate constants and the activation energies of the various reactions, were estimated by non-linear regression, using the software package Athena Visual Studio v11.0 (www.athenavisual.com). The fit criterion used is the method of least-squares minimisation, in the case of single response modelling, whereas the determinant criterion is applied for multiresponse data (van Boekel, 1996). The goodness-of-fit of the model is evaluated by scrutiny of residuals.

3. Results and discussion

3.1. Single response modelling of acrylamide concentration

To study the effect of initial reactant concentration on the kinetics of acrylamide formation and elimination, a buffered (pH 6.0) equimolar asparagine–glucose system was chosen, in which the reactant concentration was varied from 0.005 M to 0.125 M. Additionally, the initial reactant molar ratio was changed from 1:1 to 4:1 and 1:4, to evaluate the effect of an excess of one of the two main reactants on the course of acrylamide concentration. The measured net acrylamide concentration is represented in Fig. 1A–F by the symbols. In all model systems tested, the initial rate of acrylamide formation increases with increasing temperature, and the maximal acrylamide concentration attained increased consistently with increasing temperature. This increase in maximal concentration was however less pronounced for the lowest reactant concentrations tested (0.005 M and 0.01 M), especially at higher temperatures. This could possibly be related to the fact that the reactants, which are available in very low concentrations, are consumed after short heating times at these temperatures. The decrease of net acrylamide concentration, observed in all model systems tested at temperatures above 140 °C after prolonged heating, can be attributed to acrylamide elimination becoming predominant over acrylamide formation and is probably a consequence of reaction of acrylamide with components formed within the model system (EFSA, 2003). This decrease in acrylamide concentration seems to be more pronounced in the system with an excess of asparagine (Fig. 1E), resulting in lower net acrylamide levels relative to the maximum level, as compared to the equimolar system (0.025 M) (Fig. 1C) and the system with an excess of glucose (Fig. 1F). This observation confirms the results of Ehling and Shibamoto (2005), who also tested the effect of different asparagine–glucose ratios on acrylamide generation, however only for a limited range of times and temperatures. The yield of acrylamide, expressed in percentage with regard to the initial asparagine concentration, varies also with a changing initial reactant concentration and ratio. The maximal acrylamide yield, calculated for the equimolar systems, increases from 0.1% to 1.6% with an increasing initial reactant concentration from 0.005 M to 0.025 M and does not change by augmenting the initial reactant concentration further to 0.125 M. The acrylamide yield does, however, also depend on the initial reactant ratio. In the model system with an excess of asparagine, the yield observed is maximally 0.9%, which is lower than the corresponding equimolar system, whereas the maximal acrylamide yield in the model system with an excess of glucose amounts to 2.9%, being higher than the corresponding equimolar system. The maximal yield of acrylamide is, however, in all cases very low and is in the same order of magnitude as reported by Wedzicha et al. (2005).

In order to evaluate the effect of reactant concentration and ratio on the kinetic model and corresponding kinetic parameters, the experimental acrylamide concentrations were modelled using the simplified kinetic model as presented in Scheme 1. The results of the model fit are represented in Fig. 1A–F by the full lines. For all model systems tested, the fit seems adequate for the different treatment temperatures. In order to further evaluate the goodness-of-fit of the kinetic model, residuals were examined. Based on the parity plot (inserts in Fig. 1A–F), no trend was observed for either of the model systems tested. In addition a normality test (data not shown) was performed on each set of data in order to inspect the appropriateness of the least-squares fit criterion for parameter estimation (van Boekel, 1996).

The estimated parameters for the kinetic model as presented in Scheme 1, are given in Table 1 (at a reference temperature of 160 °C). The results obtained show some variation in the rate constant for acrylamide formation with changing reactant concentration and ratio, but this variation remains mostly within the 95% confidence interval, with the exception of the equimolar 0.025 M system. The same trend is observed for the corresponding activation energy, which is a measure to quantify the temperature sensitivity of the reaction rate constants, in this case of the acrylamide formation reaction. The global trend within the kinetic parameters describing the formation of acrylamide confirms that both the reaction rate constant and the corresponding activation energy are independent of the initial concentration of reactants and their ratio. Therefore, it can be stated that the kinetic model is consistent with the reaction mechanism for acrylamide formation. The reaction rate constant for acrylamide elimination, however, shows an increasing trend with increasing reactant concentration for the equimolar model systems, although not significant ($\alpha = 0.05$) in all cases, with the exception of the 0.005 M system. For this model system a larger confidence interval is observed for all parameters estimated, which can probably be attributed to the fluctuations

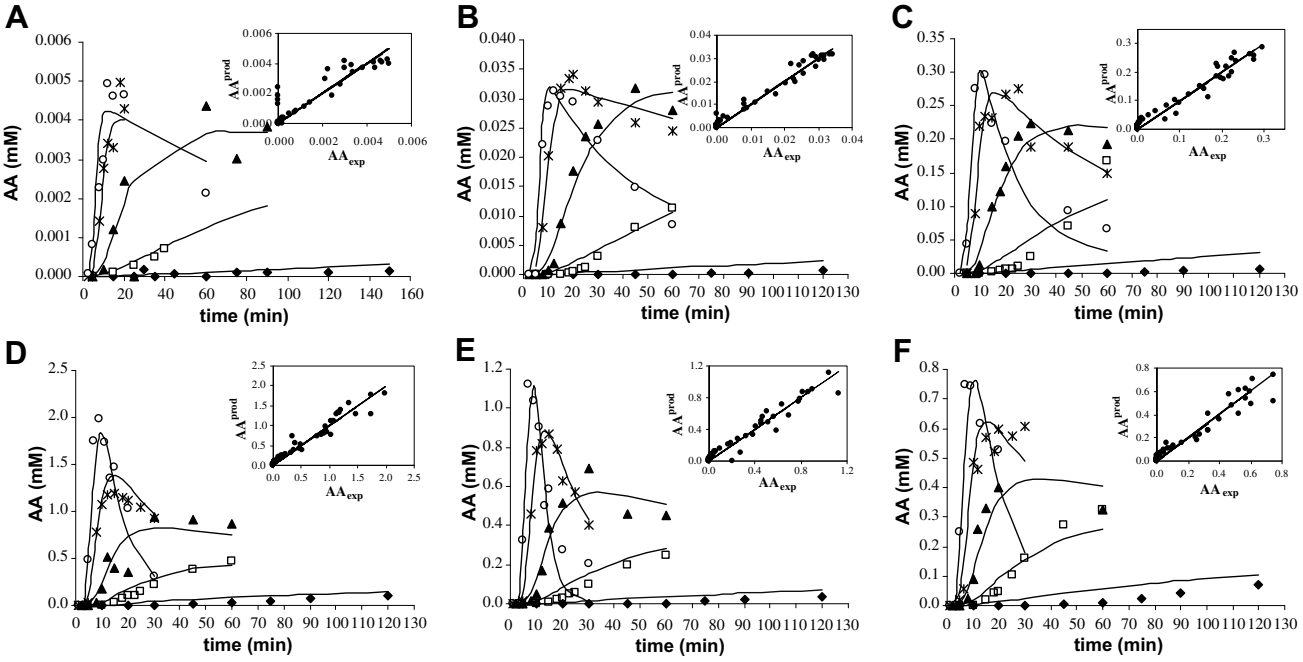


Fig. 1. Time course of acrylamide concentration in buffered (pH 6.0) asparagine–glucose mixtures with variable initial reactant concentration and ratio: (A) 1:1, 0.005 M; (B) 1:1, 0.01 M; (C) 1:1, 0.025 M; (D) 1:1, 0.125 M; (E) 4:1, 0.125–0.031 M; (F) 1:4, 0.031–0.125 M, heated at 120 °C (♦), 140 °C (□), 160 °C (▲), 180 °C (×) and 200 °C (○). The full lines represent the fit of the Scheme 1 model, while the experimental data are represented by the symbols. Inserts show the corresponding parity plot where the full lines have a slope of 1 (perfect fit).

Table 1
Estimated kinetic parameters[a] based on Scheme 1, describing acrylamide formation and elimination in asparagine–glucose model systems in citrate buffer (pH 6.0; 0.05 M), with variable initial reactant concentration and ratio, heated at temperatures between 120 and 200 °C

T_{ref} = 160 °C		k_{Fref} (M^{-1} min^{-1})	k_{Eref} (min^{-1})	E_{aF} (kJ/mol)	E_{aE} (kJ/mol)
Asn–Glc	Concentration (M)				
1:1	0.005	0.0141 ± 0.00416[a]	0.0940 ± 0.0335	173.1 ± 26.23	166.6 ± 25.71
	0.01	0.0260 ± 0.00365	0.0799 ± 0.0166	165.1 ± 10.34	165.0 ± 10.85
	0.025	0.0465 ± 0.00711	0.121 ± 0.0265	155.0 ± 11.31	136.9 ± 12.29
	0.125	0.0168 ± 0.00396	0.289 ± 0.0827	164.9 ± 13.49	115.8 ± 14.63
4:1		0.0404 ± 0.00827	0.232 ± 0.0605	180.9 ± 13.29	135.1 ± 15.33
1:4		0.0193 ± 0.00469	0.209 ± 0.0735	147.1 ± 14.22	113.2 ± 18.34

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

in acrylamide concentrations measured, which are close to the detection limit. The increase in elimination rate constant with increasing initial reactant concentration is probably related to the higher amount of reactive products formed within the system due to heating. The activation energy of the acrylamide elimination shows the opposite trend to the rate constant, as it decreases with increasing initial reactant concentration. For the model systems with an excess of one of the two reactants, the variation in the estimated kinetic parameters for the elimination of acrylamide remains mostly within the 95% confidence interval for the corresponding parameter of both the equimolar 0.025 M and 0.125 M model systems.

For all models studied, a relative high correlation was observed between k_{Fref} and k_{Eref} (0.885–0.930) and between E_{aF} and E_{aE} (0.703–0.946). This correlation will influence the uniqueness of the fit and the confidence interval of the estimated parameters, but generally does not imply an actual correlation in the physiological or biochemical mechanism underlying the data. It may simply be a consequence of performing the parameter estimation on a limited set of experimental observations or that the data points do not span a sufficiently large range of x values (Johnson, 2000; Motulsky & Ransnas, 1987).

The approach used in this section is a rather pragmatic one, in which the overall kinetics of acrylamide is considered rather than details of the complex chemistry. The use of simple reaction order for complex reaction pathways can be useful for modelling chemical changes during processing, but only reactions that have a direct effect on the response to be modelled are considered. That is why single response modelling can only be an approximation of the underlying mechanism and consequently the kinetic parameters estimated are only apparent values. In order to obtain more insight into the actual reaction mechanism and more precise parameter estimates, multiresponse modelling is recommended (van Boekel, 1996) and has been performed.

3.2. Multiresponse modelling

In order to validate the estimated kinetic parameters for acrylamide formation and elimination reactions obtained by modelling only one response, namely acrylamide, multiresponse modelling was applied. Multiresponse modelling implies that more than one response is taken into account, where the measured responses have one or more parameter in common. The advantages are that the information for the various responses is used simultaneously

resulting in more precise parameter estimation. Moreover, multiresponse modelling is helpful in obtaining insight into the actual reaction mechanism. For this purpose four additional responses were measured experimentally for three selected model systems. Next to acrylamide, the asparagine, glucose and fructose concentrations were quantified, as well as the degree of browning, expressed as the melanoidin concentration.

3.2.1. Quantification of reactants and main products

During heating of the asparagine–glucose mixture, the concentration of both reactants decreased as a function of time (Figs. 2–4B and C). As expected, the rate of reactant loss increases with increasing treatment temperature. The loss of the amino compound is actually the resultant of at least three reactions: reaction with the sugar in the initial stage of the Maillard reaction, regeneration of the amino compound in the intermediate stage and incorporation of the amino group in brown pigments in the final stage (van Boekel, 2001). Due to the high treatment temperatures applied, an apparent continuous loss of asparagine was observed (Figs. 2–4B). The loss of glucose on the other hand is not only the result of reaction with asparagine, but can also partly be explained by the isomerisation reaction forming fructose, which was detected in the heated samples (Figs. 2–4D). Moreover, since relatively high treatment temperatures were applied, sugar degradation reactions could also occur in the asparagine–glucose mixtures tested, especially in the case where an excess of sugar is initially available. The decrease of glucose, expressed relative to the initial concentration, in the model system with an excess of sugar (Fig. 4C) occurs slightly slower at each of the temperatures tested, as compared the loss of glucose from the equimolar system (Fig. 2C) with a comparable initial sugar concentration. This could indicate that the Maillard reaction between asparagine and glucose occurs at a higher rate in the equimolar model system or that sugar degradation reactions are of more importance in the equimolar system, as compared to the system with an excess of sugar. This hiatus in the understanding of the exact mechanism might be filled by modelling different responses simultaneously and by comparing the resulting estimated kinetic parameters.

Fructose seems to be the only detectable isomerisation product of glucose under the reaction conditions applied. This is in line with observations of other research groups studying the Maillard reaction or more specifically acrylamide formation (Brands & van Boekel, 2003; Knol et al., 2005; Martins & van Boekel, 2005b). Generally, the fructose concentration (Figs. 2–4D) increases and decreases again at temperatures higher than 140 °C, probably due to further reaction with other compounds. The net concentration of fructose measured was however 4–6 times smaller as compared to the initial glucose concentration. Consequently, the resulting trend in the fructose concentration is not always unambiguous.

The Maillard reaction is frequently quantified by measuring the extent of browning, originating from the formation of melanoidins. This involves a heterogeneous mixture of nitrogenous polymers and copolymers formed in the final stage of the Maillard reaction. However, when heating a sugar-amino acid mixture, the resulting browning could also partly be attributed to the formation of caramel colours (Buera, Chirife, Resnik, & Wetzler, 1987). These are sugar degradation products which are polymerized without intervention of an amino compound. Browning, measured as the absorbance at 470 nm, originating from the Maillard reaction, can be related to the number of sugar molecules incorporated into the melanoidins, by means of the molar extinction coefficient of these melanoidins, which is dependent on the type of amino acid and sugar being heated (Leong, 1999). Since browning originating from caramel colours is difficult to quantify, browning measured in this study was quantified in terms of melanoidins, implying that formation of caramel colours is negligible. This assumption seems acceptable, since it has also been made in other studies in equimolar sugar–amino acid mixtures (Knol et al., 2005; Martins & van Boekel, 2005b), but will be tested further on in this study in the modelling part. A lag-phase was observed for browning in all systems tested (Figs. 2–4E), which according to other authors (Knol et al., 2005; Martins & van Boekel, 2005b) can be attributed to the

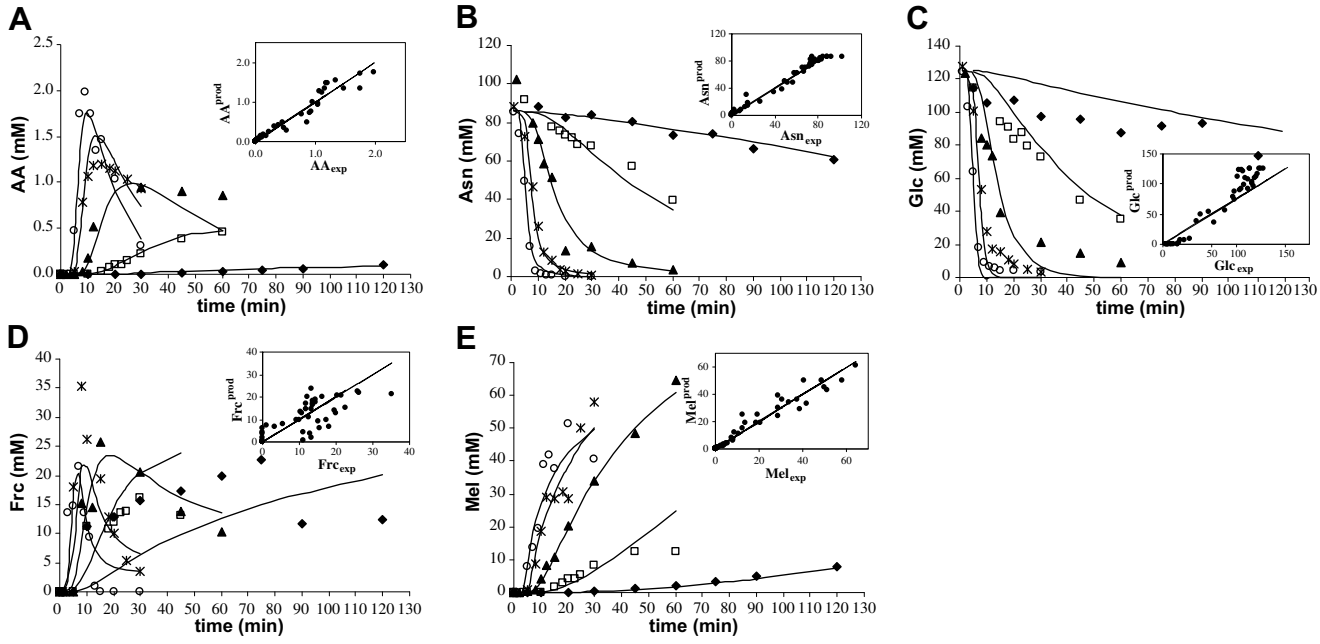


Fig. 2. Time course of (A) acrylamide; (B) asparagine; (C) glucose; (D) fructose; (E) melanoidins in a buffered (pH 6.0) equimolar (0.125 M) asparagine–glucose system, heated at 120 °C (♦), 140 °C (□), 160 °C (▲), 180 °C (×) and 200 °C (○). The full lines represent the fit of the Scheme 3 model, while the experimental data are represented by the symbols. Inserts show the corresponding parity plot where the full lines have a slope of 1 (perfect fit).

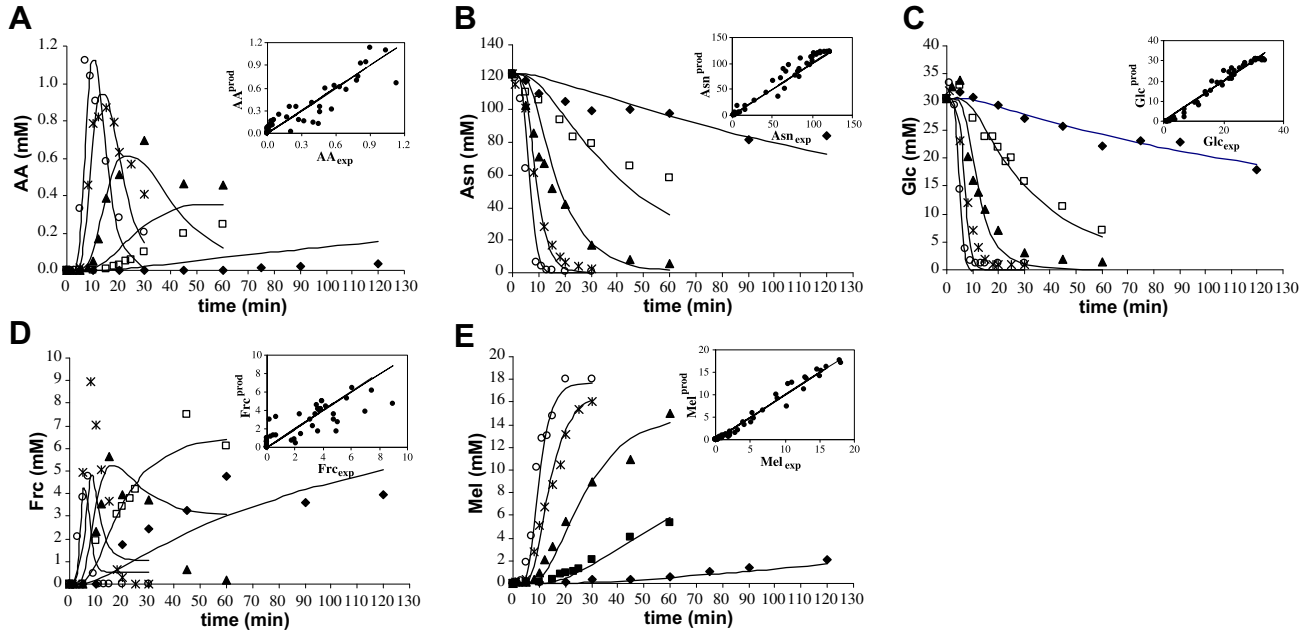


Fig. 3. Time course of (A) acrylamide; (B) asparagine; (C) glucose; (D) fructose; (E) melanoidins in a buffered (pH 6.0) asparagine–glucose system with a molar ratio 4:1 (0.125 M:0.031 M), heated at 120 °C (♦), 140 °C (□), 160 °C (▲), 180 °C (×) and 200 °C (○). The full lines represent the fit of the Scheme 3 model, while the experimental data are represented by the symbols. Inserts show the corresponding parity plot where the full lines have a slope of 1 (perfect fit).

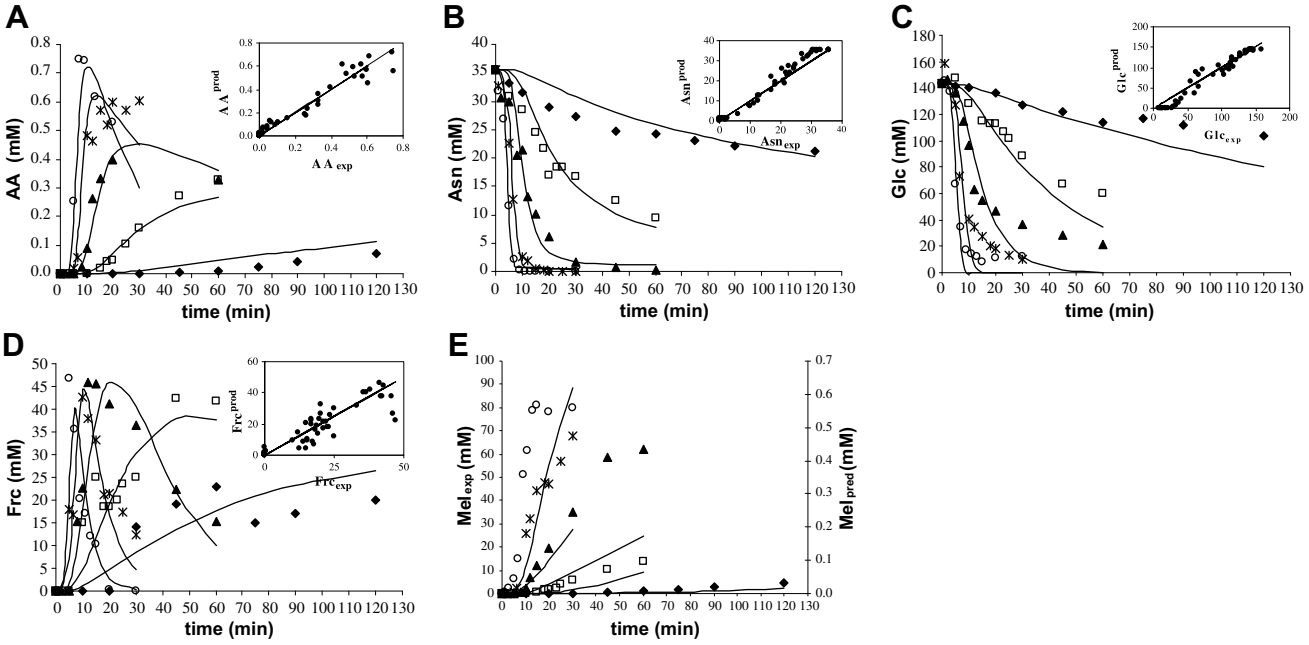


Fig. 4. Time course of (A) acrylamide; (B) asparagine; (C) glucose; (D) fructose; (E) melanoidins in a buffered (pH 6.0) asparagine–glucose system with a molar ratio 1:4 (0.031 M:0.125 M), heated at 120 °C (♦), 140 °C (□), 160 °C (▲), 180 °C (×) and 200 °C (○). The full lines represent the fit of the Scheme 4 model, while the experimental data are represented by the symbols. Inserts show the corresponding parity plot where the full lines have a slope of 1 (perfect fit).

fact that browning products are end products that require a certain time to be formed. This lag-phase could however also be a consequence of the inevitable dynamic heating conditions to which the samples were subjected. This ambiguity will be elucidated in the modelling section. As expected, the rate of browning expressed as the melanoidin concentration, increases with increasing temperature and evolves towards a plateau, which is observed after shorter heating times at higher treatment temperatures. Under the conditions of this study, the plateau was however not observed for the equimolar system, indicating that the precursors were probably not yet exhausted. The level of browning is about 4–5 times higher in the system with an excess of sugar as compared to the system with an excess of asparagine, whereas the latter system has a level of browning that is comparable to the equimolar system of 0.025 M (data not shown). This indicates that the extent of browning is highly dependent on the sugar concentration.

3.2.2. Kinetic modelling of reactions in asparagine–glucose systems

Kinetic modelling is an iterative process, in which originally a kinetic model is proposed based on the postulated reaction mechanism. This model is fitted to the experimental data using an appropriate fit criterion (van Boekel, 1996). If the fit proves adequate, this indicates consistency between the data and the model. If not, the postulated model is incorrect and should be adjusted and the fitting procedure be repeated until an acceptable model is found.

In order to model the reactions taking place in the asparagine–glucose model systems with variable initial reactant ratio, a kinetic model was constructed starting from the model proposed by Knol et al. (2005). This model was however expanded with caramelisation reactions that could play an important role, especially in systems with a large excess of sugars, as compared to the amino acid concentration. Sugar degradation products formed, as a result of these caramelisation reactions, are to a large extent similar to those formed through the initial pathways of the Maillard reaction (Martins & van Boekel, 2005b; van Boekel, 2001). They are included in the kinetic model as the second intermediaries, Int2, which can be linked by nitrogen containing components (as amino acids) to form the brown-coloured melanoidins. The reaction model, presented in Scheme 2, was translated into a mathematical model, as described in the materials and methods section.

3.2.3. Equimolar asparagine–glucose system and system with an excess of asparagine

In a first step the proposed model was fitted to the data obtained for the equimolar model system (0.125 M). The overall fit was acceptable, but the reaction rate constants for the caramelisation reactions were very small and difficult to estimate, indicating that these reactions are not of high relevance in this system. Consequently, the kinetic model was simplified by leaving out caramelisation reactions from glucose and fructose. Moreover, since no information was available on Int2, the reactions with reaction rate constants k_M and k_B were replaced by one single reaction with a corresponding reaction rate constant k_{Mel}. Finally, an additional reaction was included into the reaction network in which asparagine was consumed, since it was observed in the previous model fit that the concentration of asparagine was overestimated by the model. This extra reaction represents the chemical conversion of asparagine into aspartic acid, since during amino acid analysis a certain amount of aspartic acid was measured. This deamidation of asparagine normally takes place under more acid or basic conditions (Claeys, De Vleeschouwer & Hendrickx, 2005b), which initially were not applied in the prepared model systems in buffer at pH 6.0. Measurement of pH of the thermally treated samples after cooling to room temperature, revealed a decrease of the pH, as a function of time, at each tested temperature (Fig. 5A), which results from the production of acid during the Maillard reaction. The resulting pH decrease, especially pronounced at temperatures higher than 160 °C, presumably triggered the deamidation of asparagine to aspartic acid. This hypothesis is confirmed by an increase in pH following the observed initial decrease, which results in a significant pH-increase at 200 °C and a levelling off of the expected pH decrease at 160 and 180 °C. This unexpected increase in pH can probably be attributed to the free ammonium groups being released during the conversion of asparagine to aspartic acid, which seems to occur at a faster rate at elevated temperatures (Fig. 5A). The formation of aspartic acid is also observed at temperatures below 160 °C, but is less pronounced (data not shown). The adapted model, as given in Scheme 3, was fitted to the experimental data of the five responses simultaneously and appeared to follow the trend in the data, as can be seen from Fig. 2A–E. The same model (based on Scheme 3) was acceptable for describing the system with an excess of asparagine (Fig. 3A–E). The observed increase in pH in this model system is however more pronounced (Fig. 5B), as compared to the equimolar system, which is reflected in a higher concentration of aspartic acid (data not shown). This is likely to be related to the excess of available asparagine, in comparison to the equimolar system. The fit of the fructose concentration for both reactant ratios is not always accurate, which can be attributed to the low concentrations measured. The other responses were modelled in a more adequate way, especially for the model system with an excess of asparagine. For this system the fit of acrylamide was however less accurate as compared to the fit obtained by single response modelling for the same experimental data, especially at lower temperatures tested. The goodness-of-fit of the model was tested by inspecting the parity plot, given as an insert in the same figure for each response. For none of the responses of neither the equimolar system nor the system with an excess of asparagine was a trend observed within the residuals. However, as expected, the deviation from the line with a slope of 1 is more pronounced for the fructose concentration as compared to the other responses. The residuals for the various responses also showed a normal distribution (data not shown).

3.2.4. Asparagine–glucose system with an excess of glucose

Next, the proposed model, as presented in Scheme 2, was fitted to the experimental data for the five responses of the model system with an excess of glucose. The model seemed to describe the data accurately, although the estimated reaction rate constant k_{Mref} (from *Int*1 to Int2) was very small and the corresponding standard error on both the reaction rate constant and the activation energy of the reaction was much higher than the parameter itself. This indicates that the model was unable to estimate these parameters,

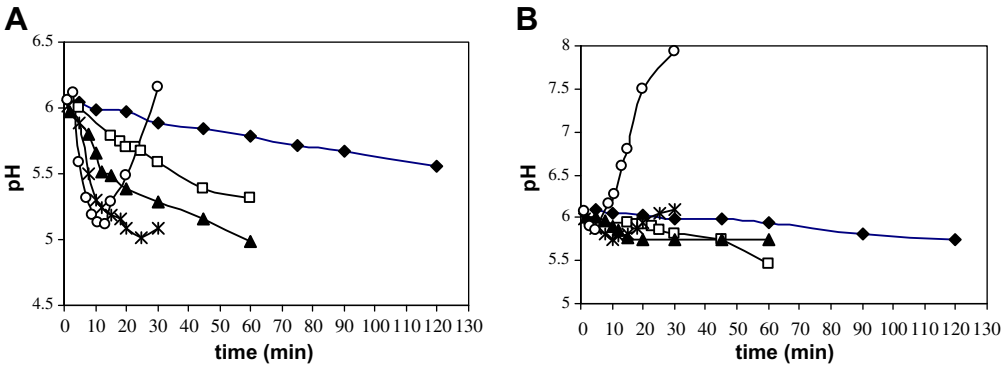
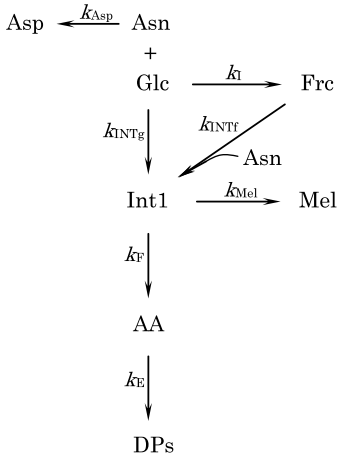
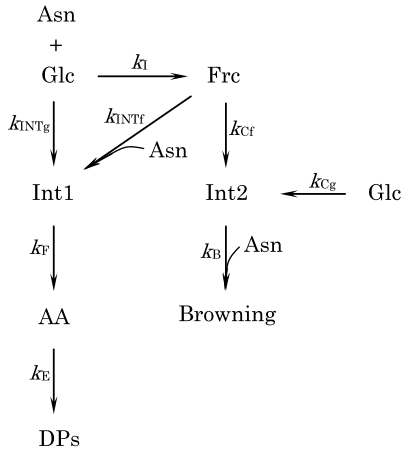


Fig. 5. Time course of pH in buffered (pH 6.0) asparagine–glucose mixtures with variable initial reactant ratio: (A) 1:1; (B) 1:0.25, heated at 120 °C (♦), 140 °C (□), 160 °C (▲), 180 °C (×) and 200 °C (○).



Scheme 3. Modified reaction network for the formation and elimination of acrylamide from asparagine and glucose through the Maillard reaction in equimolar systems and systems with an excess of asparagine.



Scheme 4. Modified reaction network for the formation and elimination of acrylamide from asparagine and glucose through the Maillard reaction in systems with an excess of glucose.

probably due to a lack of data concerning the intermediaries in the kinetic model, and that the value given has no physical meaning. For this reason, the proposed model was simplified by neglecting the reaction from Int1 to Int2 and thus the corresponding kinetic parameters k_{Mref} and E_{aM}. Due to this simplification, the degradation reaction of sugars (glucose and fructose) appears to be the primary source of brown-coloured polymers in this newly proposed model. The fit of the resulting model (Scheme 4) to the data is shown in Fig. 4A–E. The model appears to be suitable to describe the experimental data of all responses in a satisfying way, except for melanoidins. Browning, expressed as the melanoidin concentration, is strongly underestimated by the model, presented in Fig. 4E on the secondary *Y*-axis. This is probably a consequence of the fact that browning in this model system is mostly originating from caramel colours, instead of from melanoidins. Quantification of caramel colours was not possible, which is why browning was not well modelled by the kinetic model presented in Scheme 4. The fit for the fructose concentration was also again less accurate compared to the other responses. The trend observed in the fructose concentration as a function of time is however well described by the model. Also, the concentration of glucose is, especially at temperatures above 140 °C, slightly underestimated by the model. The less accurate fit of this model on the glucose and fructose concentration could also (partly) be related to the lack of fit of browning, since browning according to this model is mostly dependent on the available sugars. Another effect related to the lack of fit for browning, is that the kinetic parameters k_B and E_{aB} for the formation of brown-coloured polymers could not be estimated and consequently had to be fixed. The adequacy of the model was tested by verifying the parity plot for the different responses, which are shown as inserts in Fig. 4A–D. The closer the points are to the line with slope 1, the better the model describes the experimental data. From these figures, it can be concluded that all responses, except browning, could be modelled quite accurately, although less precise for the fructose concentration as expected. Also for this system, residuals were normally distributed for each of the responses (data not shown). It is noted that in the kinetic model used to fit the experimental data obtained in this system with an excess of glucose, the formation of aspartic acid from asparagine was left out. This assumption is acceptable since only very low concentrations of aspartic acid were detected.

The estimated parameters, being the reaction rate constants and the corresponding activation energy, corresponding to the final models (Schemes 3 and 4) used to fit the data, are listed in Table 2. The estimated reaction rate constants and activation energies for acrylamide formation and elimination are not significantly different between the equimolar system and the system with an excess in glucose. The rate constant for acrylamide formation for the model system with an excess of asparagine is however significantly higher as compared to the other systems, although in the same order of magnitude, whereas the elimination rate constant is significantly lower. This trend was also observed for single response modelling. The corresponding activation energies for the system with an excess of asparagine are less different from the corresponding values estimated for the equimolar system and the system with an excess in glucose. Comparison of the absolute value of k_{Fref} with the value obtained for single response modelling is not possible, because k_{Fref} obtained by single response modelling is a second order constant, derived directly from asparagine and glucose, whereas k_{Fref} obtained by multiresponse modelling is a first order constant, because acrylamide is assumed to be formed *via* an intermediary. The formation *via* an intermediary is more correct, but was simplified to one step for single response modelling, since no information was available about the intermediary. The acrylamide elimination rate constant is not significantly different from the one estimated *via* single response modelling for the model system with an excess of asparagine, but is higher for both the equimolar system and the system with an excess of glucose, although of the same order of magnitude. The more pronounced elimination is also apparent when comparing the figures obtained for single response modelling for these systems with the acrylamide concentration predicted using multiresponse modelling. The kinetic parameters for the isomerisation reaction from glucose to fructose are independent of the initial reactant ratio. Also other estimated parameters, as for the formation of Int1 from asparagine and glucose or fructose, are mostly within the 95% confidence interval, with the exception of the activation energy $E_{aINTf,}$ for a reactant ratio of 1:4, which is however unreliable, due to the high standard error. The estimated parameters are somewhat higher than the corresponding parameters estimated by Knol et al. (2005), but this is possibly related to the fact that in our work the dynamic conditions of the heat treatment were included in the data analysis, whereas this is not the case for the other study. Both for the equimolar system and the system with an excess of glucose, the estimated kinetic parameters for acrylamide formation and elimination showed a high intercorrelation (0.757–0.959) for each system. This implies that the corresponding estimated confidence intervals are not reliable and that it is difficult to indicate significant differences based on these intervals.

Table 2
Estimated kinetic parameters[a] based on multiresponse data, describing acrylamide formation and elimination in asparagine–glucose model systems in citrate buffer (pH 6.0; 0.05 M), with variable initial reactant concentration and ratio, heated at temperatures between 120 and 200 °C

T_{ref} = 160 °C	Asn–Glc		
	1:1 Scheme 3	4:1 Scheme 3	1:4 Scheme 4
k_{Fref} (min^{-1})	0.0107 ± 0.00423[a]	0.0361 ± 0.0106	0.00938 ± 0.00426
k_{Eref} (min^{-1})	0.665 ± 0.228	0.296 ± 0.0775	0.579 ± 0.186
k_{Iref} (min^{-1})	0.107 ± 0.0349	0.111 ± 0.0487	0.0953 ± 0.0177
$k_{INTgref}$ (M^{-1} min^{-1})	1.75 ± 0.569	2.01 ± 0.808	3.85 ± 1.24
$k_{INTfref}$ (M^{-1} min^{-1})	2.84 ± 0.975	2.61 ± 1.36	0.193 ± 0.162
k_{Melref} (min^{-1})	0.0244± 0.00275	0.0429 ± 0.00710	–*
k_{Bref} (M min^{-1})	–*	–*	Fixed
k_{Cgref} (min^{-1})	–*	–*	0.0216 ± 0.0340
k_{Cfref} (min^{-1})	–*	–*	0.0584 ± 0.0148
k_{Aspref} (min^{-1})	0.00428 ± 0.00134	0.0805 ± 0.0126	
E_{aF} (kJ/mol)	65.96 ± 22.75	51.71 ± 13.69	72.97 ± 25.58
E_{aE} (kJ/mol)	53.92 ± 22.34	22.40 ± 3.992	50.71 ± 22.43
E_{aI} (kJ/mol)	123.2 ± 15.90	130.3 ± 18.55	103.9 ± 11.58
E_{aINTg} (kJ/mol)	238.7 ± 46.81	159.6 ± 22.84	187.9 ± 30.62
E_{aINTf} (kJ/mol)	106.6 ± 21.95	131.0 ± 35.50	4.890 ± 7.444
E_{aMel} (kJ/mol)	17.80 ± 2.058	61.80 ± 10.16	–*
E_{aB} (kJ/mol)	–*	–*	Fixed
E_{aCg} (kJ/mol)	–*	–*	306.5 ± 204.0
E_{aCf} (kJ/mol)	–*	–*	61.22 ± 19.63
E_{aAsp} (kJ/mol)	2.226 ± 2.236	103.6 ± 10.28	–*

[a] ±95% highest posterior density (HPD) interval.
* Parameters not inserted in the model.

The high correlation between parameters of acrylamide formation and elimination reactions could possibly be related to the lack of information on the elimination reaction. This correlation was not observed for the model system with an excess of asparagine.

As mentioned before, it is not clear whether the observed lag-phase for the formation of browning can be attributed mostly to the dynamic heating conditions applied or to the fact that browning products are end products which may require some time to be formed. To test this, parameters estimated under dynamic conditions were fixed to model the same data but now assuming isothermal conditions, in order to elucidate the effect of the initial dynamic heating phase. The resulting fit clearly showed that the observed lag-phase for the formation of browning products is mostly dependent on the rate of precursor formation for the lower heating temperatures applied (i.e., 120 and 140 °C), whereas in case of the higher heating temperatures applied (i.e., 160–200 °C) the rate of sample heating is the decisive factor.

4. Conclusion

In this paper, the effect of varying initial reactant concentration and ratio on the kinetic parameters describing acrylamide formation and elimination was studied, using asparagine–glucose model systems at pH 6.0. It was demonstrated *via* single response modelling of the experimental data for acrylamide, obtained after thermal treatment at temperatures between 120 and 200 °C, that the effect of both the initial reactant concentration, and ratio on the kinetic parameters of acrylamide formation was negligible. The rate constant for acrylamide elimination increased linearly with increasing reactant concentration, in the range from 0.01 M to 0.125 M using equimolar model systems. By changing the initial reactant ratio, the rate constant for acrylamide elimination remained within the 95% confidence interval.

In a next step, a multiresponse approach was used. Next to acrylamide, data were obtained of both reactants, of fructose and of the extent of browning expressed as the melanoidin concentration. A general mechanistic model was constructed for the reactions taking place in the tested asparagine–glucose system. Next to the Maillard reaction, caramelisation reactions were assumed to occur, as well as the isomerisation from glucose to fructose. This is confirmed in the case where an excess of sugar is present, but caramelisation reactions were negligible for the equimolar system and the system with an excess of asparagine. The proposed models were suitable to describe the different responses measured adequately. This does not mean that these represent the exact reaction mechanisms, but it indicates that the proposed models are suitable to explain the experimental data. The corresponding estimated parameters for acrylamide formation and elimination for the different reactant ratios tested, were of the same order of magnitude, but were significantly different in the case of an excess of asparagine, based on the 95% confidence interval. Due to the high correlation between the kinetic parameters of acrylamide formation and elimination reactions, it is however difficult to assign significant differences between the parameters. Additional information on possible mechanistic routes for acrylamide elimination could help to improve the mechanistic model to predict acrylamide concentrations. Up to date, only hypotheses concerning the elimination have been formulated, since it is hypothesised that the reaction is highly dependent on compounds formed during the reaction between asparagine and sugars and therefore difficult to study. Moreover, methods to quantify caramelisation products could also be an aid in improving the accuracy of the model.

Knowledge obtained in this study is relevant to approach the dynamic reactant concentrations for acrylamide as observed in real food systems. The mechanistic model derived in this study has proven to be generic in nature and thus can be used as a tool to predict acrylamide formation in different food products. A next step involves extrapolation of kinetics from basic model systems to *in situ* conditions or real food products.

Acknowledgements

Research was funded by a Ph.D. Grant for Kristel De Vleeschouwer of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). Iesel Van der Plancken is a postdoctoral researcher funded by the Fund for Scientific Research Flanders (FWO).

References

Amrein, T. M., Bachmann, S., Noti, A., Biedermann, M., Ferraz Barbosa, M., & Biedermann-Brem, S. , et al. (2003). Potential of acrylamide formation in potatoes: A comparison of cultivars and farming systems. *Journal of Agricultural and Food Chemistry, 51*(18), 5556–5560.

Becalski, A., Lau, B. P.-Y., Lewis, D., & Seaman, S. W. (2003). Acrylamide in foods: Occurrence, sources, and modelling. *Journal of Agricultural and Food Chemistry, 51*(3), 802–808.

Biedermann, M., Biedermann-Brem, S., Noti, A., & Grob, K. (2002). Two GC–MS methods for the analysis of acrylamide in foodstuffs. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 93*, 638–652.

Blenkinsop, R. W., Copp, L. J., Yada, R. Y., & Marangoni, A. G. (2002). Changes in compositional parameters of tubers of potato (*Solanum tuberosum*) during low-temperature storage and their relationship to chip processing quality. *Journal of Agricultural and Food Chemistry, 50*(16), 4545–4553.

Brands, C. M. J., & van Boekel, M. A. J. S. (2003). Kinetic modelling of reactions in heated disaccharide-casein systems. *Food Chemistry, 83*, 13–26.

Brierley, E. R., Bonner, P. L. R., & Cobb, A. H. (1996). Factors influencing the free amino acid content of potato (*Solanum tuberosum* L) tubers during prolonged storage. *Journal of the Science of Food and Agriculture, 70*, 515–525.

Buera, M. P., Chirife, J., Resnik, S. L., & Wetzler, G. (1987). Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. *Journal of Food Science, 52*(4), 1063–1067.

Claeys, W. L., De Vleeschouwer, K., & Hendrickx, M. E. (2005a). Kinetics of acrylamide formation and elimination during heating of an asparagine–sugar model system. *Journal of Agricultural and Food Chemistry, 53*(26), 9999–10005.

Claeys, W. L., De Vleeschouwer, K., & Hendrickx, M. E. (2005b). Quantifying the formation of carcinogens during food processing: Acrylamide. *Trends in Food Science and Technology, 16*, 181–193.

Coffin, R. H., Yada, R. Y., Parkin, K. L., Grodzinski, B., & Stanley, D. W. (1987). Effect of low temperature on sugar concentrations and chip color of certain processing potato cultivars and selections. *Journal of Food Science, 52*(3), 639–645.

Davies, C. G. A., & Labuza, T. P. (1997). The Maillard reaction application to confectionery products. [http://citeseer.ist.psu.edu/cache/papers/cs/24623/http:zSzzSzcourses.che.umn.eduzSz00fscn8334-1fzSzPdf_FolderzSzmaillard-confectionary.pdf/the-maillard-reaction-application.pdf].

De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., & Hendrickx, M. (2006). Impact of pH on the kinetics of acrylamide formation/elimination reactions in model systems. *Journal of Agricultural and Food Chemistry, 54*(20), 7847–7855.

EFSA, European Food Safety Authority (2003). Workshop on acrylamide formation in food – report of the workshop. [http://www.efsa.europa.eu/etc/medialib/efsa/science/ahawdocuments/330.Par.0001.File.dat/other_01_acrylamide_report_annex_en1.pdf].

Ehling, S., & Shibamoto, T. (2005). Correlation of acrylamide generation in thermally processed model systems of asparagine and glucose with color formation, amounts of pyrazines formed, and antioxidative properties of extracts. *Journal of Agricultural and Food Chemistry, 53*, 4813–4819.

Eppendorfer, W. H. (1996). Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilisation and phosphorus and potassium deficiency. *Journal of the Science of Food and Agriculture, 71*, 449–458.

Franke, K., Sell, M., & Reimerdes, E. H. (2005). Quality related minimization of acrylamide formation – An integrated approach. In M. Friedman & D. Mottram (Eds.). *Advances in experimental medicine and biology: Chemistry and safety of acrylamide in food* (Vol. 561, pp. 357–369). New York: Springer.

Haase, N. U., Matthäus, B., & Vosmann, K. (2004). Aspects of acrylamide formation in potato crisps. *Journal of Applied Botany and Food Quality, 78*, 144–147.

IARC, International Agency for Research on Cancer (1994). Acrylamide. IARC monographs on the evaluation of the carcinogenic risks of chemicals to humans (Vol. 60 pp. 389–433). Lyon, France.

Johnson, M. L. (2000). Parameter correlations while curve fitting. *Methods in Enzymology, 321*, 424–446.

Jung, M. Y., Choi, D. S., & Ju, J. W. (2003). A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *Journal of Food Science, 68*(4), 1287–1290.

Knol, J. J., van Loon, W. A. M., Linssen, J. P. H., Ruck, A.-L., van Boekel, M. J. S., & Voragen, A. G. J. (2005). Toward a kinetic model for acrylamide formation in a glucose–asparagine reaction system. *Journal of Agricultural and Food Chemistry, 53*, 6133–6139.

Leong, L. P. (1999). *Modelling of the Maillard reaction involving more than one amino acid.* Ph.D. dissertation. Leeds, UK: University of Leeds.

Leufvén, A., & Lingnert, H. (2003). *Factors influencing acrylamide formation in food processing – Introductory model experiments performed at SIK. Public report of the Swedish Institute for Food and Biotechnology.*

Martins, S. I. F. S. (2003). *Unravelling the Maillard reaction network by multiresponse kinetic modelling. Ph.D. thesis. The Netherlands: Wageningen University.*

Martins, S. I. F. S., & van Boekel, M. A. J. S. (2005a). Kinetics of the glucose/glycine Maillard reaction pathways: Influences of pH and reactant initial concentrations. *Food Chemistry, 92*, 437–448.

Martins, S. I. F. S., & van Boekel, M. A. J. S. (2005b). A kinetic model for the glucose/glycine Maillard reaction pathways. *Food Chemistry, 90*, 257–269.

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

Motulsky, H. J., & Ransnas, L. A. (1987). Fitting curves to data using nonlinear regression: A practical and nonmathematical review. *The FASEB Journal, 1*, 365–374.

Noti, A., Biedermann-Brem, S., Biedermann, M., Grob, K., Albisser, P., & Realini, P. (2003). Storage of potatoes at low temperature should be avoided to prevent increased acrylamide formation during frying or roasting. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 94*, 167–180.

Olsson, K., Svensson, R., & Roslund, C.-A. (2004). Tuber components affecting acrylamide formation and colour in fried potato: Variation by variety, year, storage temperature and storage time. *Journal of the Science of Food and Agriculture, 84*, 447–458.

Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L., & Törnqvist, M. (2003). Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry, 51*(24), 7012–7018.

Silva, E. M., & Simon, P. W. (2005). Genetic, physiological, and environmental factors affecting acrylamide concentration in fried potato products. In M. Friedman & D. Mottram (Eds.). *Advances in experimental medicine and biology: Chemistry and safety of acrylamide in food* (Vol. 561, pp. 371–386). New York: Springer.

Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., & Guy, P. A. , et al. (2002). Acrylamide from Maillard reaction products. *Nature, 419*, 449–450.

van Boekel, M. A. J. S. (1996). Statistical aspects of kinetic modelling for food science problems. *Journal of Food Science, 61*(3), 477–486.

van Boekel, M. A. J. S. (2001). Kinetic aspects of the Maillard reaction: A critical review. *Nahrung/Food, 45*(3), 150–159.

Warmbier, H. C., Schnickels, R. A., & Labuza, T. P. (1976). Nonenzymatic browning kinetics in an intermediate moisture model system: Effect of glucose to lysine ratio. *Journal of Food Science, 41*, 981–983.

Wedzicha, B. L., Mottram, D. S., Elmore, J. S., Koutsidis, G., & Dodson, A. T. (2005). In M. Friedman & D. Mottram (Eds.). *Advances in experimental medicine and biology: Chemistry and safety of acrylamide in food* (Vol. 561, pp. 235–253). New York: Springer.

Yaylayan, V. A., Wnorowski, A., & Locas, C. P. (2003). Why asparagine needs carbohydrates to generate acrylamide. *Journal of Agriculture and Food Chemistry, 51*, 1753–1757.

Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., & Ewald, D. K, et al. (2003). Acrylamide formation mechanism in heated foods. *Journal of Agriculture and Food Chemistry, 51*, 4782–4787.