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Food Chemistry 92 (2005) 437–448
Food Chemistry
www.elsevier.com/locate/foodchem

Kinetics of the glucose/glycine Maillard reaction pathways: influences of pH and reactant initial concentrations

Sara I.F.S. Martins, Martinus A.J.S. Van Boekel *

Product Design and Quality Management, Department of Agrotechnology and Food Sciences, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Received 17 May 2004; accepted 2 August 2004

Abstract

A previously proposed kinetic model for the glucose/glycine Maillard reaction pathways has been validated by changing the initial pH (4.8, 5.5, 6.0, 6.8 and 7.5) of the reaction and reactant initial concentrations (1:2 and 2:1 molar ratios were compared to the 1:1 ratio). The model consists of 10 steps, each characterised by a rate constant. The initial pH had a different effect on the various rate constants, and the results suggest a mixture between specific acid and base catalysis. pH–rate profiles were established and, from these, a quantitative relationship was derived: $k_{obs} = k_e(10^{pD \times pH})$, in which k_{obs} is the estimated rate constants from experiments, k_e an expression for the elementary reaction, and pD the parameter describing the pH-dependence. The parameters k_e and pD were estimated from the pH–rate profiles. This equation thus expresses the pH-dependence of rate constants in much the same way as the Arrhenius equation does for the temperature-dependence of rate constants. The initial concentrations of glucose and glycine did not have an effect on the estimated rate constants, indicating that the model is robust to change in initial concentrations of the reactants. Finally, a sensitivity analysis of the model was performed to highlight the important steps, as well as finding possible redundant ones. Again, the model performed well; all steps were important and the model was consistent with the established reaction mechanism.

Keywords: Kinetics; Maillard reaction; pH–rate profile; Multiresponse modelling; Glucose; Glycine

1. Introduction

From a technological point of view, controlling food quality means controlling chemical, physical and microbiological changes during food processing and storage. However, to be able to do that, the reactions of interest need to be studied in a quantitative way. Kinetic parameters describing such changes are thus needed.

The Maillard reaction, also known as the reaction between carbonyls and amines or non-enzymatic browning, strongly affects food quality. Not only does it influence food sensorial properties, such as flavour and colour, but also nutritional and health aspects (Finot, Aeschbacher, Hurrell, & Liardon, 1990; Horiuchi, Taniguchi, Hayase, Kurata, & Osawa, 2002; Labuza, Reineccius, Monnier, O'Brien, & Baynes, 1994; Waller & Feather, 1983). Knowledge of the kinetic parameters, such as reaction order, rate constant and activation energy, is essential for predicting and controlling food quality attributes associated with the Maillard reaction. In previous studies, multiresponse kinetic modelling (i.e., taking more than one reactant and product into consideration in the modelling process) proved to be a powerful tool to model complicated consecutive and parallel reactions as occur in the Maillard reaction (Brands & Van Boekel, 2002; Brands & Van Boekel,

* Corresponding author. Tel.: +31 317 482520; fax: +31 317 483669.
E-mail address: tiny.vanboekel@wur.nl (M.A.J.S. Van Boekel).

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doi:10.1016/j.foodchem.2004.08.013

2003; Martins & Van Boekel, 2003a; Martins & Van Boekel, 2004). Such a multiresponse approach provided major guidance for understanding the reaction mechanism. 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 precisely than with the common uniresponse approach.

Kinetic model validation is often achieved by changing important reaction conditions to investigate how robust the model is and how consistent it is with the established reaction mechanism (Van Boekel, 1996, 2001). The course of the Maillard reaction can be affected by many factors, such as buffer, pH, temperature, reactant concentrations and the ratio between reactants. A buffer may exert a specific effect as catalyst of the reaction (Bell, 1997; Rizzi, 2004). With regard to the influence of pH on the Maillard reaction, Labuza and Baisier (1992) observed that the substrate loss increased with increasing pH, up to a pH of about 10, with little if any browning occurring below pH 6. Nicoli, Anese, and Lerici (1993) observed a smooth increase in browning and rate constants with pH between 2 and 8, but a rather abrupt increase between pH 8 and 10. The latter phenomenon is also obvious from work published by Ajandouz and Puigserver (1999) and Ajandouz, Tchiapke, Dalle Ore, and Puigserver (2001). Furthermore, the main degradation pathways of the Amadori compound, namely enolisation and retro-aldolisation, were shown to be strongly dependent on the reaction pH (Huyghues-Despoints & Yaylayan, 1996; Ledl & Schleicher, 1990; Smith & Thornalley, 1992). The pH-dependence of the Maillard reaction for the amino acid reagent can, at least qualitatively, be described by the effect of protonation of the amino acid. The amount of unprotonated amino group, which is considered to be the reactive species, increases obviously with increasing pH. Furthermore, pH has an effect on the reactant sugar. First, the open chain form of the sugar is considered to be the reactive species, and the amount of open chain increases with pH (Yayalayan, Ismail, & Mandeville, 1993). Second, the formation of the enediol anion is considered a key reaction in both reversible and irreversible sugar reactions (De Bruin, 1986). Reversible sugar reactions are ionisation, mutarotation, enolisation and isomerisation, and these reactions imply that the sugar moiety remains intact. Irreversible sugar reactions imply that the sugar moiety is degraded in, eventually, organic acids. Since ionisation is a rate-determining step (De Bruin, 1986), pH obviously has an influence on sugar reactions and hence on the Maillard reaction. All in all, the initial step of the Maillard reaction is highly influenced by the initial pH of the reaction. However, since the Maillard reaction consists of several reaction steps, some of which are acid–base catalysed, pH may have a considerable effect on which reaction route prevails and which products are formed. In all kinetic models published so far, including ours, pseudo rate constants, or observed rate constants, are used, which actually include pH-dependent rate constants, and do not include, for instance, sugar anions and the key intermediates, enediol anions, since these concentrations are assumed to be proportional to the total sugar concentrations (De Bruin, 1986). Since the pseudo rate constants do include pH-dependence, study of so-called pH–rate profiles can be helpful as a starting point for development of mechanistic hypotheses (Loudon, 1991). This author considered five possible options:

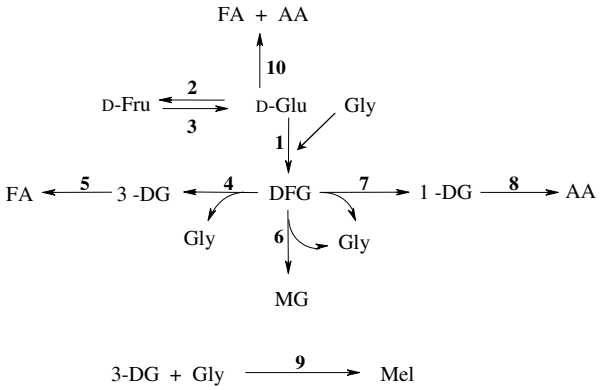
1. The observed rate constant is independent of pH: $k_{obs} = c_1$.
2. The observed rate constant is directly proportional to H^+ ions: $k_{obs} = c_2[H^+]$.
3. The observed rate constant is inversely proportional to H^+ ions (or equivalently directly proportional to $[OH^-]$): $k_{obs} = c_3/[H^+]$.
4. The observed rate constant is independent of pH at low pH and directly proportional to pH at high pH: $k_{obs} = c_4[H^+]/(c_5+[H^+])$.
5. The observed rate constant is inversely proportional to pH at low pH and independent at high pH: $k_{obs} = c_6/(c_7 + [H^+])$.

Besides pH, initial concentrations and ratio of the reactants should also have a significant impact on the reaction. Kato, Yamamoto, and Fujimaki (1969) observed that, at low concentrations of glycine, fructose browned faster than glucose, whereas, at high amino acid concentration, the reverse occurred. However, it should be noted that the *rate* of browning and the *rate constant* of the step in the reaction network that results in colour formation, are two different things. In fact, increasing the initial reactant concentrations should *not* influence the reaction rate constants since, for the reaction of sugar (S) with the amino acid (A), it follows that:

$$-\frac{d[S]}{dt} = -\frac{d[A]}{dt} = k_1[S][A].$$

The overall rate of loss of the S and the A is equal to the rate constant times their concentration. Obviously, the overall *rate* does depend on concentration.

Maillard browning of course increases with temperature. However, each step within the Maillard reaction network may have different temperature sensitivity and, as a result, temperature may strongly determine which reaction route prevails. Recently, the influence of temperature on the glucose/glycine Maillard reaction was studied and a comprehensive kinetic model was proposed, which is reproduced here as Scheme 1 (Martins & Van Boekel, 2004). Using multiresponse kinetic analysis, the temperature-dependence of the various degradation



Scheme 1. Kinetic model for the glucose/glycine Maillard reaction. D-Glucose (D-Glu); glycine (Gly); D-fructose (D-Fru);. *N*-(1-deoxy-D-fructos-1-yl)–glycine (DFG); 3-deoxglucosone (3-DG); 1-deoxyglucosone (1-DG); methylglyoxal (MG); acetic acid (AA); formic acid (FA); melanoidins (Mel).

steps in the glucose/glycine Maillard reaction was shown to be Arrhenius-like. Indeed, there were rather large differences in activation energies between the various steps. Applying the same methodology in the present study, the proposed kinetic model in Scheme 1 was strained by changing the equally important conditions, pH and reactant initial concentrations. If the proposed model is consistent with the reaction mechanism, no significant differences on the estimated parameters should be observed by changing the reactant concentrations. On the other hand, by changing the reaction pH, an indication should be obtained of the pH-dependence of each individual step in the reaction network. This is a challenge to our understanding of the mechanism of the individual steps within the Maillard reaction. Apart from the general notion that pH has a strong influence on Maillard reaction, a study of the effect of pH on the various steps in the Maillard reaction has not previously been published to our knowledge.

2. Material and methods

2.1. Preparation of reaction mixtures

Equimolar solutions of glucose and glycine (0.2 mol/l) were prepared in phosphate buffer (10 ml, 0.1 mol/l), set to have an initial pH 4.8, 5.5, 6.0, 6.8 and 7.5 at 20 °C. The solutions were filtered (0.2 μm, Schleicher & Schuell) and heated at 100 °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, prior to analyses. Each reaction mixture was prepared, heated and analysed, at least in duplicate. Because of the observed heat-induced pH drop, an additional experiment was done at 100 °C where the pH was kept constant; the initial pH was 6.8 as set at room temperature. The pH stat controller monitored the pH and, when it dropped below its set point, an auto burette (ABU901 Radiometer Copenhagen) added a solution of NaOH (1 mol/l) to the reaction mix. It is common knowledge that the pH decreases with temperature because of increased water dissociation. It is therefore stressed that the initial pH values reported are as measured at room temperature. When the pH was kept constant, this was done at the pH the solution had at 100 °C; the corresponding initial pH was 6.8 at room temperature. The temperature was monitored by a Consort R305. At predetermined times, samples were taken through a septum with a Hamilton SampleLock (10 ml) into a glass tube and immediately cooled in ice.

2.2. Identification and quantification of main intermediates

The following compounds were 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 & Van Boekel, 2004). The melanoidins concentration was calculated from the measured absorbance at 470 nm, using the extinction coefficient 0.64 ± 0.03 l mmol^{-1} cm^{-1} (Martins & Van Boekel, 2003b), and the resulting concentration reflects the amount of glucose molecules incorporated in the melanoidins.

2.3. Kinetic modelling

The proposed kinetic model (Scheme 1) was translated into a mathematical model by deriving differential equations for each reaction step, where the rate constants are the parameters to be estimated. The software package Athena Visual Workbench (www.athenavisual.com) was used for numerical integration of the differential equations, as well as for parameter estimation. The model parameters were estimated by non-linear regression, using the determinant criterion, that is to minimise the determinant of the matrix of cross-products of the various responses, the so called dispersion matrix. The determinant criterion replaces the familiar least-squares objective, because the multiresponse method requires a different statistical approach (Stewart, Caracotsios, & Sørensen, 1992). The goodness of fit test is installed in the used software package. It gives a sampling probability by which the adequacy of the model can be judged and was based on replicate experiments (Stewart, Shon, & Box, 1998).

3. Results and discussion

3.1. Influence of pH on reaction routes

To study the effect of pH on the glucose/glycine reaction kinetics, the samples were heated at 100 °C for up to 4 h at five different initial pHs (4.8, 5.5, 6.0, 6.8 and 7.5, as measured at room temperature). As the initial pH increased, glucose degradation rate also increased (Fig. 1). A maximum of 50 % degradation was observed at pH 7.5 whereas, for the same heating time, only 6% of glucose was degraded at pH 4.8. The increase of sugar reactivity was in line with the fact that the higher the pH the higher were the concentrations of the detected intermediates: D-fructose, *N*-(1-deoxy-D-fructos-1-yl)–glycine (DFG), formic and acetic acid, the α-dicarbonyl compounds 3-deoxyglucosone (3-DG), 1-deoxyglucosone (1-DG) and methylglyoxal (MG), and melanoidins. In contrast, the formation of 5-hydroxymethylfurfural (HMF) increased with decreasing pH; however, its amounts were one order of magnitude lower (i.e. in the μmolar range) than the other detected reaction products, and this compound was therefore neglected in the further analysis.

The results of the mass balance calculations showed that, for pH $\leqslant$ 6.0, the main quantified compound was the Amadori compound (DFG) (Fig. 2(a)). The observed gap (mass balance <100%) between 45 and 180 min suggested that other products, not identified in the present study, were also formed. However, the fact that we calculated 100% recovery, eventually, indicates that the acids formed are stable end-products of scission reactions, leading to C_1–C_5 reaction products. Moreover, under these reaction conditions the predominance of 3-DG over 1-DG was also observed at pH $\leqslant$ 6.0. These α-dicarbonyl compounds are unstable and undergo either benzylic acid rearrangement yielding saccharinic acids or a cleavage reaction (at the C–C bond), resulting in formic and acetic acid, respectively (De Bruin, 1986). In fact, at lower pH values, a slightly higher concentration of formic acid than acetic acid was observed at the beginning of the heating period (Fig. 3). The opposite was observed when the pH was increased. At pH 7.5, 20% of the nearly 50% of degraded glucose represented both formic and acetic acid, of which 14% was due to acetic acid (Fig. 2(b)).

The importance of carboxylic acid formation is that it is related to the observed pH drop, even though a buffer system (0.1 M) was used (Fig. 4). This phenomenon was particularly important at higher pH values. The same observation has been reported by Waller, Becker, and Adeleye (1983) when heating arginine and xylose systems with different initial pH values. Besides organic acid formation, the sugar isomerisation step, with the

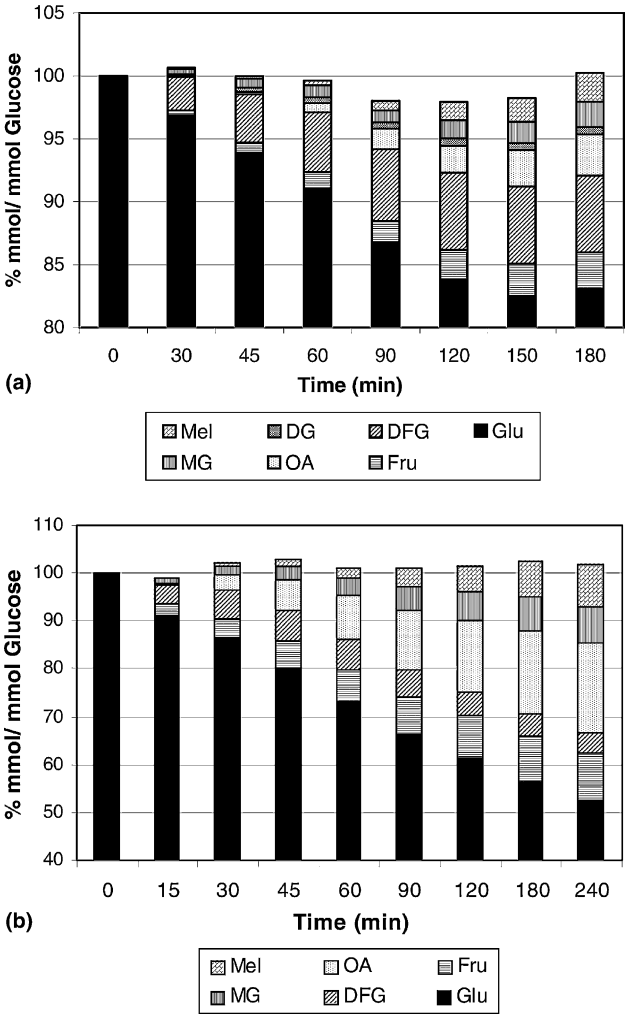


Fig. 2. Mass balance of reactants and reaction products in heated glucose–glycine reaction mixture in phosphate buffer (0.1 M) at 100 °C and (a) pH 6.0, and (b) pH 7.5. Glucose (Glu); fructose (Fru); Amadori compound (DFG); sum of 1-deoxy and 3-deoxyglucosone (DG); sum of formic and acetic acid (OA); methylglyoxal (MG); melanoidins (Mel).

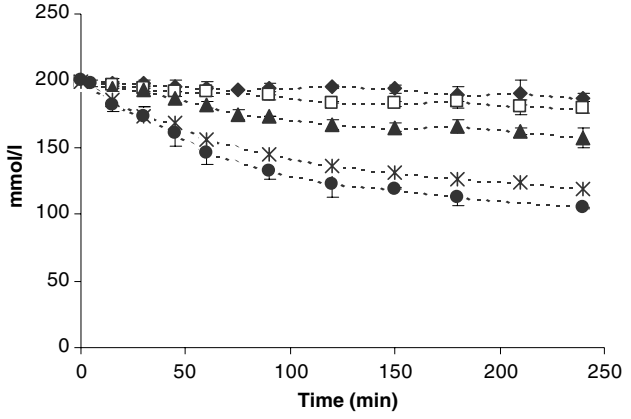


Fig. 1. Glucose degradation during heating of glucose/glycine reaction mixture in phosphate buffer (0.1 M) at 100 °C and initial pH 4.8 (◆); pH 5.5 (□); pH 6.0 (▲); pH 6.8 (∗) and pH 7.5 (●).

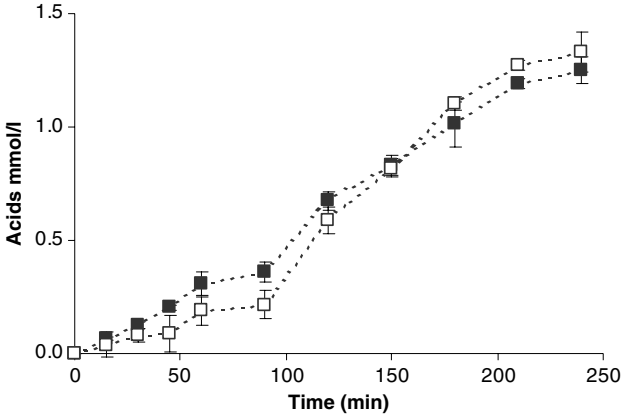


Fig. 3. Organic acids formation during heating of glucose/glycine reaction mixture in phosphate buffer (0.1 M) at 100 °C and pH 5.5. Formic acid (■); acetic acid (□).

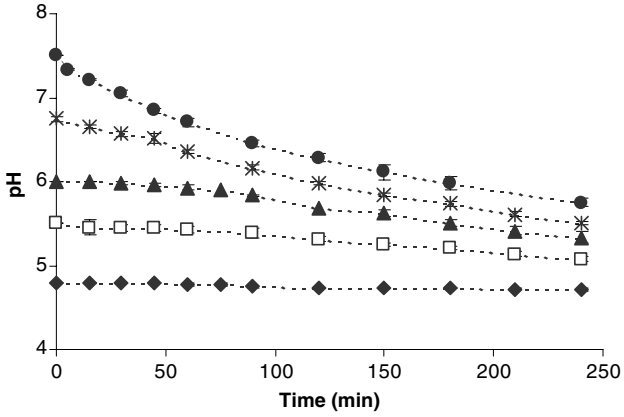


Fig. 4. pH drop during heating of glucose/glycine reaction mixture in phosphate buffer (0.1 M) at 100 °C and pH 4.8 (◆); pH 5.5 (□); pH 6.0 (▲); pH 6.8 (∗) and pH 7.5 (●).

formation of fructose, also became more evident at higher pH values.

The results clearly show that the reaction mechanism is strongly influenced by the pH, with respect to which reaction route prevails and to the type of intermediates and end-products that are formed, basically in line with literature. If consistent pH-dependence can be accounted for in a model, this is an additional indication that the model is acceptable.

3.2. pH-dependence of observed rate constants

As discussed in the introduction, the effect of pH on reactivity of both sugar and amino acid explains, qualitatively, the dependence of the Maillard reaction on pH. However, in order to be able to predict the reaction rates at various pHs, the pH-dependence has to be determined in a quantitative way. For specific acid–base catalysis, it can be derived that the observed rate constant, k_{obs}, should have the following relationship (Loudon, 1991):

$$k_{obs} = k_1[H^+] \quad \text{for acid catalysis,}$$

$$k_{obs} = k_2 K_w/[H^+] \quad \text{for base catalysis,}$$

where k_1 and k_2 are elementary rate constants for the reaction under study, and K_w is the water dissociation constant, so a plot of $\log(k_{obs})$ vs. pH should have a slope of −1 for acid catalysis and +1 for base-catalysis. De Bruin (1986) arrived at a similar relationship for base catalysis, following a complete analysis of sugar anion and enediol anion formation in alkaline reactions of carbohydrates. It was reported earlier that the Maillard reaction resembles alkaline sugar reactions to some extent (Berg & Van Boekel, 1994). Therefore, it stands to reason that similar pH-dependence as for alkaline sugar reactions can be expected within the Maillard reaction.

With respect to the proposed kinetic model, it would in principle be best to have a mechanistic base to explain the pH-dependence, on which the model would be built. However, it is not precisely known where and how H^+ ions interfere in the various steps in the Maillard reaction mechanism. Consequently, a pragmatic approach was taken and we studied how the observed rate constants (i.e. estimated from the proposed model in Scheme 1) were affected by varying the initial pH in separate experiments. Ideally, within each pH experiment, the pH should be kept constant, but this is experimentally quite difficult at temperatures at or above 100 °C. Even though a buffer was used, the pH dropped as the Maillard reaction progressed, as shown in Fig. 4. In order to have some idea of the error involved due to pH decrease, one experiment was done where the pH was kept constant during heating, using a pH Stat Controller. The used conditions were 100 °C and pH 6.8 (set at room temperature). The results of the model fits to the experimental data are shown in Fig. 5 and the estimated rate constants are presented in Table 1, along with results of an experiment where the pH was allowed to drop, but restricting the experiment to observations where the drop was less than 1 pH unit. The estimated rate constants did not change drastically whether or not the system had a constant pH. This made us conclude that it was justified – within limits – to use non-constant pH studies to study pH effects on rate constants under the studied conditions. However, to minimise the disturbing effects of non-constant pH as much as possible, only data were taken for subsequent modelling for which the pH drop was less than one unit. Table 2 shows the estimated rate constants, observed at each initial pH, for data with a pH drop <1 unit. Overall, the rate constants increased with increasing initial pH.

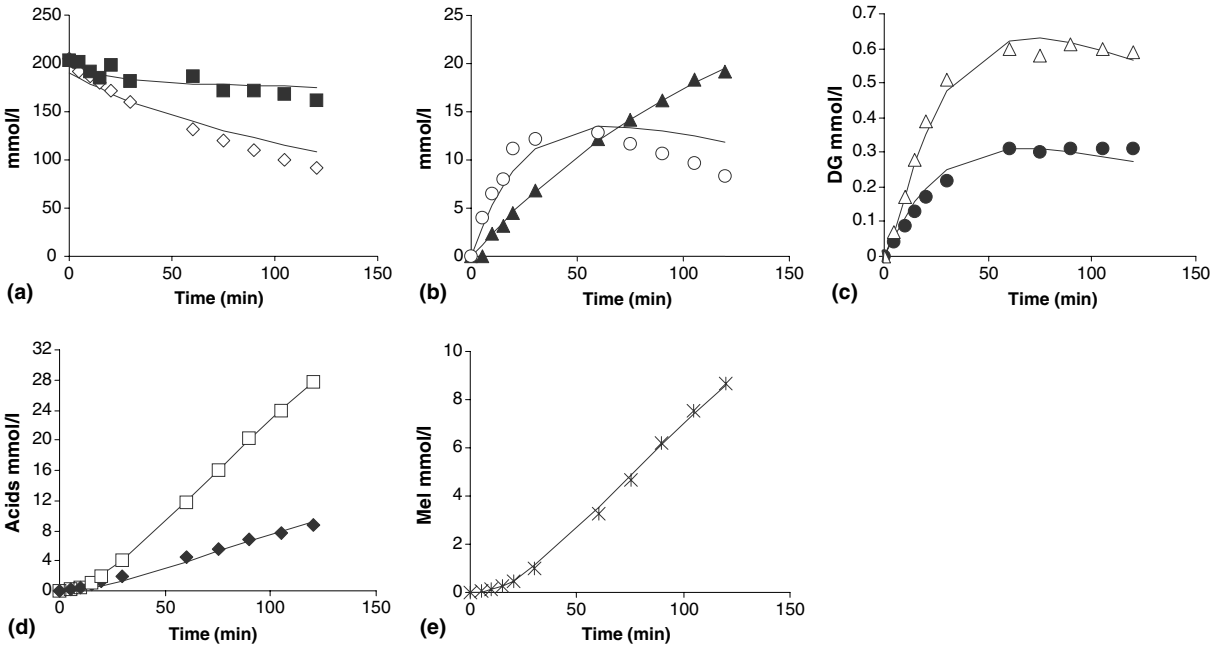


Fig. 5. Glucose–glycine Maillard reaction at 100 °C with pH kept constant by addition of 1 N NaOH (initial pH set at 6.8 at room temperature). Model fit (lines, calculated according to Scheme 1) to experimental data (markers). (a) Glucose (◇), glycine (■); (b) fructose (▲), *N*-(1-deoxy-D-fructos-1-yl)–glycine (○); (c) 1-deoxyglucosone (●), 3-deoxyglucosone (△); (d) formic acid (◆), acetic acid (□); (e) melanoidins (∗).

Table 1
Rate constants (k) estimation ± 95% HPD[a] intervals: system with observed pH drop vs. pH kept constant by NaOH addition

	pH drop $\leqslant 1$	pH constant
1 (l mol^{-1} min^{-1})	$1.1\times10^{-5}\pm4\times10^{-6}$	$1.8\times10^{-5}\pm1\times10^{-6}$
2 (min^{-1})	$8.9\times10^{-4}\pm4\times10^{-5}$	$1.3\times10^{-3}\pm1\times10^{-4}$
3 (min^{-1})	$5.9\times10^{-3}\pm8\times10^{-4}$	$2.5\times10^{-3}\pm1\times10^{-3}$
4 (min^{-1})	$6.5\times10^{-3}\pm3\times10^{-4}$	$1.3\times10^{-2}\pm9\times10^{-4}$
5 (min^{-1})	$4.1\times10^{-3}\pm5\times10^{-4}$	$1.3\times10^{-1}\pm1\times10^{-2}$
6 (min^{-1})	$5.2\times10^{-3}\pm2\times10^{-4}$	n.a.
7 (min^{-1})	$1.3\times10^{-2}\pm4\times10^{-4}$	$2.0\times10^{-2}\pm2\times10^{-3}$
8 (min^{-1})	$1.3\times10^{+0}\pm2\times10^{-1}$	$8.7\times10^{-1}\pm5\times10^{-2}$
9 (min^{-1})	$9.1\times10^{-4}\pm2\times10^{-5}$	$7.8\times10^{-4}\pm3\times10^{-5}$
10 (min^{-1})	$1.0\times10^{-4}\pm5\times10^{-5}$	$5.7\times10^{-4}\pm4\times10^{-5}$

Samples heated at 100 °C, pH 6.8; n.a. not analysed (formation of methylglyoxal).
[a] Highest posterior density.

As a next step, the pH-dependence was analysed quantitatively by making a so-called pH rate profile, i.e. a plot of $\log(k_{obs})$ vs. pH. A general equation for a pH rate profile is:

$$\log(k_{obs}) = \log a + b \times \mathrm{pH},$$

where a and b are regression coefficients. The coefficient a reflects one or more elementary rate constants and this coefficient is therefore called k_e, while b reflects the pH-dependence, which will be called pD. The pH-dependence of observed rate constants can then be expressed as:

$$k_{obs} = k_e(10^{\mathrm{pH}\times\mathrm{pD}}).$$

This equation basically fulfils the same function as the Arrhenius equation does for the temperature-depend-

Table 2
Rate constants (k) estimation by the proposed glucose/glycine kinetic model (Scheme 1)

K	pH 4.8	pH 5.5	pH 6.0	pH 6.8	pH 7.5
1 (l mol^{-1} min^{-1})	$7.9\times10^{-7}\pm2\times10^{-8}$	$2.3\times10^{-6}\pm5\times10^{-8}$	$4.9\times10^{-6}\pm1\times10^{-7}$	$1.1\times10^{-5}\pm4\times10^{-6}$	$1.8\times10^{-5}\pm8\times10^{-7}$
2 (min^{-1})	$8.8\times10^{-6}\pm1\times10^{-6}$	$5.5\times10^{-5}\pm4\times10^{-6}$	$2.3\times10^{-4}\pm3\times10^{-3}$	$8.9\times10^{-4}\pm4\times10^{-5}$	$2.1\times10^{-3}\pm7\times10^{-5}$
3 (min^{-1})	0.00	0.00	$2.8\times10^{-3}\pm2\times10^{-3}$	$5.9\times10^{-3}\pm8\times10^{-4}$	$1.4\times10^{-2}\pm9\times10^{-4}$
4 (min^{-1})	$1.5\times10^{-3}\pm7\times10^{-5}$	$3.1\times10^{-3}\pm1\times10^{-4}$	$4.5\times10^{-3}\pm3\times10^{-4}$	$6.5\times10^{-3}\pm3\times10^{-4}$	$1.5\times10^{-2}\pm1\times10^{-3}$
5 (min^{-1})	0.00	$6.3\times10^{-3}\pm2\times10^{-3}$	$2.0\times10^{-2}\pm2\times10^{-3}$	$4.1\times10^{-3}\pm5\times10^{-4}$	$1.9\times10^{-2}\pm1\times10^{-2}$
6 (min^{-1})	$5.0\times10^{-4}\pm8\times10^{-5}$	$9.1\times10^{-4}\pm1\times10^{-4}$	$2.7\times10^{-3}\pm1\times10^{-4}$	$5.2\times10^{-3}\pm2\times10^{-4}$	$1.1\times10^{-2}\pm9\times10^{-4}$
7 (min^{-1})	$7.7\times10^{-4}\pm7\times10^{-5}$	$4.0\times10^{-4}\pm1\times10^{-4}$	$3.1\times10^{-3}\pm3\times10^{-4}$	$1.3\times10^{-2}\pm4\times10^{-4}$	$1.5\times10^{-2}\pm1\times10^{-3}$
8 (min^{-1})	$9.9\times10^{-2}\pm9\times10^{-3}$	$6.2\times10^{-2}\pm2\times10^{-2}$	$1.0\times10^{-1}\pm1\times10^{-2}$	$1.3\times10^{+0}\pm2\times10^{-1}$	$4.5\times10^{-1}\pm4\times10^{-2}$
9 (min^{-1})	$5.0\times10^{-5}\pm4\times10^{-6}$	$2.1\times10^{-4}\pm1\times10^{-5}$	$2.4\times10^{-4}\pm2\times10^{-5}$	$9.1\times10^{-4}\pm2\times10^{-5}$	$5.3\times10^{-4}\pm5\times10^{-5}$
10 (min^{-1})	0.00	$2.1\times10^{-5}\pm3\times10^{-6}$	$6.3\times10^{-5}\pm6\times10^{-4}$	$1.0\times10^{-4}\pm5\times10^{-5}$	$3.1\times10^{-4}\pm6\times10^{-5}$

Samples heated at 100 °C, in phosphate buffer (0.1 M); to avoid an interfering affect of pH drop on the reaction rate inhibition, only data points were taken for which the pH drop was not higher than 1 unit.

ence of rate constants. For specific acid catalysis, k_e reflects the elementary rate constant for the acid-catalysed step and b, or pD should be -1 in that case. For pure specific base-catalysed reactions, k_e reflects the product of the water dissociation constant K_w and the elementary rate constant for the base-catalysed step, while b or pD should equal +1. If there is no pH-dependence (pD 0), then the observed rate constant reflects the elementary rate constant. The two parameters k_e and pD need to be determined by experiment, as we have done here.

The pH–rate profiles are shown in Fig. 6, and the resulting estimates of the parameters k_e and pD in Table 3. It is striking that only one rate constant comes close to pure base catalysis, namely the one that describes sugar isomerisation (k_2). This is in line with results of De Bruin (1986) for reactions of sugars under alkaline conditions. One rate constant does not really depend on pH (k_5), while the slopes for k_3 and k_8 do not significantly differ from zero (or are too imprecise to be estimated well). The slopes for the other rate constants vary between 0.4 and 0.6, indicating some mix between pure acid and pure base catalysis. It is quite remarkable that the rate constant describing melanoidin formation (k_9) is not very strongly pH-dependent. This means that the frequently reported pH-dependence of browning is due to reactions earlier on in the reaction.

Table 3
Estimates ± 95% confidence intervals of parameters k_e and pD from pH rate profiles

Rate constant k_{obs}	pD	Log(k_e)
1	0.50 ± 0.14	−8.43 ± 0.91
2	0.88 ± 0.27	−9.14 ± 1.68
3	0.46 ± 0.46	−5.36 ± 3.26
4	0.35 ± 0.10	−4.46 ± 0.60
5	0.09 ± 1.10	−2.59 ± 7.54
6	0.51 ± 0.14	−5.75 ± 0.85
7	0.60 ± 0.52	−6.24 ± 3.25
8	0.40 ± 0.60	−3.12 ± 2.40
9	0.40 ± 0.38	−6.03 ± 2.35
10	0.54 ± 0.36	−7.56 ± 2.35

There are not too many literature data to compare these results, and moreover, what is known pertains mainly to browning as an overall reaction, whereas our results are more detailed for intermediate steps. Nevertheless, it could be interesting to compare the few results that are available for the same pH range as studied here. Dworschák and Örsi (1977) determined a slope of 0.38 for the pH rate profile describing the loss of methionine and 0.13 for tryptophan in the Maillard

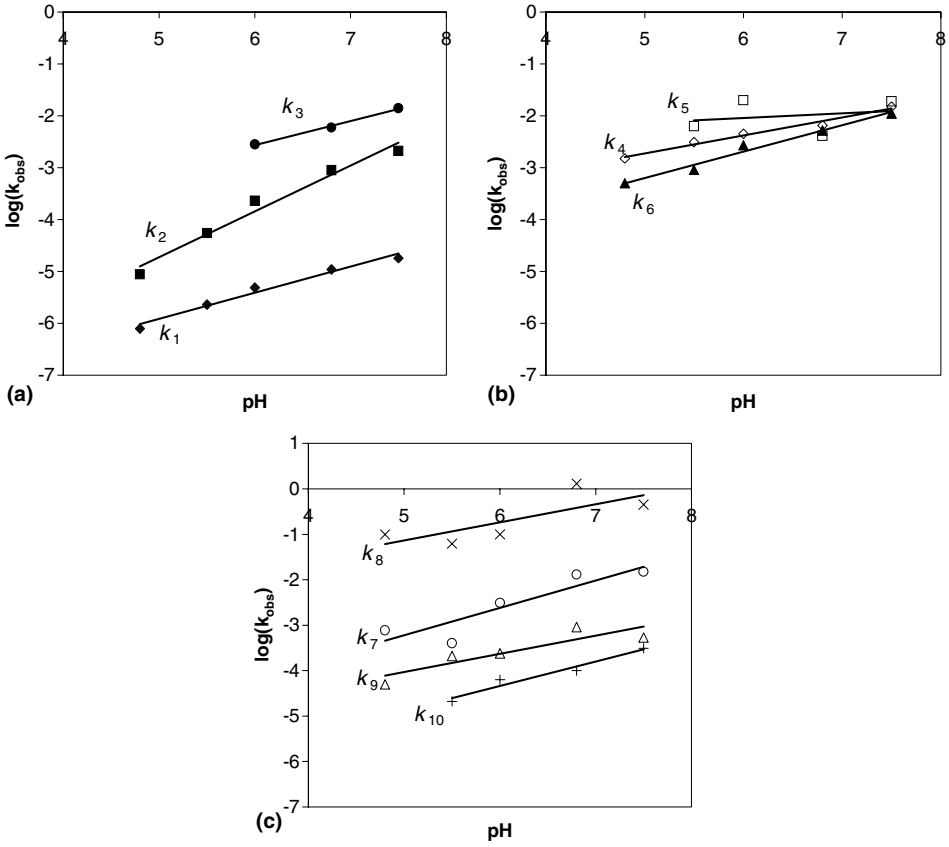


Fig. 6. pH–rate profiles for the estimated rate constants describing reactions depicted in Scheme 1. k_1 (◆), k_2 (■), k_3 (●), k_4 (◇), k_5 (□), k_6 (▲), k_7 (○), k_8 (×), k_9 (△), k_{10} (+).

reaction with glucose. From results published by Pílková, Pokorný, and Davídek (1990) for browning of Heyns compounds, likewise, a slope of 0.27 can be derived. From results of Ashoor and Zent (1984) a slope of 0.64 can be calculated for browning. From results of Nicoli et al. (1993), a slope of 0.37 can be calculated from browning data, a slope of around 0.1 for the loss of glucose and glycine, and a slope of 0.1 for production of CO_2 in the Strecker reaction. Even though these literature results are not directly comparable to our results because of the different approach taken, it is clear that the pH-dependence of most rate constants is less than that for pure base-catalysed reactions. In any case, we now have quantitative data with which we can model the pH-dependence of the Maillard reaction.

Taking into account the thus determined pH-dependence, the model presented in Scheme 1 was fitted to all the data simultaneously for all the studied pH experiments.

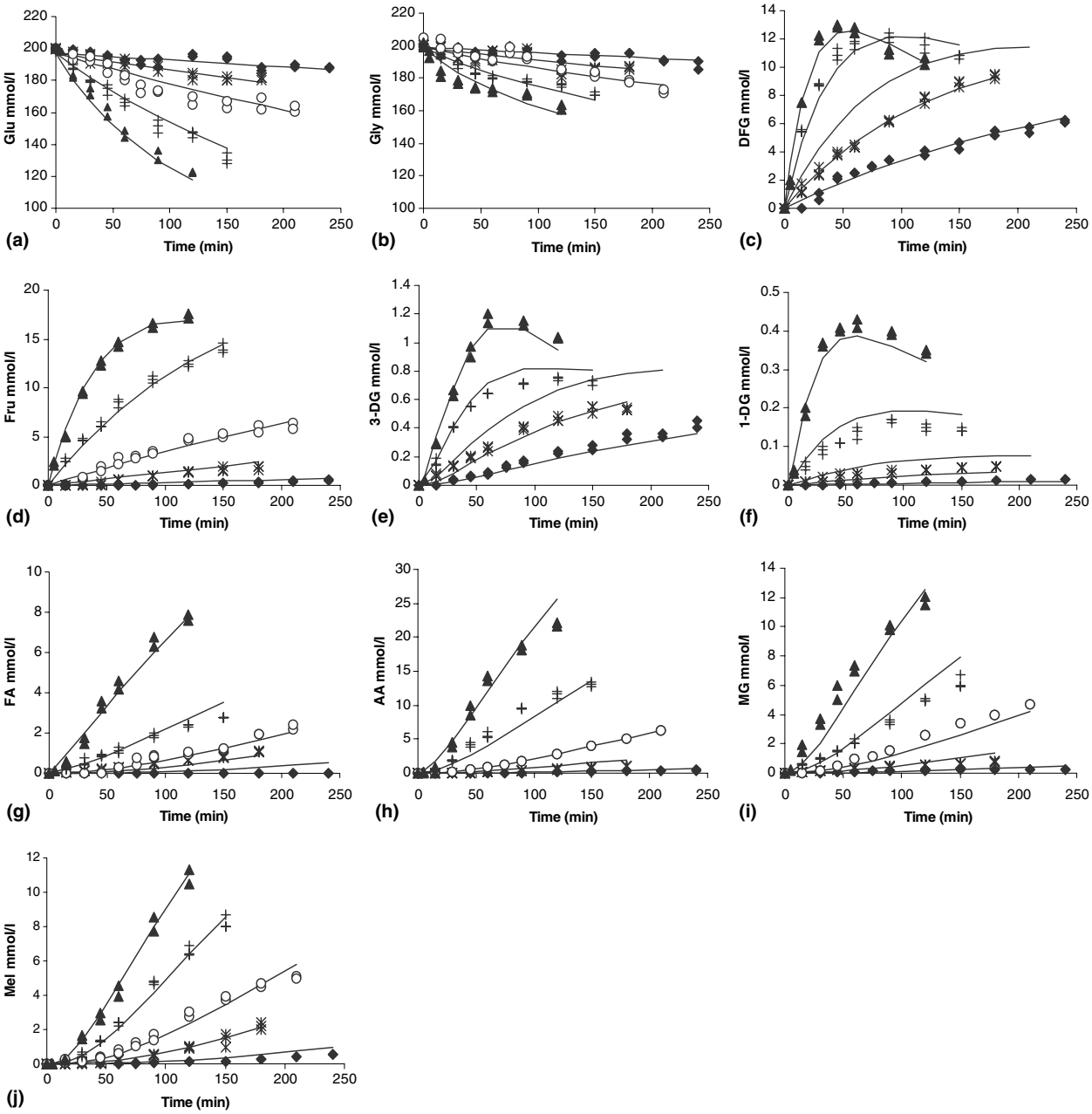


Fig. 7. Model fit (lines, calculated according to Scheme 1) to experimental data (markers) of glucose/glycine aqueous system heated at 100 °C and initial pH 4.8 (◆); pH 5.5 (∗); pH 6.0 (○); pH 6.8 (+) and pH 7.5 (▲). (a) Glucose (Glu); (b) glycine (Gly); (c) *N*-(1-deoxy-D-fructos-1-yl)–glycine (DFG); (d) fructose (Fru); (e) 3-deoxyglycosone (1-DG); (f) 1-deoxyglucosone (1-DG). (g) formic acid (FA); (h) acetic acid (AA); (i) methylglyoxal (MG); (j) melanoidins (Mel). The initial pH values are reported as set at room temperature.

The results of the fit are shown in Fig. 7. As can be observed, the deduced power law relationship captured the pH-dependence well. The model performed well and seemed to be in line with the proposed mechanism.

3.3. Influence of reactant initial concentrations

The glucose/glycine equimolar system at 100 °C and pH 6.8 was used as control and the concentrations of glucose and glycine were changed to give molar ratios of 2:1 and 1:2, respectively. If the kinetic model is consistent with the reaction mechanism, the reaction rate constants should be independent of the concentration of the reactants. As observed in Table 4, the estimated parameters show no significant difference. Some variation (as is to be expected because of experimental error) is observed, but on the whole it could be concluded that the variation in the values remained within the 95% confidence intervals. Also, the resulting plots show that the model is consistent with changing the reactant initial concentrations (Figs. 8 and 9).

3.4. Sensitivity analysis

The kinetic model has now been tested for temperature (Martins & Van Boekel, 2004), pH and initial concentrations (this paper) and seems to be quite robust. Obviously, additional experiments are needed to further strain this model. A useful aid in this respect is a sensitivity analysis, which shows the sensitivity of a response towards a parameter, expressed as the partial derivative of that response to a chosen parameter. Such an analysis

Table 4
Estimated rate constants (k) ± 95% HPD[a] interval at different initial concentrations of the reactants, expressed as molar ratio glucose/glycine

k	1:1	2:1	1:2
1 ($l\,mol^{-1}\,min^{-1}$)	$1.1\times10^{-5}\pm4\times10^{-6}$	$1.5\times10^{-5}\pm1\times10^{-6}$	$1.4\times10^{-5}\pm8\times10^{-7}$
2 (min^{-1})	$8.9\times10^{-4}\pm4\times10^{-5}$	$1.2\times10^{-3}\pm2\times10^{-4}$	$1.1\times10^{-3}\pm2\times10^{-4}$
3 (min^{-1})	$5.9\times10^{-3}\pm8\times10^{-4}$	$5.1\times10^{-3}\pm3\times10^{-3}$	$3.5\times10^{-3}\pm2\times10^{-3}$
4 (min^{-1})	$6.5\times10^{-3}\pm3\times10^{-4}$	$8.0\times10^{-3}\pm9\times10^{-4}$	$8.5\times10^{-3}\pm9\times10^{-4}$
5 (min^{-1})	$4.1\times10^{-3}\pm5\times10^{-4}$	$3.1\times10^{-3}\pm1\times10^{-4}$	$2.9\times10^{-3}\pm2\times10^{-4}$
6 (min^{-1})	$5.2\times10^{-3}\pm2\times10^{-4}$	$5.3\times10^{-3}\pm6\times10^{-4}$	$8.5\times10^{-3}\pm6\times10^{-4}$
7 (min^{-1})	$1.3\times10^{-2}\pm4\times10^{-4}$	$1.1\times10^{-2}\pm1\times10^{-3}$	$1.3\times10^{-2}\pm3\times10^{-3}$
8 (min^{-1})	$1.3\times10^{+0}\pm2\times10^{-1}$	$9.3\times10^{-1}\pm9\times10^{-2}$	$9.6\times10^{-1}\pm9\times10^{-2}$
9 (min^{-1})	$9.1\times10^{-4}\pm2\times10^{-5}$	$1.4\times10^{-3}\pm3\times10^{-4}$	$1.0\times10^{-3}\pm1\times10^{-4}$
10 (min^{-1})	$1.0\times10^{-4}\pm5\times10^{-5}$	$1.0\times10^{-4}\pm6\times10^{-5}$	$2.1\times10^{-4}\pm5\times10^{-5}$

Samples heated at 100 °C and reaction initial pH 6.8.
[a] Highest posterior density.

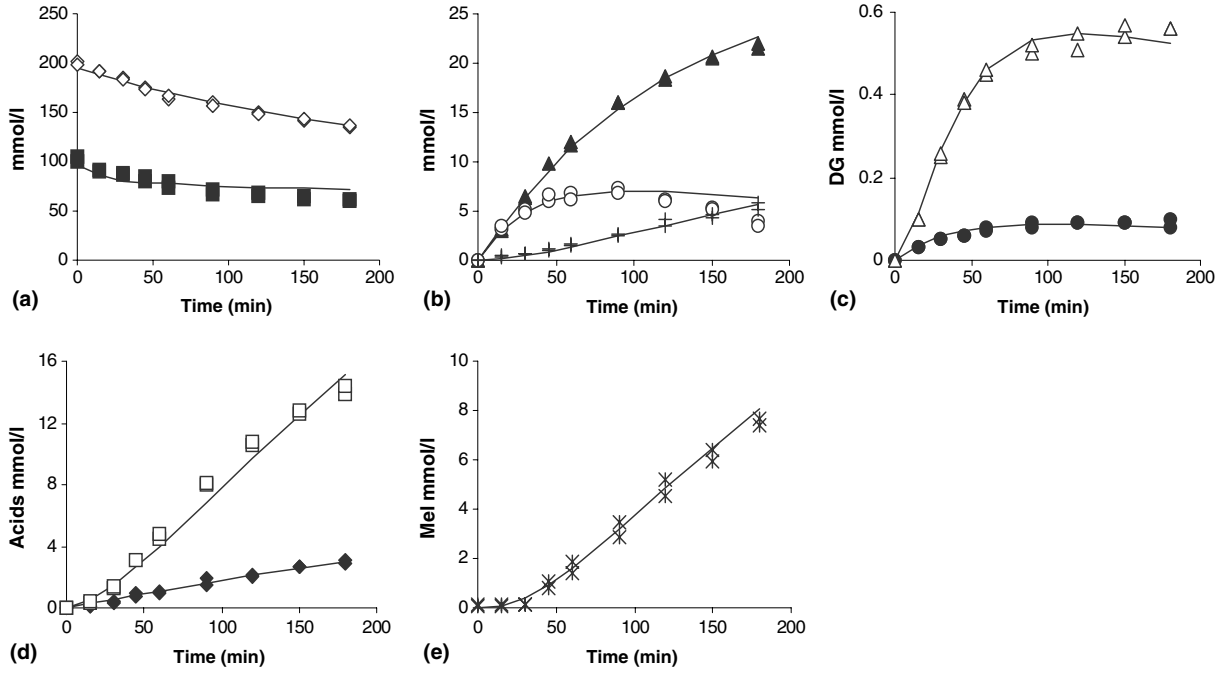


Fig. 8. Glucose/glycine molar ratio 2:1. Model fit (lines, calculated according to Scheme 1) to experimental data (markers) for the glucose/glycine aqueous system heated at 100 °C and pH 6.8. (a) Glucose (◇), glycine (■); (b) fructose (▲), *N*-(1-deoxy-D-fructos-1-yl)–glycine (○), methylglyoxal (+); (c) 1-deoxyglucosone (●), 3-deoxyglucosone (△); (d) formic acid (◆), acetic acid (□); (e) melanoidins (∗).

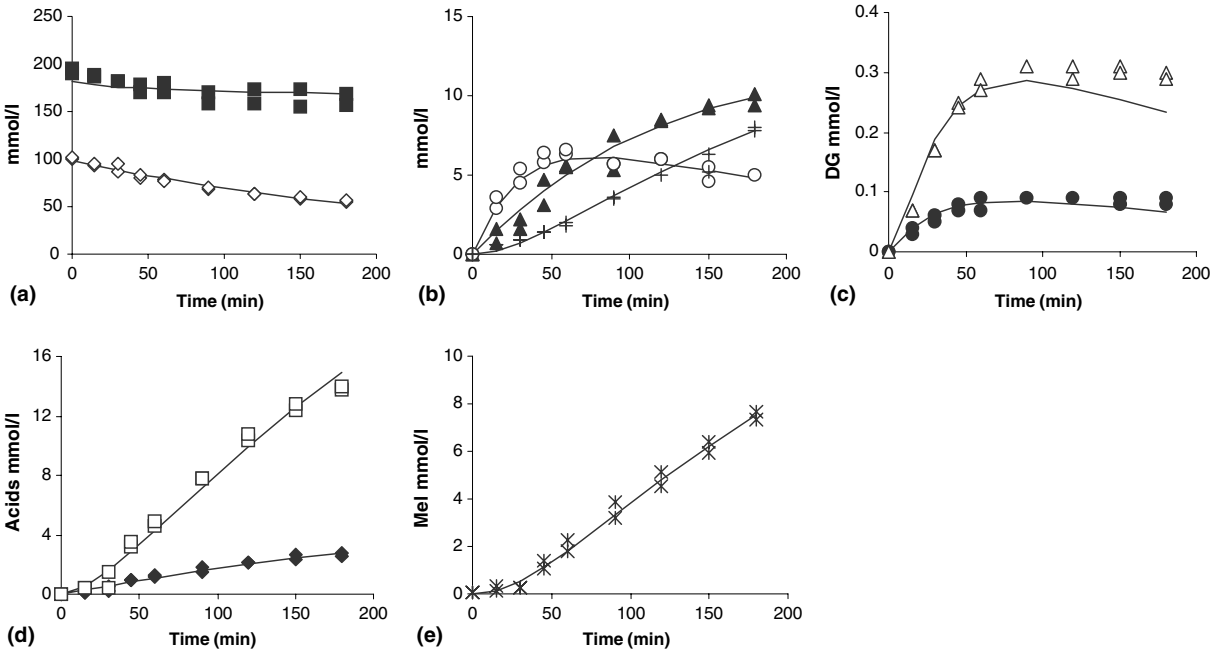


Fig. 9. Glucose/glycine molar ratio 1:2. Model fit (lines, calculated according to Scheme 1) to experimental data (markers) for the glucose/glycine aqueous system heated at 100 °C and pH 6.8. (a) Glucose (◇), glycine (■); (b) fructose (▲), *N*-(1-deoxy-D-fructos-1-yl)–glycine (○), methylglyoxal (+); (c) 1-deoxyglucosone (●), 3-deoxyglucosone (△); (d) formic acid (◆), acetic acid (□); (e) melanoidins (∗).

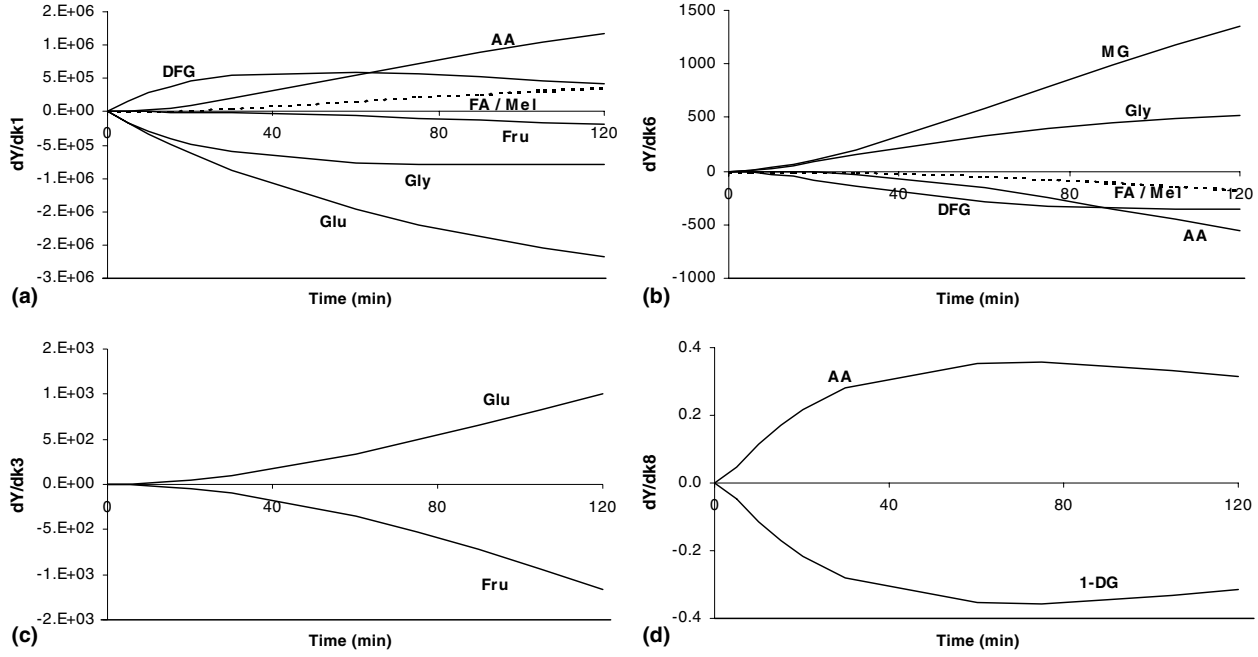


Fig. 10. Sensitivity analysis of the responses depicted in the proposed kinetic model (Scheme 1) for rate constants k_1, k_3, k_6 and k_8. Within each graph, the sensitivity was zero for the responses not shown. Glucose (Glu); glycine (Gly); fructose (Fru); *N*-(1-deoxy-D-fructos-1-yl)–glycine (DFG); acetic acid (AA); formic acid (FA); 1-deoxyglucosone (1-DG); 3-deoxyglucosone (3-DG) and melanoidins (Mel).

gives an idea of which parameters are most influential and may help to determine unimportant reactions, in which case the model may be further simplified. The same software package (www.athenavisual.com) as used for the parameters estimation supplies this information. The results for k_1, k_3, k_6 and k_8 are shown in Fig. 10. These steps were chosen, first to see the impact of k_1 on the overall mechanism, and second to determine the importance of certain steps which might cast some doubts, such as the isomerisation of fructose into glu-

cose (k_3), the retro-aldolisation of the Amadori compound (k_6), as well as the formation of acetic acid from 1-deoxyglucosone (k_8). As expected, k_1 has a strong influence in the main products formed throughout the Maillard reaction. Moreover, together with k_4 and k_7 (DFG enolisation step), the DFG retro-aldolisation step (k_6) appeared not to be a redundant parameter. Besides methylglyoxal formation, it has a positive influence in glycine regeneration. Also, the isomerisation step of fructose into glucose (k_3) becomes more evident for longer heating periods, which may explain its dependence on the pH drop. In acetic acid formation, besides k_1, k_7 (1-DG formation from DFG) also shows a strong positive influence (results not shown), which explains the low sensitivity of acetic acid to k_8. This result is well in line with the observed low pH-dependence of k_8. The limiting factor on the amount of acetic acid seems to be the formation of 1-DG.

4. Conclusions

The previously established model performed reasonably well when testing the conditions of temperature, pH and initial reactant concentrations. It shows consistency with the chemistry behind it. The estimated kinetic parameters (Table 3) showed that the sugar isomerisation and degradation steps 2, 3 and 10, respectively, have high pH-dependence, stronger than the initial Maillard reaction step 1. In fact, a strong increase in formic acid formation was observed at pH 7.5 (Fig. 7(g)). Moreover, in the enolisation step of the Amadori compound (DFG), the 1,2-enaminol route, with the formation of 3-deoxyglucosone (step 4), was found to be less pH-dependent than the 2,3-enaminol route (step 7). In line with what has been reported in earlier studies, 1-deoxyglucosone is favoured at higher pHs (Fig. 7(f)). However, its degradation into acetic acid was determined not to be pH-dependent (step 8). This suggests that 1-DG is a very reactive α-dicarbonyl compound in scission reactions leading to stable end-products, such as acetic acid. Moreover, 3-DG also showed low pH-dependence in formic acid formation (step 5). Accordingly, formic acid was detected in slightly higher amounts than acetic acid at pH 5.5 (Fig. 3). For higher pH values (pH $\geqslant$ 7.5), the sugar became the main source of formic acid, which is in agreement with the observation that sugar, when heated alone, degrades preferably into formic acid (Brands & Van Boekel, 2001; Martins, Marcelis, & Van Boekel, 2003c). 3-DG degraded (preferably into melanoidin formation) by reaction with glycine (step 9) (Fig. 7(j)) and the rate constant (k_9) was shown not to be strongly pH-dependent. Retroaldol reactions become more important at higher pH values, that is the formation of methylglyoxal from the Amadori compound (step 6). Also Huyghues-Despoints and Yaylayan (1996) reported that, under basic conditions, ARP could generate methylglyoxal and other lower carbohydrate fragments, such as glyceraldehydes, in addition to free amino acid.

The proposed model is now able to deal with varying temperature, initial concentrations of the reactants, as well as varying initial pH. For pH drops $\leqslant$1 unit, the pH-dependence was well captured by a power law relationship. The results suggest a mixture of specific acid and base catalysis. This could be a starting point for further mechanistic studies. Moreover, the observed consistency for most of the estimated parameters when the pH was kept constant gives an additional indication that the deduced pH-dependence is quite accurate. All in all, the model seemed to perform well and to be consistent with the established reaction mechanism.

Acknowledgements

The authors are grateful to the Fundação para a Ciência e Tecnologia, Ministério da Ciência e Tecnologia (Portuguese foundation for science and technology, Ministry of Science and Technology) for its financial support. The authors also thank Ruud van Gorp for performing the heating experiment at constant pH.

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