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A kinetic model for the glucose/glycine Maillard reaction pathways

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Abstract

A comprehensive kinetic model for the glucose/glycine Maillard reaction is proposed based on an approach called multiresponse kinetic modelling. Special attention was paid to reactants, intermediates and end products: D-fructose, *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG), 1-deoxy-2,3-hexodiulose and 3-deoxy-2-hexosulose, formic and acetic acid, methylglyoxal and 5-hydroxymethylfurfural (HMF). The organic acids were found to be stable end products, 3-deoxy-2-hexosulose was found to be involved in colour formation by reaction with glycine. It is suggested to consider acetic acid as an indicator of the progress of the Maillard reaction at pH 6.8. The significance of reversibility of formation of DFG was studied by kinetic model discrimination. The results suggested that the reaction path from DFG into its parents, glucose and glycine, is not important from a quantitative point of view, even though it does happen. The proposed model was updated and strained by varying one of the most important reaction conditions, the temperature. The estimated rate constants showed an Arrhenius type temperature dependence and the model performed well for all studied temperatures (80, 90, 100, 110 and 120 °C). Striking differences were found in temperature dependencies of the various reaction steps. More than just a fitting procedure, multiresponse modelling was shown to be a powerful tool in unravelling complicated reaction routes as occur in the Maillard reaction.

Keywords: Maillard reaction; Kinetics; Amadori rearrangement reversibility; Colour formation; Organic acids; Deoxyosones; Multiresponse modelling

1. Introduction

The Maillard reaction is a type of non-enzymatic browning of prime importance to both food scientists and food processors, as it affects the quality of processed food products, in particular the sensory attributes, like colour, flavour and taste. It results from an initial reaction of a reducing sugar with an amino compound, followed by a cascade of consecutive and parallel reactions to form a variety of coloured and colourless products which range from flavour volatiles (low molecular weight) to melanoidins (low and high molecular weight, brown, nitrogenous chromophores). Brown pigment formation is desired during some types of food processing (baking, cocoa and coffee roasting, cooking of meat) while it is undesirable in other technologies (milk drying, thermal treatments for the stabilization of milk, fruit juices and tomatoes). Besides having an effect on the sensory attributes of foods, the Maillard reaction can also have negative effects on the nutritional value (amino acids and protein unavailability for the human metabolism) as well as on the formation of mutagenic compounds (Brands, Alink, Van Boekel, & Jongen, 2000) or even potentially carcinogenic, as the recently discovered acrylamide (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002).

In the first coherent scheme of the Maillard reaction (Hodge, 1953) the Amadori compound had a main role in the formation of intermediates. Since then, new pathways have been established which question the central importance of the Amadori product, in particular in relation to colour formation (Leong & Wedzicha, 2000; Tressl, Nittka, & Kersten, 1995; Yaylayan, 1990). A kinetic model was proposed for glucose/glycine systems at pH 5.5, in which 3-deoxyglucosone was the key

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Nomenclature

List of symbols

AA	acetic acid
C_n	unidentified carbohydrate fragments
DFG	*N*-(1-deoxy-D-fructos-1-yl)-glycine (Amadori product of glucose and glycine)
1-DG	1-deoxyglucosone
3-DG	3-deoxyglucosone
E_1 and E_2	unidentified key compounds involved in rate-determining steps (possibly 1,2-enaminol or 2,3-enaminol, respectively)
FA	formic acid
Fru	fructose
Glu	glucose
Gly	glycine
Mel	melanoidins
MG	methylglyoxal
OA	organic acids

intermediate in colour formation and it was supposedly formed from the 1,2-enaminol, precursor of the Amadori compound (Leong & Wedzicha, 2000). Moreover, the reversibility of the Amadori product into its parent sugars and amino compound remains a question concerning the Maillard reaction pathways. The formation of parent sugars from the corresponding Amadori compound has been reported under physiological conditions (Acharya & Sussman, 1984), pyrolysis conditions (Yaylayan & Huyghues-Despointes, 1996) and under conditions relevant to food processing (Davidek, Clety, Aubin, & Blank, 2002; Martins, Marcelis, & Van Boekel, 2003a). In most of those studies the hypothesis of reversible Amadori rearrangement was confirmed. However, the mechanism of reversion is still not fully understood nor is it known whether it is quantitatively of relevance in the Maillard reaction.

To control the Maillard reaction, the reaction steps of interest need to be studied in a quantitative way. With knowledge of kinetics it becomes possible to describe and predict changes in a quantitative way at any time/temperature combination. Several authors have tried to study the kinetics of the Maillard reaction just by fitting simple kinetic models (like a zero-, first- or second-order reaction) to one selected reaction, for instance, formation of colour, or the degradation of reducing sugar. Bell, Touma, White, and Chen (1998) reported that glycine loss followed a second-order reaction model in a glucose/glycine mixture in a glassy state at 25 °C. However, no real distinction could be made between the first- and the second-order plot (Van Boekel, 2001). Also, Carabasa-Giribet and Ibarz-Ribas (2000) reported that both zero- and first-order reaction models gave acceptable fits for brown colour formation in glucose and amino acid systems heated at four different temperatures. Due to the complexity of the Maillard reaction mechanism, changes of one compound in time cannot be linked to mechanisms using simple kinetics, since the estimated reaction rate constants obtained via simple kinetics reflect a mixture of many elementary rate constants. The simple kinetics approach as applied to the Maillard reaction is actually just a fitting procedure and it gives no insight on the reaction mechanism. In contrast, applying multiresponse modelling techniques to estimate kinetic parameters helps in building mechanistic models (Van Boekel, 1996, 2001). A response in this context refers to the change in concentration of reactants and products as a result of some changed external condition. The basic idea of multiresponse modelling is to take simultaneously into account all measured reactants, intermediates and end product concentration changes, as opposed to only one response in simple kinetics. The main advantages of this approach are that models can be tested more rigorously and, once the goodness of fit is deemed acceptable, estimation of the parameters can be done much more precise. This is so because applying the appropriate statistical techniques optimally uses the information that is contained in all the available data. The multiresponse modelling approach has been successfully used for modelling the Maillard reaction in monosaccharide–casein systems (Brands & Van Boekel, 2002), in disaccharide–casein systems (Brands & Van Boekel, 2003) as well as for modelling the degradation pathways of *N*-(1-deoxy-D-fructos-1-yl)-glycine (Martins & Van Boekel, 2003b).

The aim of the present study was to develop a comprehensive kinetic model for the whole glucose/glycine Maillard reaction pathways using the above described multiresponse modelling approach. First, the main reaction products in heated glucose/glycine systems were identified and quantified, and the main reaction pathways established. The central importance of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) in colour formation was studied, as well as the kinetic significance of DFG reversibility in the early stages of the Maillard reaction. To show the power of multiresponse modelling, the iterative process of kinetic modelling (propose a model, confront it with experiments, criticise the model, adjust the model and confront it with experiments again) was gone through until a satisfactory model was obtained. The proposed model was then tested at five different

temperatures (80, 90, 100, 110 and 120 °C) simultaneously, and the model parameters (rate constants and activation energies) estimated. The effects of pH and initial concentration of reactants on the kinetics of this system are the topic of a forthcoming paper.

2. Material and methods

2.1. Preparation of reaction mixtures

Equimolar solutions of D-glucose and glycine (0.2 mol L^{-1}) were prepared in phosphate buffer (10 mL, 0.1 mol L^{-1}, pH 6.8), filtered (0.2 μm, Schleicher & Schuell) and heated at 80, 90, 100, 110 and 120 °C in an oil bath, in screw-capped glass tubes (Schott, 16 × 160 mm). At predetermined heating times, samples were taken and immediately cooled in ice water, prior to analyses. The drop in temperature upon cooling was such that the reaction virtually stops by this procedure. Each reaction mixture was prepared, heated and analysed at least in duplicate.

2.2. Identification and quantification of main intermediates

The following compounds have been identified and quantified: Glycine, D-glucose, D-fructose, *N*-(1-deoxy-D-fructos-1-yl)glycine, 1-deoxy-2,3-hexodiulose, 3-deoxy-2-hexosulose, formic acid, acetic acid, methylglyoxal and 5-(hydroxymethylfurfural). The used methodology has been described previously (Martins et al., 2003a).

2.3. Melanoidins quantification

The intensity of brown colour of the heated reaction mixtures was determined by measuring the absorbance at 470 nm with a spectrophotometer (Pharmacia Biotech). The absorbance was then expressed as the concentration of melanoidins using the equation of Lambert–Beer. The extinction coefficient formed in the glucose/glycine reaction has been determined before via radioactive labelling and was found to be 0.64 ± 0.03 L $mmol^{-1}$ cm^{-1} at 470 nm (Martins & Van Boekel, 2003c). The concentration of melanoidins is thus expressed as moles of sugar incorporated in the brown pigments.

2.4. Kinetic modelling

Based on the established reaction pathways a kinetic model was proposed and translated into a mathematical model by deriving coupled differential equations for each reaction step, as explained previously (Van Boekel, 1996). These equations contain rate constants as parameters. The software package Athena Visual Workbench (www.athenavisual.com) was used for numerical integration of the coupled differential equations as well as for parameter estimation. The model parameters, i.e., the rate constants and activation energies, were estimated by non-linear regression using minimisation of the so-called determinant criterion. This criterion replaces the commonly used least-squares minimisation, to comply with the statistical demands typically for multiresponse modelling (Stewart, Caracotsios, & Sørensen, 1992; Van Boekel, 1996). To discriminate between various proposed models, a multivariate test of goodness of fit was used together with two model discrimination tests: the posterior probability (PPB) (Stewart, Shon, & Box, 1998) and the Akaike Information Criterion (AIC) (Burham & Anderson, 1998). The model with the highest PPB and the lowest AIC was defined as the most likely one in the present study.

3. Results and discussion

3.1. Identification and quantification of reactants and main products formed

In the glucose/glycine model system at pH 6.8 the main detected reaction products were the corresponding isomer of glucose (D-fructose), *N*-(1-deoxy-D-fructos-1-yl)-glycine, α-dicarbonyls (methylglyoxal, 1-deoxy-2,3-hexodiulose, 3-deoxy-2-hexosulose) and organic acids (formic and acetic acid). In Fig. 1 the decrease of reactants and formation of reaction products is shown as averages of duplicates at 100 °C. Independently of the studied temperature (80, 90, 100, 110 or 120 °C), the loss of glucose was always faster than that of glycine (results not shown). The same observation has been reported in previous studies. Vernin et al. (1987) and Debrauwer, Vernin, Metzger, Siouffi, and Larice (1991) attributed this to the formation of diglucosylamine due to reaction of the sugar with the Amadori compound. Van Boekel and Martins (2002), on the other hand, attributed the observed difference in glucose and glycine reactivity to glycine regeneration from the initial condensation products (like the Amadori rearrangement product) as well as to the parallel reaction of the sugar into its isomer fructose.

In view of sugar isomerisation, Brands and Van Boekel (2001) reported that a main reaction product detected in a heated glucose/casein system at 120 °C under neutral conditions was D-fructose. This was confirmed in the present study. The formation of D-fructose became relatively more prominent at higher temperature (Fig. 2), in line with the corresponding increase in glucose loss. However, after correcting for isomerisation into fructose, the loss of glucose was still higher than that of glycine, as shown in Fig. 1(a). It is

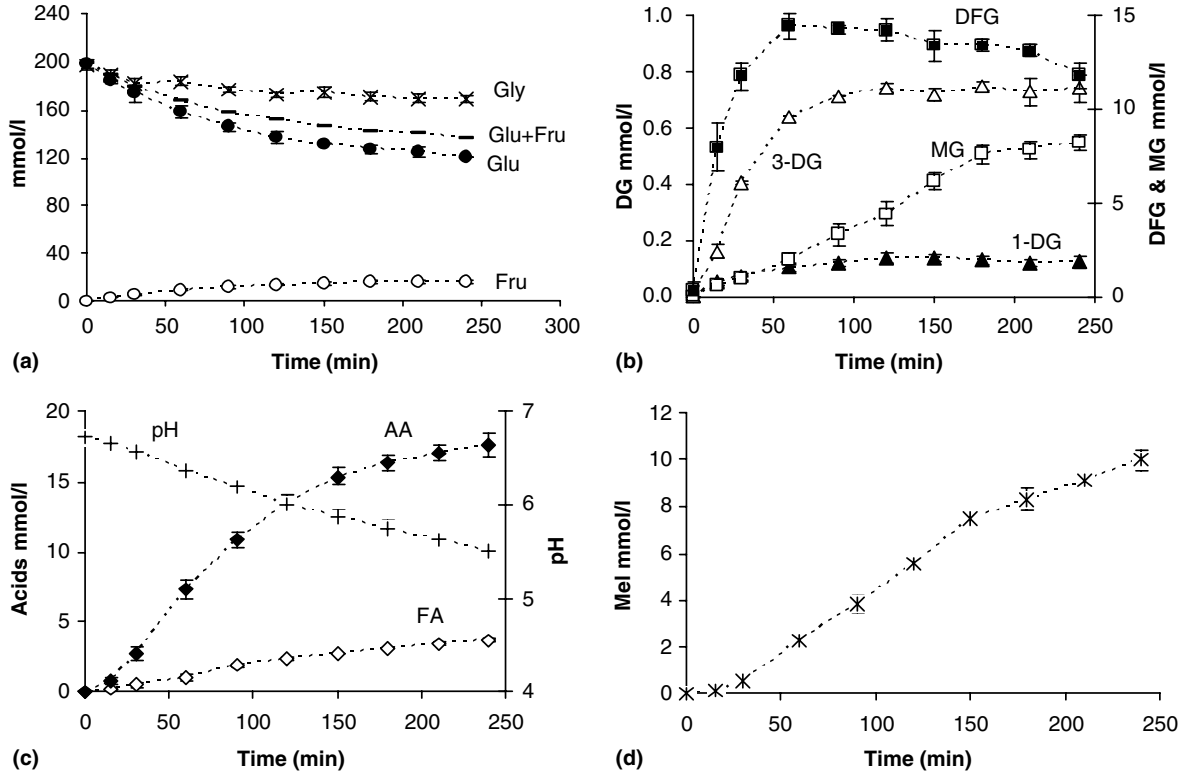


Fig. 1. Reactants and main products formed in heated glucose/glycine equimolar solution (0.2 M) at 100 °C and pH 6.8. (a) D-Glucose (Glu) (●), glycine (Gly) (×), D-fructose (Fru) (○), the sum of D-glucose and D-fructose (Glu + Fru) (-); (b) *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) (■), methylglyoxal (MG) (□), 1-deoxyglucosone (1-DG) (▲), 3-deoxyglucosone (3-DG) (△); (c) formic acid (FA) (◇), acetic acid (AA) (◆), pH (+); (d) melanoidins (Mel) (∗).

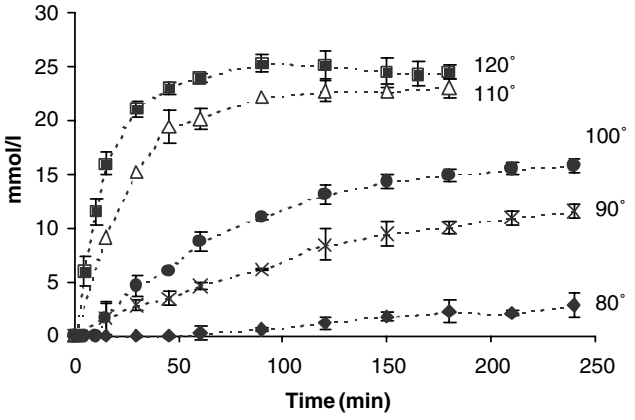


Fig. 2. Formation of D-fructose during thermal degradation of D-glucose (0.2 M) in an equimolar glucose/glycine aqueous solution at pH 6.8. 80 °C (◆); 90 °C (∗); 100 °C (●); 110 °C (△); 120 °C (■).

clear therefore, that besides isomerisation, sugars are more strongly involved, from a quantitative point of view, in the Maillard reaction than amino acids, as was also suggested by Vernin et al. (1987) and Debrauwer et al. (1991).

Another main intermediate identified and quantified in the glucose/glycine Maillard reaction was the Amadori compound *N*-(1-deoxy-D-fructos-1-yl)-glycine. Its behaviour is typical of an intermediate: at first a built up followed by a slow or fast decrease depending on the temperature (Fig. 3). The degradation pathways of DFG were studied in more detail previously, where DFG was used as reactant rather than as an intermediate at an initial concentration of 10 mM (Martins et al., 2003a; Martins & Van Boekel, 2003b). The reaction products using DFG as reactant were the same as for glucose/glycine but the amounts and relative ratios were obviously different.

Regarding organic acids formation in the glucose/glycine system, independent of temperature, acetic acid was always formed in higher concentrations than formic acid (Fig. 1(c)). After heating the system for 4 h at 100 °C and pH 6.8, 25% of the degraded D-glucose was transformed into acetic acid whereas only 5% was formic acid. The same results were observed when DFG was used as reactant (Martins et al., 2003a), but not when the sugar was heated alone. In fact, when D-glucose was heated without glycine at 100 °C and pH 6.8, besides the sugar isomer, organic acids, namely formic and acetic acid, were also detected. However, formic acid was then formed in higher amounts than acetic acid, 1.2% and 0.65% mol, respectively (results not shown). These results indicate that acetic acid is preferably formed during the Maillard reaction. The im-

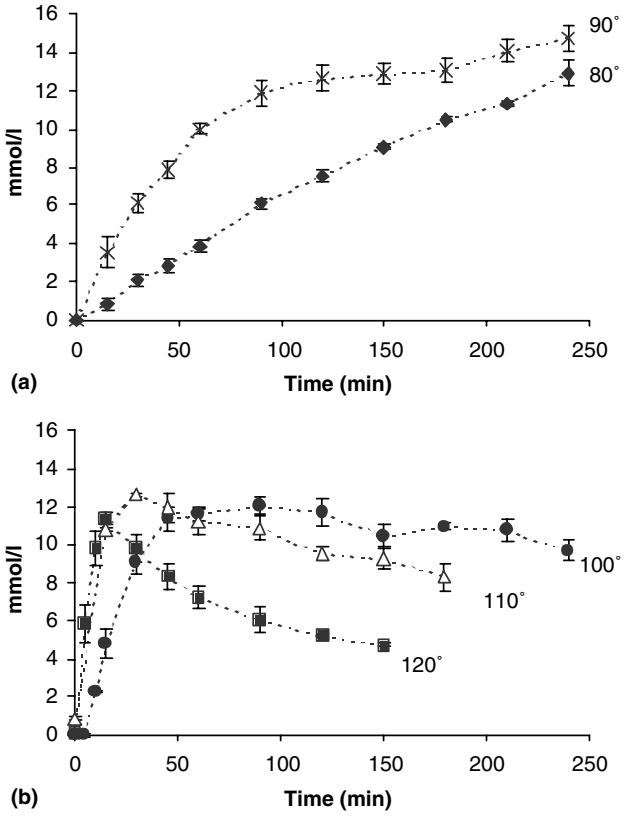


Fig. 3. Amadori compound *N*-(1-deoxy-D-fructos-1-yl)-glycine formation upon thermal treatment of glucose/glycine equimolar (0.2 M) solution at pH 6.8. (a) 80 °C (◆), 90 °C (∗); (b) 100 °C (●), 110 °C (△), 120 °C (■).

portance of carboxylic acids is that they cause considerable reduction in pH, and consequently cause a slowing down of the reaction. This phenomenon is particularly apparent at higher temperatures. Until now, the possible effects of a non-constant pH have not been included in modelling work. As will be reported quantitatively in a forthcoming paper, pH does have an effect on the kinetics. However, it was also found that if the pH decrease during an experiment stays within one pH unit, the effects are not considerable. In principle, it would of course be better to work at a constant pH, but this is experimentally very difficult at high temperatures. The use of buffers is only partially effective; moreover, buffers do have an effect on the Maillard reaction as well (Bell, 1997; Rizzi, 2004). Heat-induced pH decrease is also occurring in real foods, for instance in milk (Berg & Van Boekel, 1994), and in coffee (Ginz, Balzer, Bradbury, & Maier, 2000).

Concerning α-dicarbonyl compounds, for all the temperatures studied at pH 6.8, 3-deoxyglucosone (3-DG) was always formed in higher amounts than 1-deoxyglucosone (1-DG). At 100 °C, as shown in Fig. 1(b), the concentration of 1-DG was four times lower than that of 3-DG after some time. Deoxyosones have also

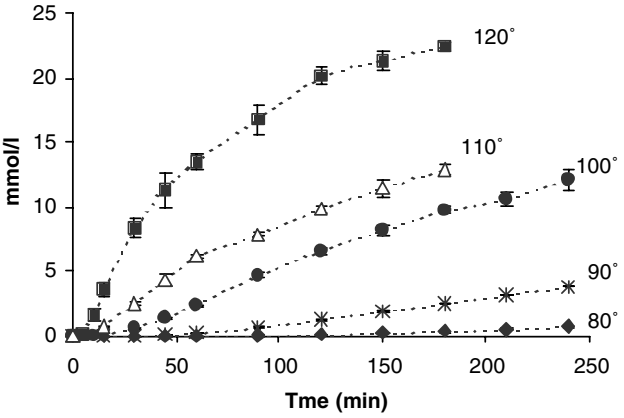


Fig. 4. Melanoidin formation upon thermal treatment of glucose/glycine equimolar (0.2 M) solution at pH 6.8. 80 °C (◆); 90 °C (∗); 100 °C (●); 110 °C (△); 120 °C (■).

been determined quantitatively as breakdown products of glucose/L-alanine aqueous solution heated under reflux at pH 7 (Hofmann, 1999). In that study the concentration of 3-DG was three times higher than that of 1-DG, which is in the same range as observed in the present study. In parallel with the increase of deoxyglucosones, the concentration of methylglyoxal (MG) also increased, reaching a yield of 4.1% after a reaction time of 4 h (Fig. 1(b)).

The concentration of melanoidins (low and high molecular weight, brown, nitrogenous chromophores), also known as Maillard reaction end products responsible for colour formation, was calculated from absorbance measurements and is actually expressed in terms of the molecular concentration of glucose converted into melanoidins. The effect of temperature on melanoidin formation is shown in Fig. 4 and is in line with the literature. However, melanoidin formation is the result of many preceding reactions and the temperature dependence of these preceding reactions is discussed in more details below.

3.2. Mass balance

The results of the mass balance calculations (Fig. 5) showed an almost negligible amount of missing compounds up to 4 h of heating at 100 °C and pH 6.8. The sum of all the intermediates identified and quantified reached 96% of the initial D-glucose concentration. In the Maillard reaction numerous products are formed, however the fact that almost 100% recovery was obtained indicates that the acids formed are stable end products of scission reactions leading to C_1–C_5 reaction products. Moreover, the complete loss of glycine could be accounted for by the sum of the Amadori product and melanoidin concentration, on the assumption of a 1:1 molar ratio of glucose:glycine in the melanoidins, as reported before (Van Boekel & Martins, 2002).

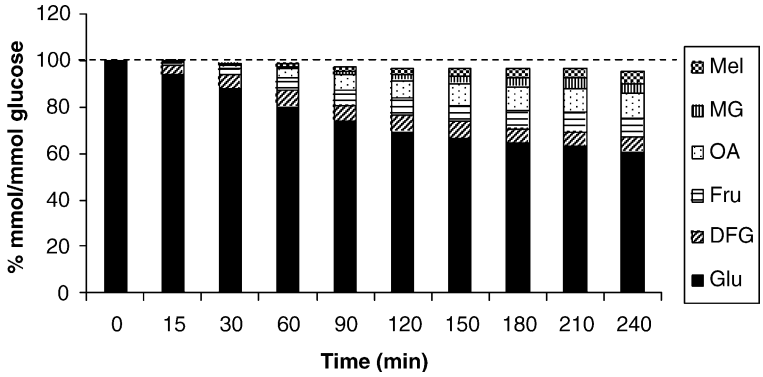
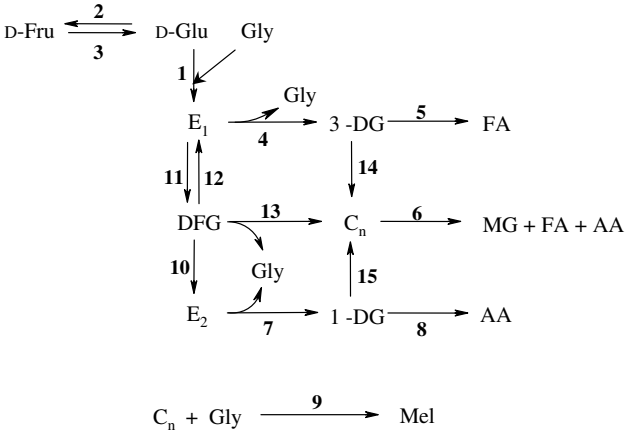


Fig. 5. Mass Balance of reactants and reaction products in heated glucose/glycine equimolar (0.2 M) solution at 100 °C and pH 6.8. D-Glucose (Glu); *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG); D-fructose (Fru); organic acids (OA) (formic acid + acetic acid); methylglyoxal (MG); melanoidins (Mel).

3.3. Building a reaction network

The reactions described above were put together into a model for D-glucose and glycine reactions; the starting point was the kinetic model derived for degradation of the Amadori compound (Martins & Van Boekel, 2003b): see Scheme 1. D-Glucose can undergo two types of transformation: (1) the Lobry de Bruyn–Alberda van Ekenstein (LA) transformation (Speck, 1985) and (2) the Maillard reaction. Via the LA transformation (reactions 2 and 3 in Scheme 1) D-glucose and D-fructose can isomerise into one another through the 1,2-enediol anion. Through this reaction pathway D-mannose can also be formed; it was however not detected in the heated model system. Moreover, the 1,2-enediol anion can undergo β-elimination to yield 3-deoxy-2-hexosulose that by α-dicarbonyl cleavage can form 2-deoxyribose and formic acid, or it can undergo retroaldolisation reaction to form glyoxal, or it can cyclise to form HMF. This 3-deoxy-2-hexosulose route is favoured under slightly acidic conditions. Under the pH conditions used in the present study formation of HMF was consequently very low (0–20 $\mu mol\,L^{-1}$). This reaction pathway was therefore not considered in Scheme 1.



Scheme 1. Kinetic model no. 1 proposed for the glucose/glycine Maillard reaction based on the established network for *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal degradation (Martins & Van Boekel, 2003b).

Sugar degradation products are to a large extent similar to those formed through Amadori degradation pathways. In the Maillard reaction the reducing sugar reacts with an amino group to form an *N*-substituted-glycosylamine, which is in equilibrium with its 1,2-enaminol (summarized in step 1, Scheme 1). This 1,2-enaminol can undergo the same reactions as described above for 1,2-enediol. Furthermore, the *N*-substituted-glycosylamine can rearrange into the Amadori rearrangement product (steps 11 and 12, Scheme 1), which is subject to further degradation via its 2,3-enaminol (step 10, Scheme 1). By release of the amino group, 1,2-enaminol and 2,3-enaminol can form their respective C_6 reactive intermediates, 3-deoxy-2-hexosulose and 1-deoxy-2,3-hexodiulose (steps 4 and 7, Scheme 1). These α-dicarbonyl compounds are unstable and undergo either benzilic acid rearrangement yielding saccharinic acids or a cleavage reaction (at the C–C bond, steps 5 and 8, Scheme 1) resulting in both formic and acetic acid, respectively (De Bruin, 1986).

Methylglyoxal was the predominant α-dicarbonyl fragment detected in quasi-water free mixtures of glucose/glycine and glucose alone systems heated at 100 °C (Hollnagel & Kroh, 1998). However, its formation was greatly enhanced when glycine was present in the reaction mixture. These authors concluded that methylglyoxal was mainly formed from intermediates that arise during the Maillard reaction, such as the 1-deoxyglucosone or the Amadori rearrangement product. These results are well in line with previous work (Martins & Van Boekel, 2003b) where multiresponse kinetic analysis suggested that methylglyoxal formation occurred mainly through Amadori retro-aldolization (steps 13 and 6, Scheme 1).

The reaction network model of Scheme 1 proposes that the glucose/glycine reaction results in an interme-

diate, designed as E_1, which can be the 1,2-enaminol, the Schiff's base or the cation form of the Schiff's base with the amino acid still incorporated (Coleman III & Chung, 2002). This intermediate is in equilibrium with DFG and due to its reactivity it hasn't been isolated yet from the Maillard reaction; however for modelling purposes it has been included as an intermediate step. The central importance of DFG in colour formation as well as its reversibility into parent sugars was studied by model discrimination, as will be discussed in the following section.

3.4. Kinetic modelling of glucose and glycine reactions

To fit the kinetic model to the experimental data, the reaction network presented in Scheme 1 was translated into a mathematical model by deriving differential equations for each reaction step using the law of mass action. These coupled differential equations were solved by numerical integration and fitted to the data. The results of the fits are shown in Fig. 6. The model of Scheme 1 did not describe all reactions in the glucose/glycine system very well. Besides the organic acids, a lack of fit is seen for both methylglyoxal and melanoidin formation. While the model predicted a longer induction phase in the formation of both compounds, this was not observed in the experimental data. Similar results were obtained at higher and lower temperatures. These results forced us to reconsider the proposed kinetic model.

From the literature, the mechanism of formation of brown colour is not fully understood and the structure of melanoidins is largely unknown. Recent studies (Cämmerer & Kroh, 1995; Yaylayan & Kaminsky, 1998) suggested that the melanoidin skeleton was mainly built up from sugar degradation products, formed in the early stages of the Maillard reaction, polymerized through aldol-type condensation and linked by amino compounds, such as amino acids. The hypothetical melanoidin structure proposed by Cämmerer and Kroh (1995) was based on the reactions of α-dicarbonyl compounds, in particular 3-deoxyglucosone. In the model proposed for *N*-(1-deoxy-D-fructos-1-yl)-glycine thermal degradation (Martins & Van Boekel, 2003b) the results showed that 3-DG more than 1-DG was involved in the formation of carbohydrate fragments (C_n) responsible for colour formation. These findings together with the induction phase predicted in melanoidin formation made us reconsider step 9 in Scheme 1. As an alternative, 3-DG was assumed to be the main precursor of colour formation by reaction with glycine (Scheme 2). Moreover, the degradation step of DFG into methylglyoxal through a carbohydrate fragment (C_n, step 13 and 6 in Scheme 1) was assumed to be a fast step without a rate-determining step in between, so step 13 was skipped in Scheme 2.

The revised model showed an improvement in melanoidin formation as well as in the MG formation (results not shown). However, the fit for both the organic acids and 1-DG formation was clearly not adequate, as

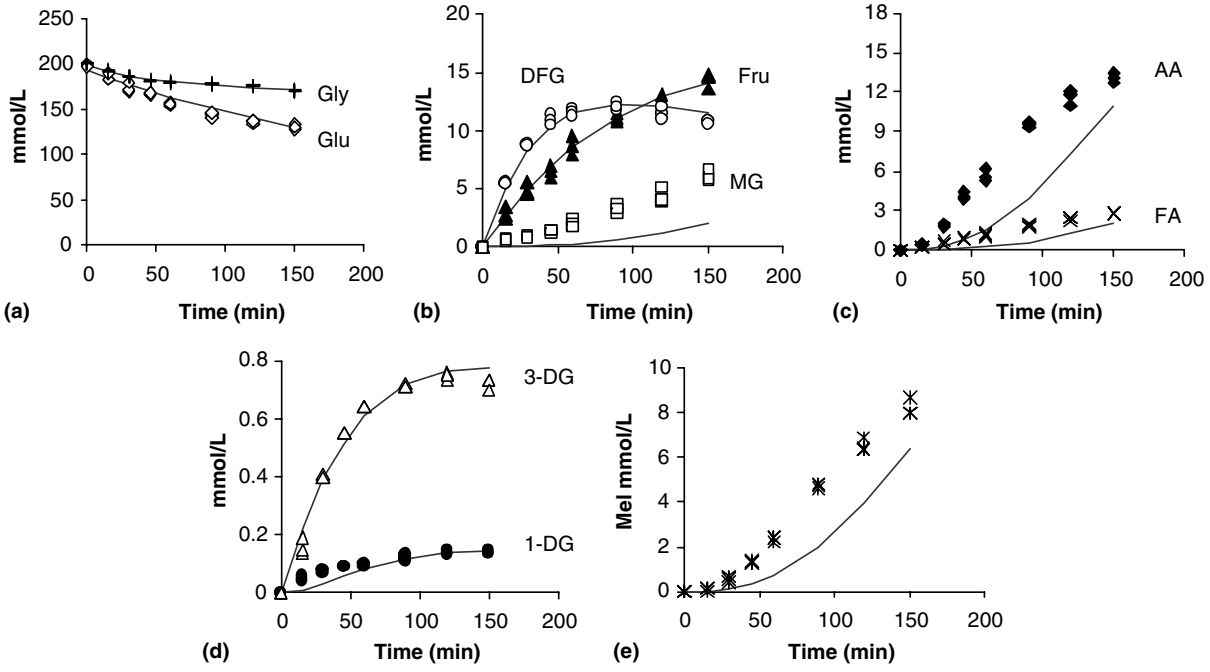
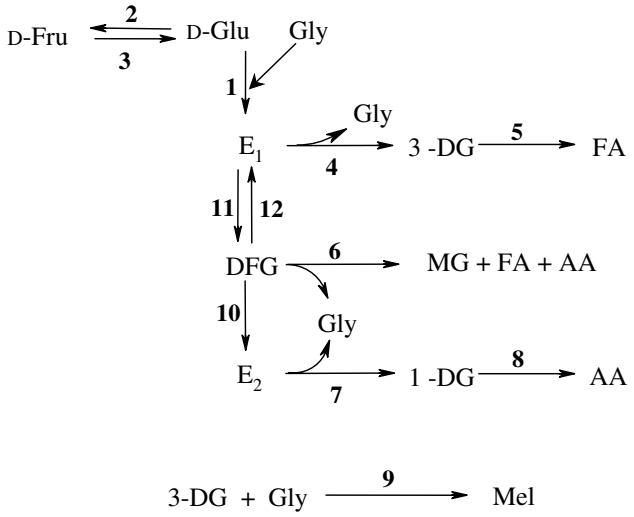
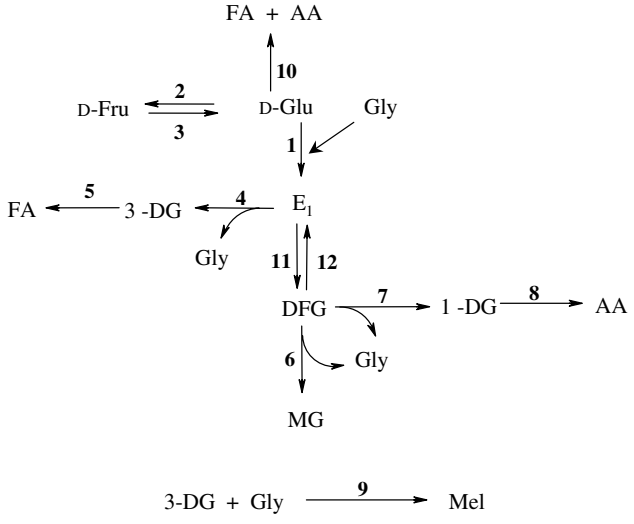


Fig. 6. Fit of the kinetic model of Scheme 1 to the experimental data for glucose/glycine system heated at 100 °C and pH 6.8. Simulations (drawn lines). (a) D-Glucose (Glu) (◇), glycine (Gly) (+); (b) D-fructose (Fru) (▲), *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) (○), methylglyoxal (MG) (□); (c) formic acid (FA) (×), acetic acid (AA) (◆); (d) 1-deoxyglucosone (1-DG) (●), 3-deoxyglucosone (3-DG) (△); (e) melanoidins (Mel) (∗).



Scheme 2. Kinetic model no. 2 for the glucose/glycine Maillard reaction.

was also observed with the previous model (Fig. 6). Both formic and acetic acid, as well as 1-DG were underestimated at the early stages of the reaction. In fact, direct cleavage of the Amadori product into formic and acetic acid (step 6, Scheme 2) is from a chemical point of view not very likely to happen. This exercise shows the power of the iterative modelling approach: more than just a fitting procedure and data analysis, multiresponse modelling gives insight on the reaction mechanism and helps to differentiate between assumptions. From the sugar isomerisation and degradation products it was observed that besides the sugar isomer, formic and acetic acid were also formed. A revised model was once more suggested in which the degradation of glucose into the organic acids was included (Scheme 3). Also, the



Scheme 3. Kinetic model no. 3 for the glucose/glycine Maillard reaction.

observation that at the beginning of the reaction 1-DG was underestimated led us to the assumption that 1-DG formation from DFG was a fast step, without E_2 as a rate-determining intermediate. This assumption, considering that the initial pH of the system used in this study was 6.8, is in line with the fact that this reaction step is favoured at pH $\geqslant$ 7 (Tressl et al., 1995).

The model in Scheme 3 was fitted to the experimental data, again by deriving differential equations for each reaction step as before. The results of the fit of Scheme 3 are shown in Fig. 7. A major improvement was observed. For most of the identified and quantified intermediates, the model described the experimental data quite well. Moreover, a multivariate test of goodness-of-fit was used, which was possible because replicates were available. This test ascertained that the model was acceptable also from a statistical point of view. However, since E_1 was not measurable, the question arises whether the reaction network in Scheme 3 can be further simplified for modelling purposes. The reason to look for further simplification is the general rule in modelling that models should be as simple as possible but no simpler than that, after a famous saying of Einstein. This also applies to kinetic models: as long as the model is able to explain experimental results, simplification is allowed and in fact necessary, so this was studied as described in the next section.

3.5. Model discrimination

The significance of E_1 and DFG reversibility was studied by model discrimination. It has been suggested that at physiological conditions the ketoamine undergoes enolization and rearrangement to form the Schiff base (Acharya & Sussman, 1984). Recently Ge and Lee (1997) reported that the formation of the Schiff base, formed prior to the Amadori compound, was reversible into the parent sugar and amino acid. Keeping this in mind, from the model presented in Scheme 3, three possible reaction mechanisms arose for the early stage of the glucose/glycine Maillard reaction:

Hypothesis A $\quad glu + glu \rightarrow E_1 \rightleftharpoons DFG$

Hypothesis B $\quad glu + glu \rightleftharpoons E_1 \rightarrow DFG$

Hypothesis C $\quad glu + glu \rightarrow E_1 \rightarrow DFG$

The model discrimination tests used were the so-called PPB, which requires replicates or an estimation of experimental uncertainty, and the AIC. PPB is given by the software used (www.athenavisual.com). The PPB is a concept from Bayesian statistics and measures how likely a model is, based on the data and on previous knowledge. The model with the highest PPB performs the best. The AIC on the other hand can be expressed in the case of least-squares (SS) approximation as:

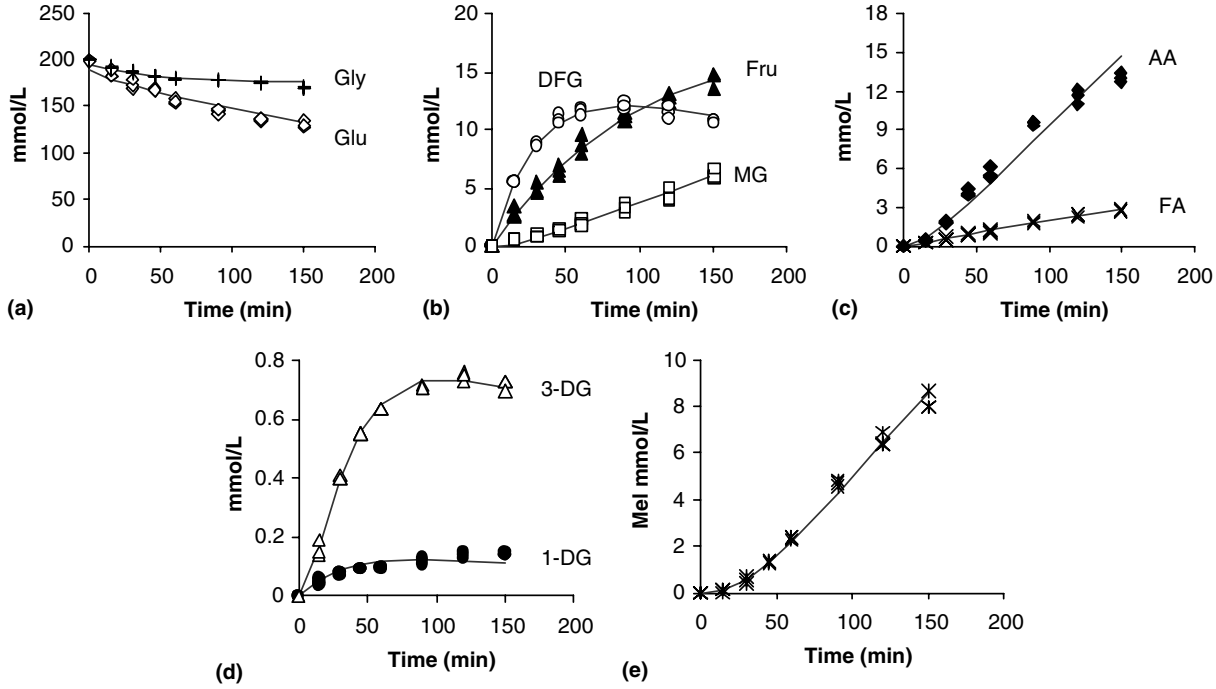


Fig. 7. Fit of the kinetic model of Scheme 3 to the experimental data for glucose/glycine system heated at 100 °C and pH 6.8. Simulations (drawn lines). (a) D-Glucose (Glu) (◇), glycine (Gly) (+); (b) D-fructose (Fru) (▲), *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) (○), methylglyoxal (MG) (□); (c) formic acid (FA) (×), acetic acid (AA) (◆); (d) 1-deoxyglucosone (1-DG) (●), 3-deoxyglucosone (3-DG) (△); (e) melanoidins (Mel) (∗). The same fit was obtained with the kinetic model of Scheme 4.

Table 1
Model discrimination tests: posterior probability (PPB) and Akaike Information Criterion (AIC) for hypothesis A, B and C

Hypothesis	p	SS	n	AIC_c	Δ_{AICc}	PPB
A	13	$8.07 \times 10^{+2}$	720	110.13	0	−19.05
B	13	$1.20 \times 10^{+3}$	720	397.59	276.00	−24.11
C	13	$1.18 \times 10^{+3}$	720	386.13	287.46	−23.91

p, Number of parameters; SS, residual sum of squares; n, number of data points including the replicates; Δ_{AIC}, AIC difference taking the smallest value as reference.

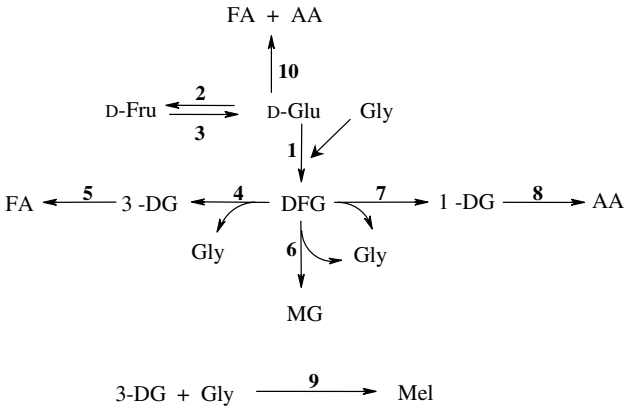
$$AIC = n \ln\left(\frac{SS}{n}\right)^2 + 2(p+1), \quad (1)$$

where n is the number of data points and p the number of estimated parameters. Because it is on a relative scale it is common practice to calculate Δ_{AIC} by difference, taking the model with the lowest value as reference (Burham & Anderson, 1998). The model with the lowest Δ_{AIC} performs the best from a statistical point of view.

In Table 1 the results of the model discrimination tests are shown. Both tests support hypothesis A, where it is suggested that a key intermediate is formed prior to the DFG formation but formation back into the parent sugar and amino acid is not important. Model discrimination is a relative concept, not an absolute one. It only provides information about the statistically most plausible model, not necessarily the true one. Taking into consideration the model discrimination results the proposed model was even further simplified (Scheme 4). Assuming steady state behaviour of the enaminol, its concentration can be taken to be directly proportional to the Amadori compound concentration. The same fitting procedure to the experimental data was performed. The model fitted the data equally well (the same results as depicted in Fig. 7). From a quantitative point of view these results suggest that E_1 is not a rate-determining step in the glucose/glycine Maillard reaction at pH 6.8.

3.6. Kinetic model validation: influence of the temperature

To strain the proposed model (Scheme 4), it was fitted to all five temperatures simultaneously. The Maillard reaction depends greatly on temperature with respect to which reaction route prevails and what pattern of intermediates and end products are formed. Consistent temperature dependence is an additional indication that a model is acceptable. In order to be able to predict the reaction rates at various temperatures, the temperature dependence had to be determined. The relationship between the rate constant (k) and temperature (T) is frequently indicated by the Arrhenius equation



Scheme 4. Kinetic model no. 4 (simplified version of kinetic model no. 3) for the glucose/glycine Maillard reaction.

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right), \tag{2}$$

where k_0 is the so-called frequency factor (same dimensions as k), R the gas constant (8.314 J mol^{-1} K^{-1}) and E_a the activation energy (J mol^{-1}). The estimated rate constants obtained from the model at each temperature separately showed that the temperature dependence was indeed Arrhenius like, in the sense that a straight line was obtained when $\ln k$ was plotted against $1/T$ (results not shown). When estimating the activation energies the experimental range of studied temperatures is small compared to the absolute temperature range over which the Arrhenius equation would apply. As a result the equation should be reparameterised, which can be done as follows:

$$k = X \exp(-YE_a), \tag{3}$$

where

$$X = k_0 \exp\left(\frac{-E_a}{RT_{av}}\right), \tag{4}$$

$$Y = \frac{1}{R}\left(\frac{1}{T} - \frac{1}{T_{av}}\right), \tag{5}$$

$$T_{av} = \frac{\sum T}{n}. \tag{6}$$

Taking into account the temperature dependence by including the reparameterised Arrhenius equation (Eq. (3)) at the five heating temperatures simultaneously, the k-values in the proposed model were replaced by Eq. (3) and fitted to all the data at once. Such a procedure gives a much better precision in the final estimates than first deriving rate constants and then fitting these to the Arrhenius model (Van Boekel, 1996). In Fig. 8 the results of the fit for the systems heated at 80, 100 and 120 °C are shown as example. The estimates of the activation energies and their 95%-highest posterior density

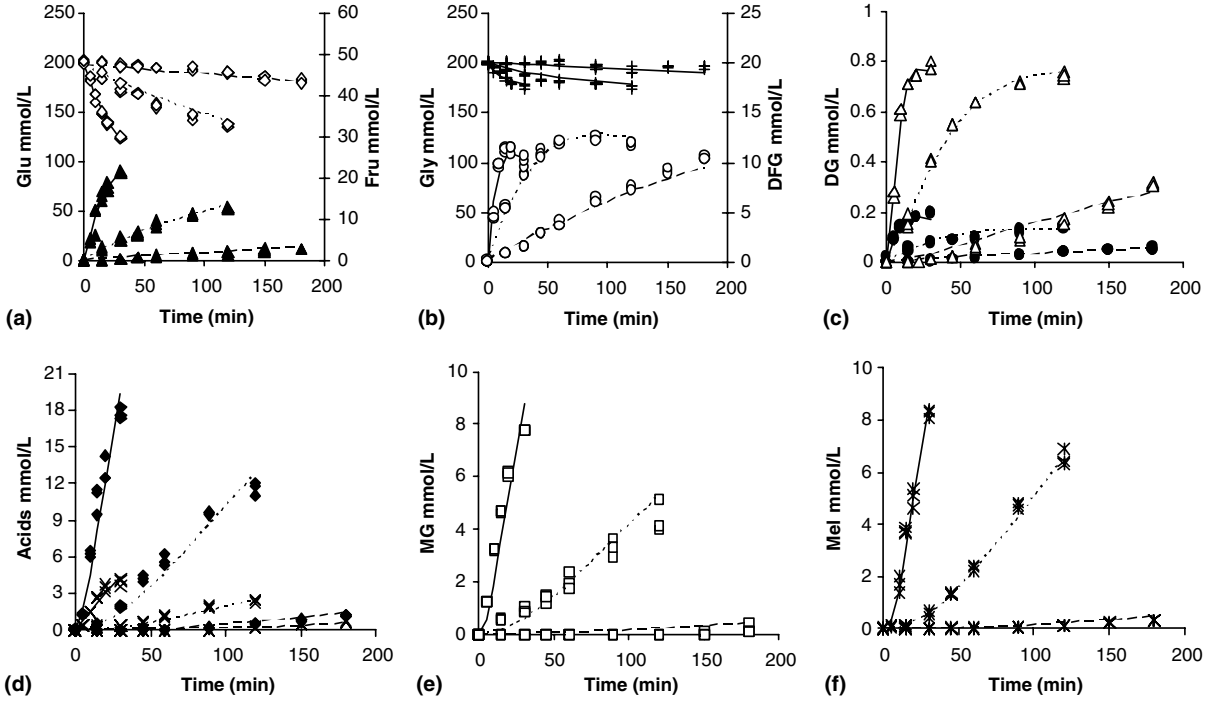


Fig. 8. Kinetic model validation. Simulations (drawn lines) based on the kinetic model of Scheme 4 for glucose/glycine system heated at 80 °C (– – –), 100 °C (- - -) and 120 °C (—). (a) D-Glucose (Glu) (◇), D-fructose (Fru) (▲); (b) glycine (Gly) (+), *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) (○); (c) deoxyglucosones (DG), 1-deoxyglucosone (1-DG) (●), 3-deoxyglucosone (△); (d) formic acid (×), acetic acid (◆); (e) methylglyoxal (MG) (□); (f) melanoidins (Mel) (∗).

Table 2
Estimated parameters (X and E_a) with temperature dependence included as found by kinetic modelling for the proposed model in Scheme 4

Reaction step	X[a]	E_a (kJ mol^{-1})[b]
1	$1.6 \times 10^{-5} \pm 3.3 \times 10^{-7}$	96.8 ± 2.8
2	$1.6 \times 10^{-3} \pm 1.0 \times 10^{-4}$	122.6 ± 5.2
3	$9.2 \times 10^{-3} \pm 1.9 \times 10^{-3}$	93.4 ± 1.9
4	$1.1 \times 10^{-2} \pm 4.0 \times 10^{-4}$	97.1 ± 1.7
5	$3.5 \times 10^{-2} \pm 6.4 \times 10^{-3}$	29.6 ± 8.5
6	$7.1 \times 10^{-3} \pm 4.6 \times 10^{-4}$	124.5 ± 4.7
7	$1.6 \times 10^{-2} \pm 6.8 \times 10^{-4}$	107.3 ± 7.3
8	$1.4 \times 10^{0} \pm 6.8 \times 10^{-2}$	75.7 ± 3.8
9	$8.1 \times 10^{-4} \pm 1.7 \times 10^{-5}$	95.2 ± 2.3
10	$4.4 \times 10^{-5} \pm 3.6 \times 10^{-5}$	236.7 ± 63.4

[a] Reparameterised Arrhenius equation as shown in Eq. (4).
[b] Activation energy.

intervals (i.e., the Bayesian equivalent of confidence intervals) are shown in Table 2. The consistent temperature dependence together with how well the model fitted the data is an additional but important indication that the model is valid. Also, the precision of the estimates is seen to be very high, except perhaps for step 10 in Scheme 4, which probably indicates that there is not enough information in the data to estimate this reaction step well.

An increase in temperature leads to a higher loss of the reactants and an increase in the formation of the reaction products. The assumption that glycine was regenerated from the Amadori compound degradation steps was well predicted by the model (Fig. 8(b)), as well as that colour formation was mainly related with the reaction between 3-DG and glycine (Fig. 8(f)). Moreover, under these reaction conditions DFG degradation showed lower temperature dependence in 3-DG and 1-DG formation (97.1 and 107.3 kJ mol^{-1}, respectively) and higher in MG formation (124.5 kJ mol^{-1}). It is suggested that DFG degrades first through the enolisation step. This result is well in line with the model discrimination results where DFG, in the Maillard reaction, is believed to be in equilibrium with an intermediate (Schiff's base, the cation form of the Schiff's base or the 1,2-enaminol) formed previously and responsible for the formation of 3-DG, the main colour precursor. This result is supported by the relatively low tendency for DFG to form brown colour under the same reaction conditions (Martins et al., 2003a; Molero-Vilchez & Wedzicha, 1997).

Concerning the sugar isomerisation step, D-fructose was one of the main products formed from D-glucose and its formation appeared to be quite highly temperature dependent (122.6 kJ mol^{-1}). The reverse step, on the other hand, appeared to be less temperature dependent (93.4 kJ mol^{-1}). Ketoses are generally more reactive than aldoses, but no decrease was observed in D-fructose concentration at any temperature studied. It is quite likely that fructose reactions were quantitatively of less importance because there was much more glucose present. Therefore, no degradation pathway for fructose was included in the proposed model, because this would then undoubtedly cause model estimation difficulties due to too many parameters. However, the authors are aware that the kinetics and the mechanism of D-fructose on the Maillard reaction may be different from D-glucose. Comparison with a study where D-fructose is used as the main reactant with glycine instead of glucose would be quite informative, as has been done previously in sugar–casein systems (Brands & Van Boekel, 2002).

The results suggest that glucose degradation into the organic acids (step 10 in Scheme 4) becomes more important at higher temperatures ($E_a = 237$ kJ mol^{-1}), but it must be acknowledged that this step was not estimated very precisely (cf. Table 2). In contrast, formation of 3-DG and 1-DG showed much lower temperature dependence in formic (29.6 kJ mol^{-1}) and acetic acid (75.7 kJ mol^{-1}) formation, respectively. The low amount of formic acid detected relatively to acetic acid at pH 6.8 suggested that 3-DG degraded preferably into melanoidin formation ($E_a = 95.2$ kJ mol^{-1}) as the temperature increased whereas 1-DG degraded mainly into acetic acid. Moreover, evidence is obtained that acetic acid is a main end product from the Maillard reaction (Fig. 8(d)). Acetic acid could therefore be proposed as an indicator of the progress of the Maillard reaction at pH 6.8.

4. Concluding remarks

The iterative process of proposing a model, confronting it with experimental data and criticizing the results, has lead to a kinetic model that is largely consistent with all results obtained. These results suggest that the reaction path from DFG into its parents, glucose and glycine, is not important from a quantitative point of view, even though the step prior to DFG formation is reversible. Moreover, the significance of DFG in colour formation is questioned. Circumstantial evidence is obtained that DFG is in equilibrium with an intermediate compound that is responsible for the formation of 3-DG, the main colour precursor. However, more research is needed to substantiate this. Furthermore, it should be noted that for extended heating periods a levelling-off was observed for most identified intermediates and end products, which was not predicted by the model. This could be a result of the observed pH drop, which may inhibit the reaction rate. A kinetic analysis should take this into account. However, this issue will be addressed in more detail in a subsequent paper where the influence of pH in the glucose/glycine system will be reported. In the present study, the effect of a non-constant pH, if any, is concealed in the rate constants.

The authors would like to stress that in the present study the identified compounds have been related to each other in a quantitative way via the proposed kinetic model. Products derived from the Strecker degradation, for instance, were not included because no characteristic Strecker compound was determined. However, the fact that we could quantitatively account for the loss of glycine in the proposed model suggests that Strecker degradation products are not very important in a quantitative sense in the glucose/glycine reaction, or if they are, they are accounted for in melanoidin formation.

The approach of numerical integration of differential equations, representing a model, followed by fitting to experimental data is flexible because different models can easily be tested by changing relevant differential equations and fitting them again to the experimental data. More than just a fitting procedure, multiresponse modelling was shown to be both helpful in deriving relevant kinetic parameters (rate constants and activation energies) as well as in obtaining insight in the reaction mechanism.

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