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Kinetic modelling of Amadori *N*-(1-deoxy-D-fructos-1-yl)-glycine degradation pathways. Part II—Kinetic analysis

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

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

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Abstract

A kinetic model for *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal decomposition was proposed. Two temperatures (100 and 120 °C) and two pHs (5.5 and 6.8) were studied. The measured responses were DFG, 3-deoxyosone, 1-deoxyosone, methylglyoxal, acetic acid, formic acid, glucose, fructose, mannose and melanoidins. For each system the model parameters, the rate constants, were estimated by non-linear regression, via multiresponse modelling. The determinant criterion was used as the statistical fit criterion. Model discrimination was performed by both chemical insight and statistical tests (Posterior Probability and Akaike criterion). Kinetic analysis showed that at lower pH DFG 1,2-enolization is favoured whereas with increasing pH 2,3-enolization becomes a more relevant degradation pathway. The lower amount observed of 1-DG is related with its high reactivity. It was shown that acetic acid, a main degradation product from DFG, was mainly formed through 1-DG degradation. Also from the estimated parameters 3-DG was found to be the main precursor in carbohydrate fragments formation, responsible for colour formation. Some indication was given that as the reaction proceeded other compounds besides DFG become reactants themselves with the formation among others of methylglyoxal. The multiresponse kinetic analysis was shown to be both helpful in deriving relevant kinetic parameters as well as in obtaining insight into the reaction mechanism.

Keywords: Amadori compound; *N*-(1-Deoxy-D-fructos-1-yl)-glycine; Maillard reaction; Multiresponse kinetic analysis

1. Introduction

The control of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) degradation pathways is a subject in chemical kinetics and can be treated in the same way as any chemical reaction, that is by measuring the concentrations of the reactants, intermediates and products with time. General kinetic data describing Maillard reaction pathways are lacking. Also, most studies apply simple kinetics, which does not provide any understanding of the reaction mechanism.[1,2] The use of kinetic simulation techniques for predicting and controlling properties of interest for the chemical and food industry is becoming increasingly important. The kinetic approach is useful in the sense that it describes not only the way in which the rate of the slowest step of a reaction changes with reaction variables, e.g., temperature and pH, but also that those changes can be predicted in a quantitative way.

Concerning Amadori compound decomposition kinetics only few studies have been published,[3–6] from which only the first had ARP as starting reactant and still simple kinetics was applied. The multiresponse kinetics analysis considers reaction pathways in more detail. It provides extra information about the reaction mechanism since the reactants degradation is analyzed simultaneously with the intermediates formation. The advantage is that the information in various responses can be used simultaneously so that more precise parameter estimates and more realistic models can be determined. The following steps should be taken into account:[7] (i) identification and quantification of the reactants and main products formed; (ii) identification

* Corresponding author. Tel.: +31-317-484281; fax: +31-317-483669.
E-mail address: tiny.vanboekel@wur.nl (M.A.J.S. Van Boekel).

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of reaction pathways based on reaction conditions; (iii) differentiate between primary and secondary reaction routes; (iv) propose a kinetic model based on the established reaction network; (v) test the hypothesized mechanism; (vi) estimate the rate constants. In Part I[8] the chemistry behind the degradation pathways of DFG was extensively discussed, dealing with the initial three steps. The present paper (Part II) is a follow up for the complete multiresponse kinetic analysis of the thermal degradation pathways of DFG. It deals with the last three steps: the kinetic analysis of the reaction network model proposed. One should keep in mind that kinetic modelling is an iterative process: propose a model, confront it with experimental data, criticize the model, adjust the model and confront the adapted model with experiments again, until an acceptable model results. What acceptable is, is of course debatable and we will discuss it by comparing two different possible models.

This paper can be seen as a stepping stone for a complete kinetic analysis of the whole Maillard reaction, as will be described in subsequent papers.

2. Material and methods

2.1. Kinetic modelling

Based on the established reaction network a kinetic model was proposed and translated into a mathematical model by setting-up differential equations for each reaction step. The software package Athena Visual Workbench[9] was used for numerical integration as well as for parameter estimation. The model parameters, the rate constants, were estimated by non-linear regression using the determinant criterion,[10] that is to minimize the determinant of the matrix of cross-products of the various responses, so called dispersion matrix. To discriminate between models, a multivariate test of goodness-of-fit was used together with two model discrimination tests. 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. The model discrimination tests used were the posterior probability (PPB),[11] which requires replicates or an estimation of experimental uncertainty, and the Akaike Criterion (AIC).[12]

3. Results and discussion

Most literature reports regarding the kinetics of Maillard reaction use simple kinetics to describe reactants degradation or products formation. Therefore, a comparison with multiresponse kinetic analysis was made in the present study.

3.1. Simple kinetics

Disregarding the actual mechanism or the number of steps involved, Yaylayan and Forage[3] assumed a pseudo-first-order reaction in determining the degradation kinetics of Amadori product of tryptophan and D-Glc. Taking this result into account, when applying simple kinetics to the thermal degradation of DFG we observed that DFG disappearance followed indeed a first-order reaction model; that is $C = C_0 \exp(-kt)$, where C is the concentration of the reactant (mmol l^{-1}), C_0 is the initial reactant concentration, k the reaction rate constant and t time (min). However, no real distinction could be made between other kinetic orders, in particular for the reaction conditions at 100 °C and pH 5.5 (Fig. 1). First- and second-order plots are almost the same. In fact, when optimising the fit of the curve to the data by minimizing the sum of squares, the reaction order was estimated to be 1.6 with a 95% confidence interval of 0.83–2.4. These results show that the use of simple kinetics is very limited. It is important to realize that the simple kinetics approach is actually only a mathematical fit procedure disregarding the actual mechanism or the number of steps involved.

3.2. Complex kinetics—multiresponse kinetic analysis

A step further in the kinetic analysis is to consider reaction paths in more detail. The chemistry behind the degradation pathways of DFG has been extensively discussed in Part I[8] and from this, a reaction network model was established which is summarized in Scheme 1. The main pathways (primary routes) for the DFG degradation are presented together with alternative pathways that at this stage are considered as secondary. However, confronted with the results some of the

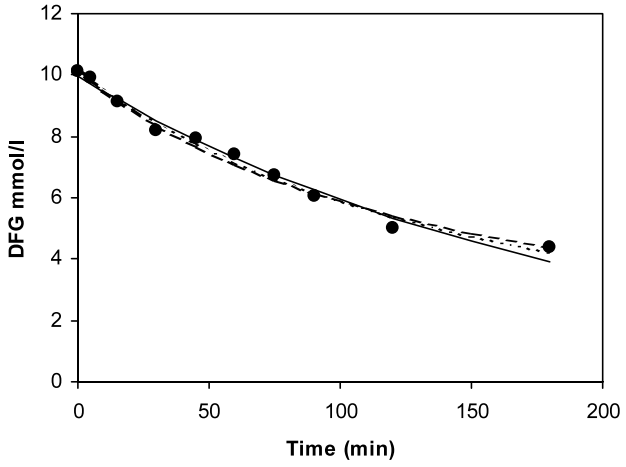
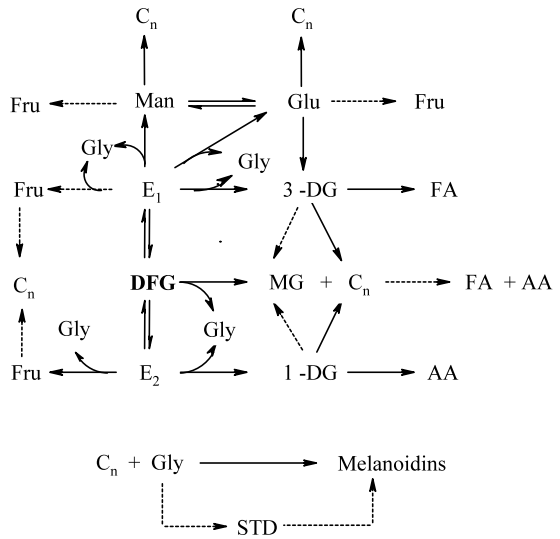


Fig. 1. Simple kinetics analysis of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal degradation at 100 °C, pH 5.5. Comparison of a first-order (—), estimated order of 1.6 (---) and second-order (– –) plot.



Scheme 1. Established reaction network for DFG thermal degradation. Primary routes (—); Secondary routes (---). E_1 and E_2 are unidentified key compounds involved in rate-determining steps that can be the Schiff's base, the cation form of the Schiff's base, the 1,2 enaminol or the 2,3-enaminol, respectively. Glycine (Gly); methylglyoxal (MG); Glc; Man; fructose (Fru); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG); unidentified carbohydrate fragments (C_n); Strecker degradation products (STD).

secondary routes might turn into primary routes and vice-versa. As stated above, one should be aware that kinetic modelling is an iterative process.

3.3. Propose a kinetic model based on the established reaction network

For modelling purposes the reaction network model presented was simplified. The degradation of DFG through enolization requires two intermediates E_1 (1,2-enolization) and E_2 (2,3-enolization) which was assumed to have the amino acid still incorporated.[13,14] Due to their reactivity these compounds have not been isolated yet from the Maillard reaction. However, in terms of modelling it is an important step to take into account. Moreover, besides the enolization step, DFG also degrades through retro-aldolization reaction with the formation of methylglyoxal (MG) together with unidentified carbonyl compounds (C_n) and release of the amino acid.[15,16] It was assumed to be a fast step with no intermediate in between. The intermediates E_1 and E_2 by release of glycine lead to the formation of 3-deoxyosone (3-DG) and 1-deoxyosone (1-DG), respectively. 3-DG and 1-DG, due to their reactive functional group, can easily degrade to produce reactive C_n ($n \leq 6$) carbonyl compounds as well as organic acids.[17] As we mentioned in Part I,[8] the acids formation occurs preferably by direct cleavage of 3-DG and 1-DG with formic and acetic acid formation, respectively. Concerning the sugars it was assumed that Glc and Man were formed through the same intermediate (E_1) whereas fructose (Fru) was preferably formed by E_2, even though theoretically it can also be formed through E_1. The main reason for this is that Fru was not detected at the lower pH studied and, while E_1 is favoured at lower pH, E_2 is favoured at higher pH.[18,19] However, for modelling purposes both hypotheses were compared. Moreover, Man and Glc can isomerise into each other as well as degrade into C_n ($n \leq 6$) carbonyl compounds. In the sugar isomerization step it was concluded that neither Glc nor Man isomerised into Fru, since no lag phase was observed in its formation. From a previous study[20] where the kinetics of monosaccharides isomerization was studied in alkaline conditions it was reported that Man isomerised into Glc at a rate of 18×10^{-3} min^{-1} while the reverse was approximately half (7×10^{-3} min^{-1}), as well as the degradation of Man into C_n ($n \leq 6$) compounds. Also MacLaurin and Green[21] came to the same conclusion. These literature results suggest that Man degrades preferably into Glc rather than into carbonyl compounds. Concerning Glc the rate of its degradation into C_n ($n \leq 6$) compounds was found to be higher than its rate of isomerization into Man.[20,21] As a result the degradation step of Glc into Man was neglected. The degradation of glycine was assumed to occur only by reaction with carbonyl compounds leading to melanoidins (Mel) formation. The Strecker degradation (STD) to produce the corresponding amines, carboxylic acids and Strecker aldehydes was considered to be a minor step. These assumptions lead us to the model presented in Scheme 2.

To fit the model to the experimental data, the reaction network presented in Scheme 2 needs to be translated into a mathematical model. This is done by setting-up differential equations for each reaction step, using the law of mass action:

$$\frac{d[DFG]}{dt} = -k_1[DFG] - k_2[DFG] - k_3[DFG] \quad (1)$$

$$\frac{d[E_1]}{dt} = k_1[DFG] - k_4[E_1] - k_{10}[E_1] - k_{11}[E_1] \quad (2)$$

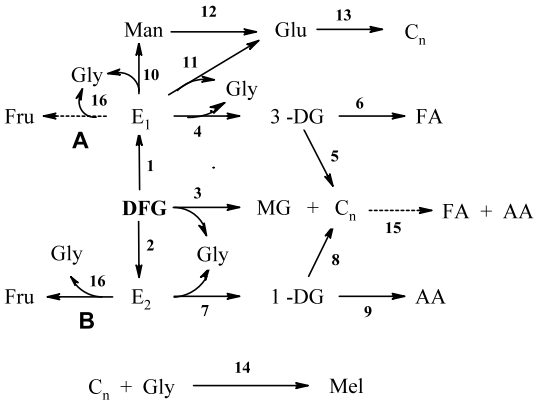
$$\frac{d[E_2]}{dt} = k_2[DFG] - k_7[E_2] - k_{16}[E_2] \quad (3)$$

$$\frac{d[MG]}{dt} = k_3[DFG] \quad (4)$$

$$\frac{d[3DG]}{dt} = k_4[E_1] - k_5[3DG] - k_6[3DG] \quad (5)$$

$$\frac{d[1DG]}{dt} = k_7[E2] - k_8[1DG] - k_9[1DG] \quad (6)$$

$$\frac{d[Man]}{dt} = k_{10}[E_1] - k_{12}[Man] \quad (7)$$



Scheme 2. First proposed kinetic model, Model 1 (M_1), based on the established reaction network. Primary routes (—); Secondary routes (---). E_1 and E_2 are unidentified key compounds involved in rate-determining steps that can be the Schiff's base, the cation form of the Schiff's base, the 1,2 enaminol or the 2,3-enaminol, respectively. Glycine (Gly); methylglyoxal (MG); glucose (Glu); mannose (Man); fructose (Fru); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG); unidentified carbohydrate fragments (C_n); melanoidins (Mel). **A** (secondary route) and **B** (primary route) hypothesis for fructose formation.

$$\frac{d[Glc]}{dt} = k_{11}[E_1] + k_{12}[Man] - k_{13}[Glc] \quad (8)$$

$$\frac{d[Fru]}{dt} = k_{16}[E_2] \quad (9)$$

$$\frac{d[FA]}{dt} = k_6[3DG] \quad (19)$$

$$\frac{d[AA]}{dt} = k_9[1DG] \quad (11)$$

$$\frac{d[Gly]}{dt} = k_3[DFG] + k_4[E_1] + k_{10}[E_1] + k_{11}[E_1] + k_7[E_2] + k_{16}[E_2] - k_{14}[Gly][C_n] \quad (12)$$

$$\frac{d[Cn]}{dt} = k_3[DFG] + k_5[3DG] + k_8[1DG] + k_{13}[Glc] - k_{14}[Gly][C_n] \quad (13)$$

$$\frac{d[Mel]}{dt} = k_{14}[Gly][C_n] \quad (14)$$

These coupled differential equations are difficult to solve analytically but can be solved by numerical integration. Nowadays different software packages are able to this, like Nsolve or Mathematica.[5] In the present study, the used subroutine was DDAPLUS, which is available in the software package Athena Visual Workbench.[9] The results of the fit for the experimental data at 120 °C are presented in Fig. 2 (A and B).

For the reaction conditions at pH 5.5 the model fitted the data reasonably well (Fig. 2A). Note that the observations were done in at least duplicate. Glycine was slightly overestimated, while formic acid and melanoidins were underestimated at the beginning and overestimated at the end of heating period. Similar results were obtained at 100 °C, pH 5.5. When the pH was increased to 6.8 (Fig. 2B) there was clearly a miss fit for the organic acids formation, namely for formic acid, as well as for Glc. Both compounds were underestimated. Also a lack of fit was observed for MG formation. It was overestimated at the beginning and underestimated as the reaction proceeded. The results forced us to reconsider the kinetic model. This shows the power of the iterative modelling approach. To begin with, an extra step was added to the model. Incorporating step 15 as a primary route would induce both formic acid (C_1) and acetic acid (C_2) formation from MG (C_3). Not only the underestimation of formic acid could be solved as well as it would induce 3-DG, instead of Glc, to form more C_n ($n \leq 6$). However, it appeared that the estimation of this parameter lead to an indeterminate result for all the studied systems, which means that this step is not important for the model. Also it suggests that the organic acids formation does not result from MG degradation.

From a chemical point of view it is clear that the model is not completely correct. Isbell and co-workers[22] have postulated the existence of cis- and trans-isomers of the 1,2-endiol to explain the transformation of various sugars. It was suggested that both enolization and ring opening might be involved in the rate-determining steps of the process. Moreover, the relative rates of enolization for Glc and Man were found to be 1.0 and 0.5 h^{-1}, respectively. If that is the case then Glc has a higher ability to transform into 1,2-endiol and not so much to degrade into sugar fragments (C_n) involved in colour formation. Apparently, the transformation step of Glc into 3-deoxyosone that initially has been considered as a secondary route might be in fact a primary route. Also, the observation that at the beginning of the reaction MG was overestimated leads us to the assumption that a rate-determining intermediate might be formed previously to MG. The initial assumption that MG formation from DFG was a fast step might not be correct.

When calculating the reaction mass balance (evolution of each intermediate towards the reactant (DFG) initial concentration) we observe that at the initial stage of the reaction the products identified and quantified in the present study do not count for the total DFG degradation. However, as the degradation reaction proceeded, within experimental error, 100% was reached. In Fig. 3, we can observe the results for pH 6.8. Besides melanoidins, and glycine, the other main end products obtained were the organic acids, in particular acetic acid, as mentioned in Part I.[8] A possible explanation for the observed gap is that, as the reaction proceeds besides DFG other compounds

formed during its degradation become reactants themselves with the formation of the same end products.

As a result a modified simplified model is proposed, as shown in Scheme 3. To fit the model to the experimental data, the same procedure was taken by coupling differential equations to each reaction step. The approach of numerical integration followed by fitting to the data is flexible because changing relevant differential equations and fitting them to experimental data can easily test different models. The results of the fit for the experimental data taken at all the studied systems are presented in Figs. 4–7.

A major improvement in the organic acids fit was observed, as well as in the sugars and MG formation. Independently of the reaction conditions the model seems to fit the experimental data quite well. However, an important question is still not clear: is Fru preferably formed through E_1 or E_2? To answer this question both hypotheses (A and B, respectively) were tested for both proposed models, through model discrimination.

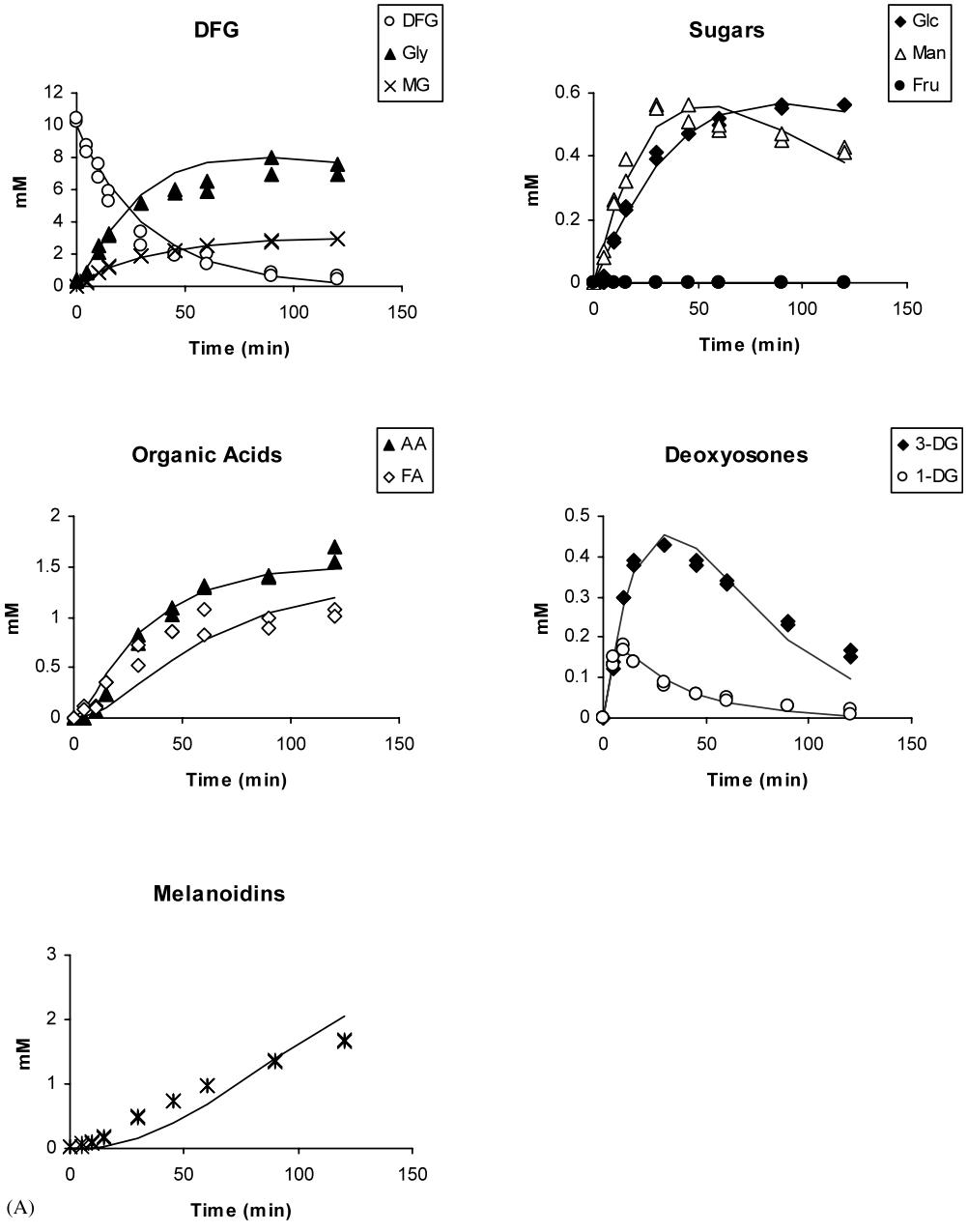


Fig. 2. Model 1 fit (lines) to experimental data (dots) of DFG thermal degradation at 120 °C: pH 5.5 (A) and 6.8 (B). Glycine (Gly); methylglyoxal (MG); Glc; Man; fructose (Fru); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG).

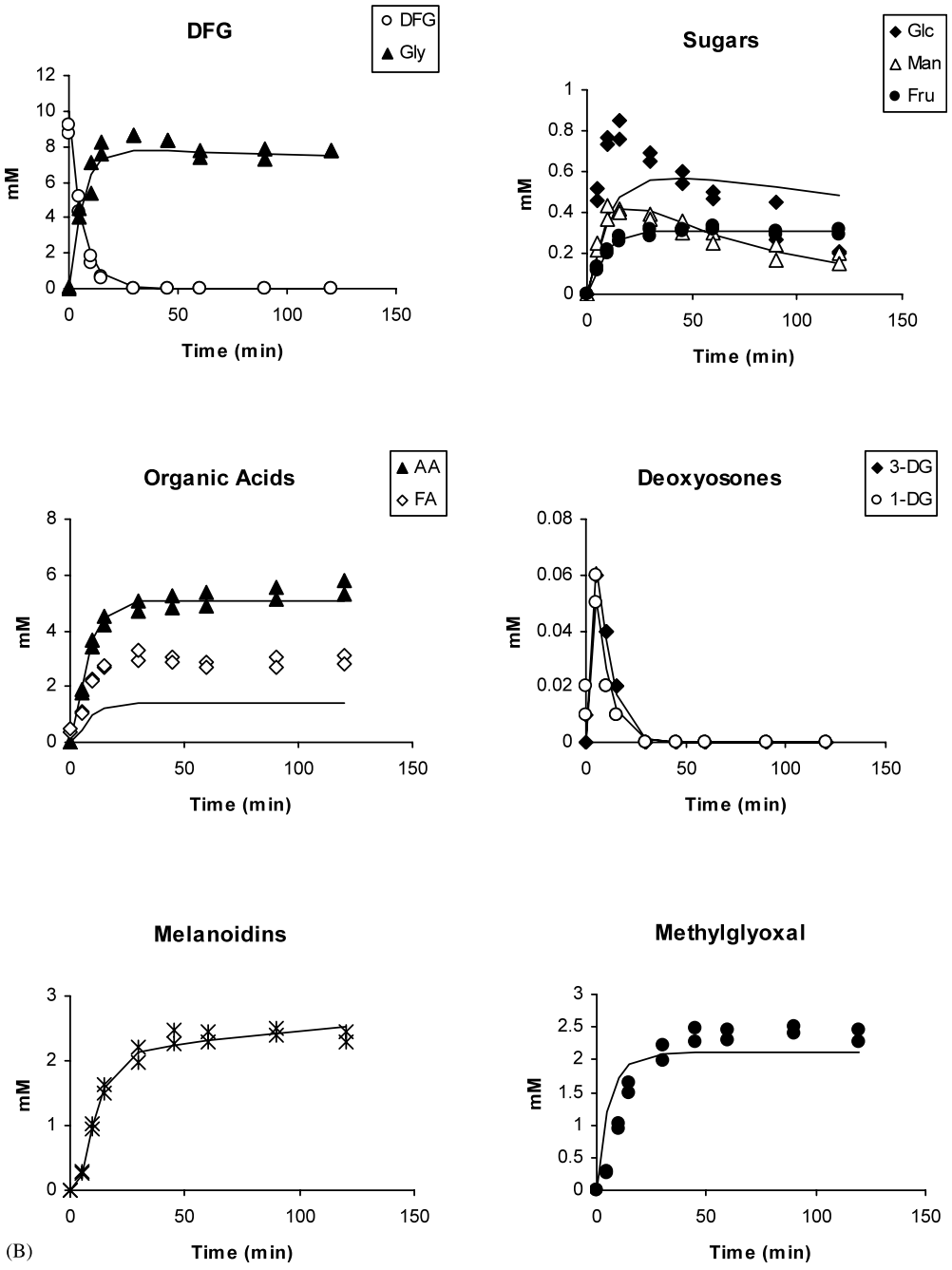


Fig. 2 (*Continued*)

3.4. Test the hypothesized mechanism (goodness-of-fit and model discrimination)

The question how well the proposed model describes the experimental data must be addressed from a statistical point of view. One could be led to choose a poor model because it was *not as bad* as the other models. To avoid such a mistake it is recommended to use a multivariate test of goodness-of-fit (or its counterpart lack-of-fit).[11] This test is installed in the same software package as before[9,10] and was based on replicate experiments. Apart from each model there is an error associated with the data, the experimental error. If this is too high any model is in principle able to fit the data. However, if the experimental error is small the discrimination between the data and the model estimation gives a better understanding how good the model fits the data. As can be seen in the Figs. 4–7, the scatter in replicates was not very high which allowed a goodness-of-fit test. The quality of experimental data is therefore very important.

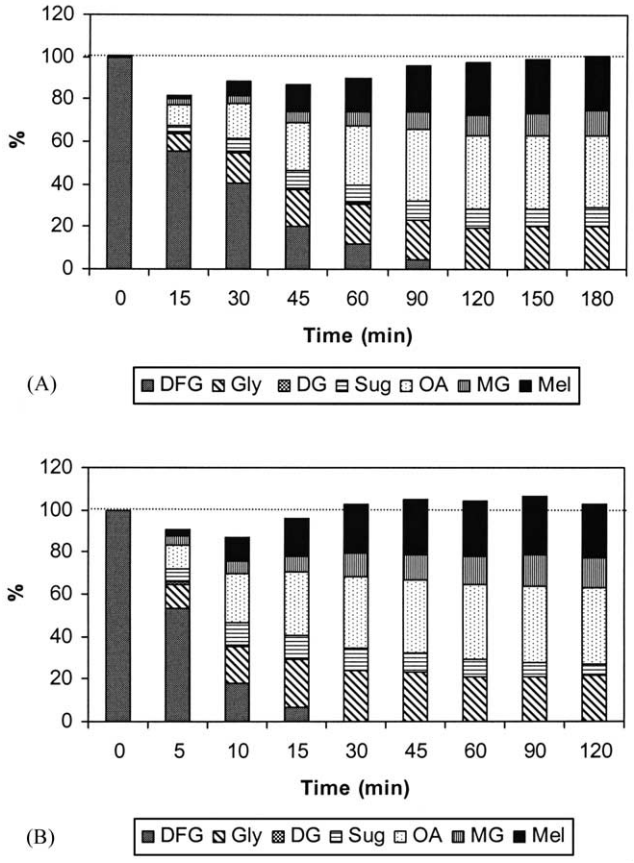
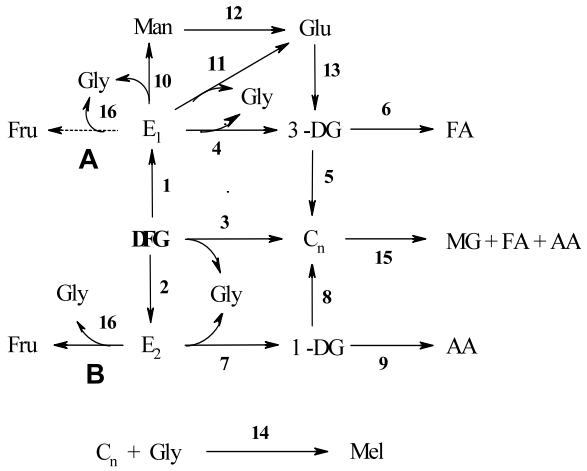


Fig. 3. Mass balance: evolution of each intermediate towards the reactant initial concentration in heated *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) at 100 °C, pH 6.8 (A) and 120 °C, pH 6.8 (B). Glycine (Gly); Deoxyosones (DG); Sugars (Sug); organic acids (OA); methylglyoxal (MG); melanoidins (Mel).

The fit of the mathematical model to the data was done simultaneously with parameter estimation, which will be addressed in Section 3.5.

As mentioned before, kinetic modelling is an iterative process: propose a model, confront it with experimental data, criticise the model, adjust the model and confront the adapted model with experiments again, until an acceptable model results. What is acceptable though, is of course debatable. Alternative models may be formed from a candidate model by adding or deleting parameters. Once models show an acceptable fit, there may still be many models left, so the next step is model discrimination. According to the Bayesian concept,[23] on which Athena Visual Workbench is based, the plausibility of a model results from the combination of likelihood (given by the data) with prior probability (given by previous results or personal belief). This combination is called Posterior Probability (PPB). The model with the highest posterior probability performs then the best. It is required to have replicates (or an estimate of experimental uncertainty) in order to execute model discrimination. It is a relative concept, not an absolute one, meant for comparison. It provides information about the most plausible model, not necessarily the true one. In model construction additions of parameters are favored when they yield higher posterior probabilities; deletions are favored when they do not make the PPB appreciably worse.



Scheme 3. Second proposed kinetic model, Model 2 (M_2), based on the established reaction network. E_1 and E_2 are unidentified key compounds involved in rate-determining steps that can be the Schiff's base, the cation form of the Schiff's base, the 1,2 enaminol or the 2,3-enaminol, respectively. Glycine (Gly); methylglyoxal (MG); Glc; Man; fructose (Fru); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG); unidentified carbohydrate fragments (C_n); melanoidins (Mel). **A** (secondary route) and **B** (primary route) hypothesis for fructose formation.

However, it is also known that the higher the number of parameters the better fit the fit will be.[1] In order to have a second opinion the model discrimination was also performed by using the Akaike criterion (AIC)[12] which, can be expressed in the case of least-squares approximation as:

$$\mathrm{AIC} = n \ln(\sigma^2) + 2(p+1) \tag{15}$$

and the maximum likelihood estimator for the variance is:

$$\sigma^2 = \frac{\mathrm{SS}}{n} \tag{16}$$

in which p is the number of estimated parameters ($+1$ to include the variance estimate). The $2p$ term is the penalty in the Akaike criterion for the use of more parameters. When the number of data points n is relatively small compared to p (say $n/p < 40$) the corrected AIC should be used:

$$\mathrm{AIC_c} = n \ln(\sigma^2) + 2(p+1)\left(\frac{n}{n-p}\right)$$

Because the AIC criterion is on a relative scale it is common practice to calculate AIC differences, taking

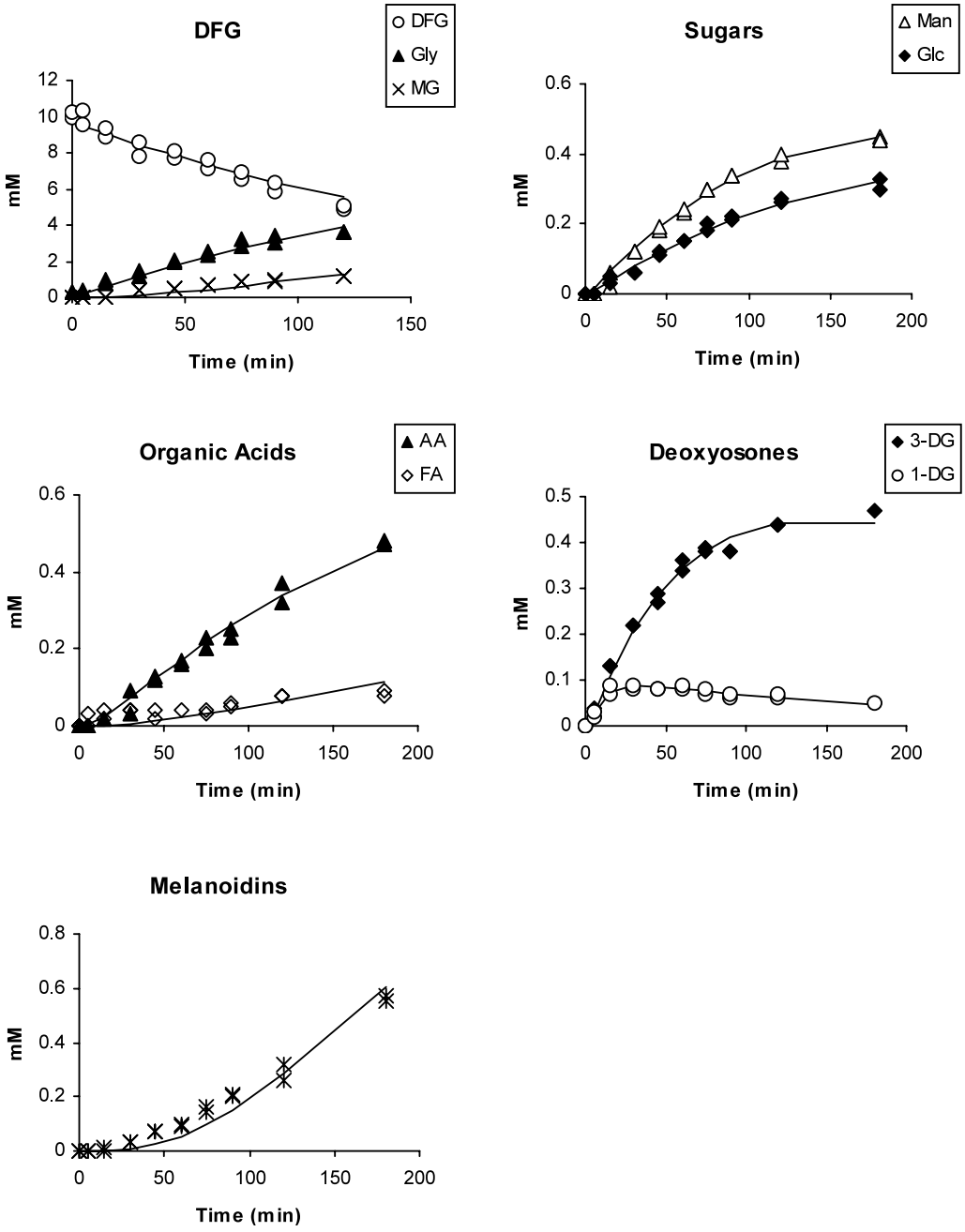


Fig. 4. Model 2 (M2) fit (lines) to experimental data (dots) of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal degradation at 100 °C and pH 5.5. Glycine (Gly); methylglyoxal (MG); Glc; Man; acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG).

the model with the lowest value (AIC_{min}) as the reference:

$$\Delta_{AIC} = AIC - AIC_{min} \quad (17)$$

A rule of thumb is that models with $\Delta_{AIC} \leq 2$–3 are worthwhile to consider, values of Δ_{AIC} between 4 and 7 indicate that models are less supported, and values higher than 10 indicate that models may be discarded.

In Tables 1 and 2 the results of the model discrimination tests are shown for M_1 (Scheme 2) and M_2 (Scheme 3) using PPB and the AIC criterion. At lower pH (Table 1) according to the Akaike criterion the results are contradictory. At 100 °C, M_2 is less supported whereas at 120 °C M_1 can be discarded, which was also supported by the PPB values. It should be taken into account that under these conditions (pH 5.5), for the studied heating period, the DFG degradation rate is quite small as well as the amount of products formed, which gives the models more flexibility to fit the experimental data. When the pH was increased to 6.8

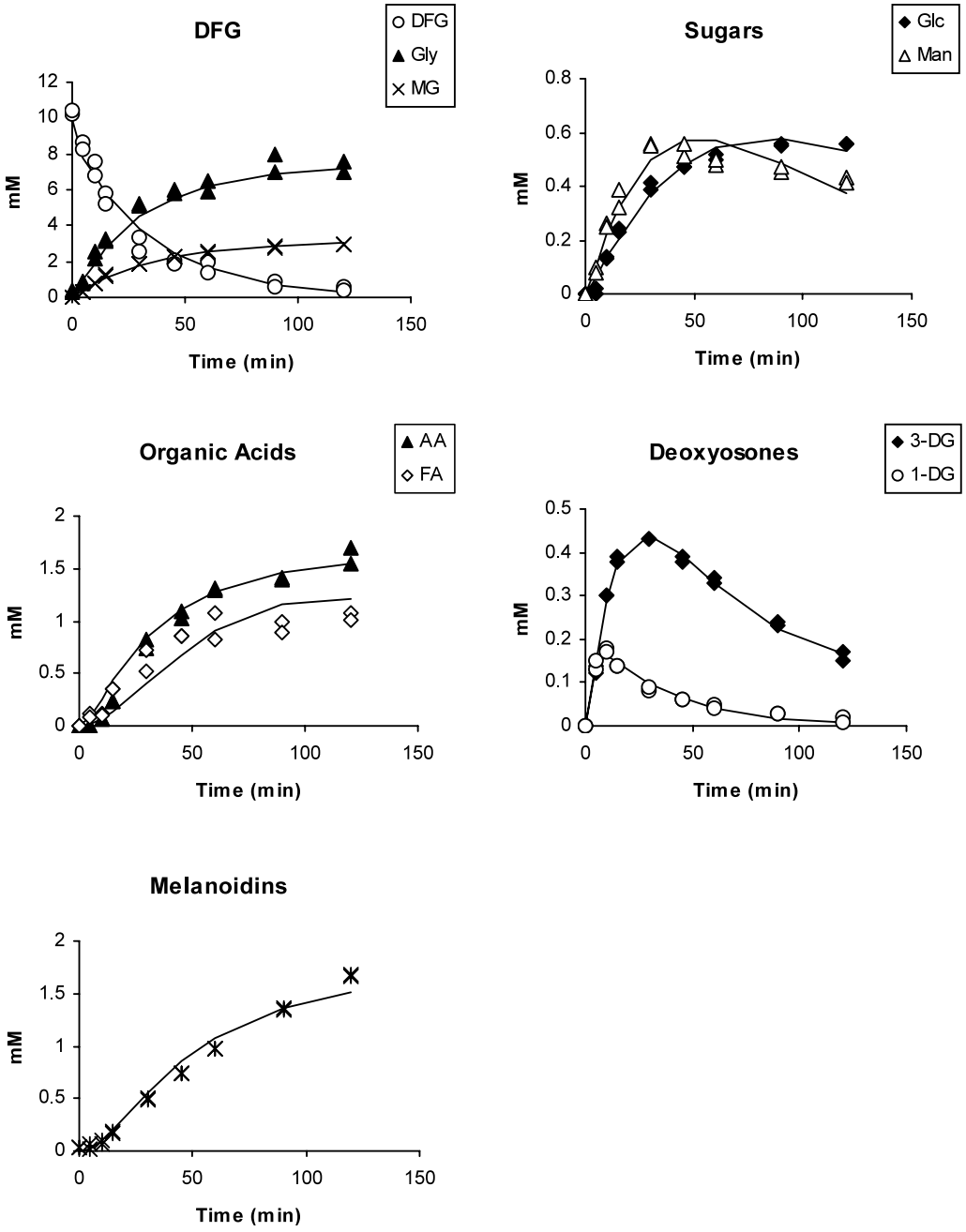


Fig. 5. Model 2 (M2) fit (lines) to experimental data (dots) of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal degradation at 120 °C and pH 5.5. Glycine (Gly); methylglyoxal (MG); glucose (Glu); mannose (Man); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG).

(Table 2), besides M_1 and M_2 model discrimination, hypotheses A and B for Fru formation via E_1 and E_2, respectively, were also tested. Note that this test is only relevant for the systems at pH 6.8, since at lower pH Fru was not detected. According to the Akaike criterion, independently of the chosen hypothesis A or B, M_2 always performed better than M_1. The results for M_1 indicated that this model could be discarded ($\Delta_{AIC} \geq 10$). These findings are confirmed by the obtained PPB values that were always higher in M_2. As for both model discrimination tests, it was clear that M_2 performed better than M_1. Also the obtained fits at pH 6.8 come into agreement with this conclusion. When comparing hypotheses A and B for M_2 the PPB values as well as the AIC values of hypothesis B are higher than those of hypothesis A.

These results suggest that fructose was mainly formed through the intermediate E_2, supporting the assumption made in Part I.[8] Also that model M_2 is more likely than model M_1, giving some evidence that the transformation

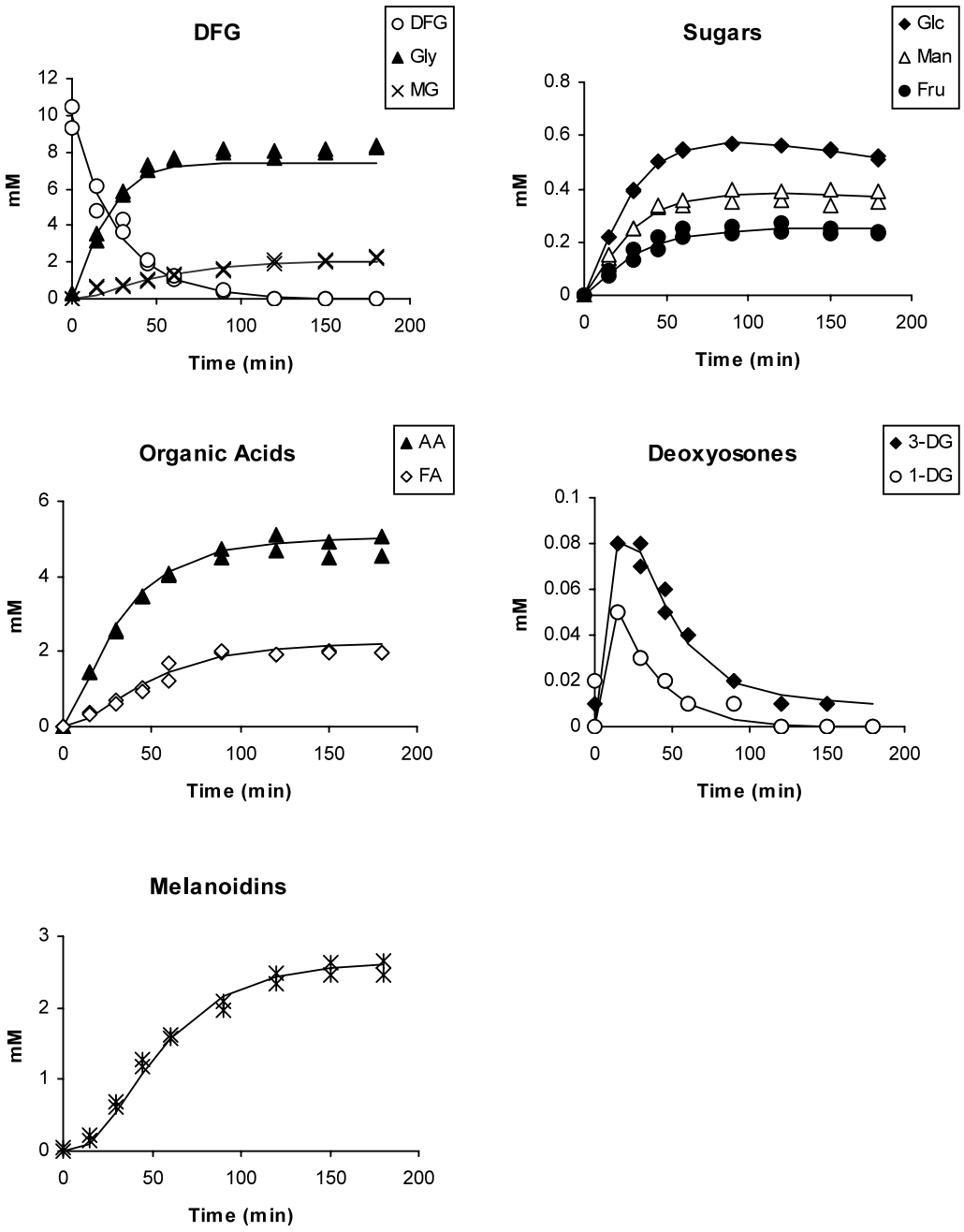


Fig. 6. Model 2 (M2) fit (lines) to experimental data (dots) of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal degradation at 100 °C and pH 6.8. Glycine (Gly); methylglyoxal (MG); glucose (Glu); mannose (Man); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG).

step of Glc into 3-deoxyosone that initially has been considered as a secondary route might be in fact a primary route and that the initial assumption that MG formation from DFG was a fast step was indeed not correct.

3.5. Estimate the rate constants

As mentioned before, once the basic form of the functional part of the model is established, the next step is to fit the model to the experimental data to obtain estimates for the model parameters, i.e., the rate constants. In general, this is accomplished by solving an optimization problem in which the objective function (the function being minimized or maximized) relates the response variable and the functional part of the model, in a way that will produce parameter estimates that will be closest to the true, unknown parameter values. In previous studies[4,5] with multi-step kinetic analysis of glucose–amino acid Maillard reaction the approach

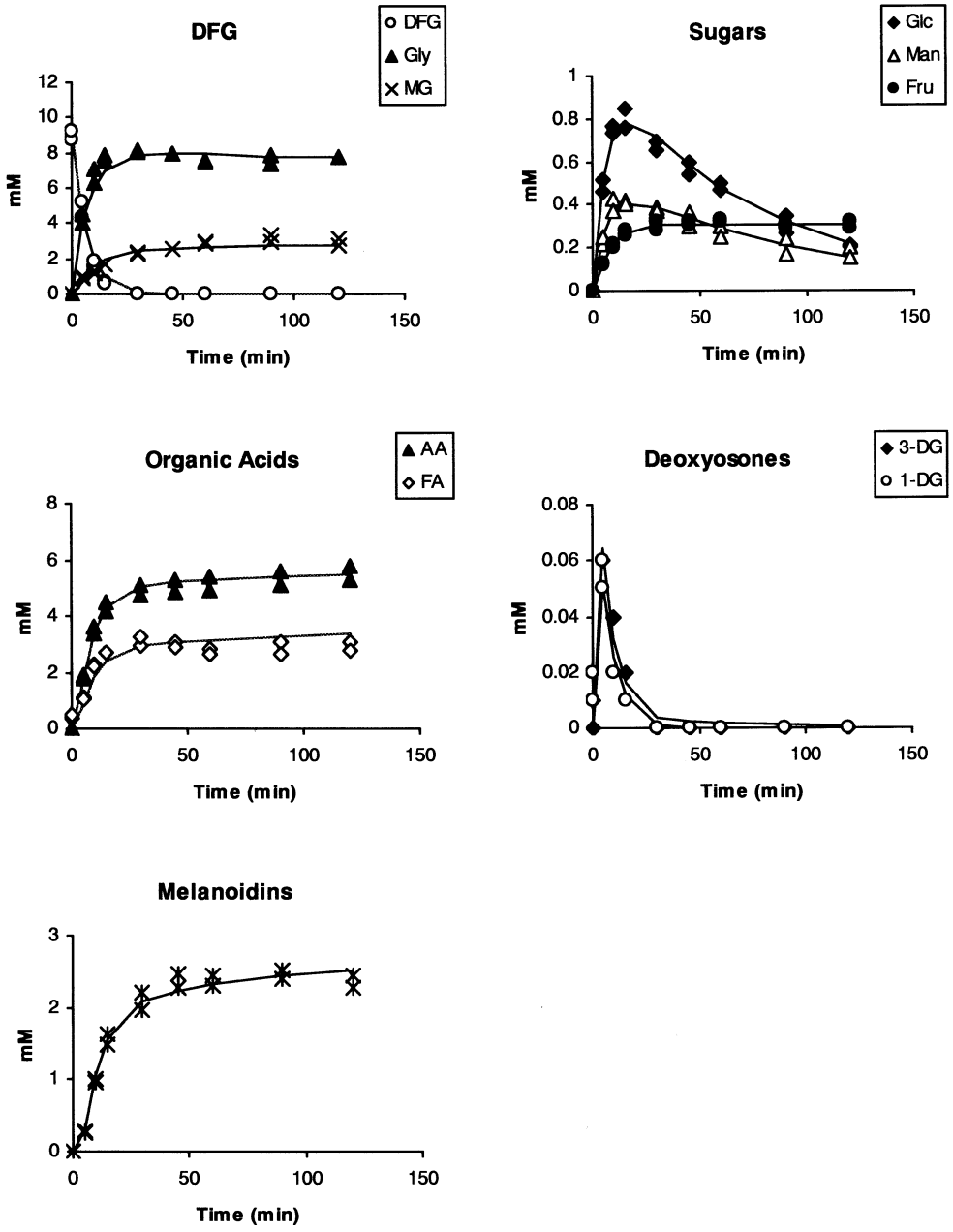


Fig. 7. Model 2 (M2) fit (lines) to experimental data (dots) of *N*-(1-deoxy-D-fructos-1-yl)-glycine (DFG) thermal degradation at 120 °C and pH 6.8. Glycine (Gly); methylglyoxal (MG); glucose (Glu); mannose (Man); acetic acid (AA); formic acid (FA); 3-deoxyosone (3-DG); 1-deoxyosone (1-DG).

Table 1
Model discrimination tests for the systems studied at pH 5.5

System	Model [a]	Parameters	SS	*n*	AIC_c	Δ_{AICc}	PPB
A (100 °C)	M_1	14	5.14	100	−261.92	0	34.11
	M_2	15	5.58	100	−250.96	10.96	32.28
B (120 °C)	M_1	13	17.01	90	−117.22	11.50	14.78
	M_2	15	14.05	90	−128.72	0	17.65

[a] Model 1 (M_1) presented in Scheme 2; Model 2 (M_2) presented in Scheme 3.

Table 2
Model discrimination tests for the systems studied at pH 6.8

System	Hypotheses	Model *	Parameters	SS	n	AIC_c	Δ_{AICc}	PPB
C (100 °C)	A	M_1	14	50.05	99	−32.60	173.32	16.20
		M_2	14	8.69	99	−205.92	0	19.80
D (120 °C)	A	M_1	13	49.85	99	−35.69	127.86	15.78
		M_2	15	12.96	99	−163.55	0	21.25
C (100 °C)	B	M_1	12	754.67	99	230.67	423.88	Indt. [a]
		M_2	15	9.61	99	−193.21	0	29.09
D (120 °C)	B	M_1	13	49.08	99	−37.22	123.62	14.59
		M_2	15	13.32	99	−160.85	0	21.77

* Model 1 (M_1) presented in Scheme 2; Model 2 (M_2) presented in Scheme 3.
[a] Indeterminate (low trust region).

used was to minimize the overall residual sum of squares (RSS) from all the responses. However, the RSS criterion is based on the assumption that the data on each response have the same variance and there is no correlation between the variances of the individual measurements of the response, which is not very realistic. In the present study several responses were measured from which some were measured from the same sample (e.g., formic and acetic acid) and with different degrees of precision. For cases of multiresponse modelling the fit criterion to be used depends on the experimental error structure of the data. Box and Draper[23] provided a solution for this problem assuming normally distributed errors. The best-fit criterion is the minimization of the determinant of the matrix of cross-products of the various responses, the so-called dispersion matrix from the responses. If the determinant of the dispersion matrix is minimized, the most probable estimates of the parameter will be found. The resulting parameter estimates for the hypothesized B mechanism in Scheme 3 (M_2) using the determinant criterion are shown in Table 3. The assumptions made in Part I[8] can now be confronted with the estimated rate constants.

DFG was assumed to degrade preferably through E_1 (1,2-enolization) at lower pH. In fact at pH 5.5 step 1 prevailed to steps 2 and 3, especially when the temperature was increased. At higher pH (6.8) on the other hand step 2 gained importance, which is evident at lower temperature, suggesting that 2,3-enolization becomes more relevant by increasing the pH. Moreover, from DFG enolization step it becomes clear that independently of the reaction conditions, deoxyosones formation prevail to sugars formation. The rate constant for step 4 is always higher than for step 10 and for step 11. Also, Fru formation is only a minor step from the 2,3-enolization pathway. The rate constant for step 7 is always higher than for step 16. Moreover, the results suggest that sugar formation is mainly pH dependent. At lower pH Man formation was favored towards Glc and no other sugar was detected, whereas at higher pH

Table 3
Rate constants (10^{-2}) ± 95% highest posterior density (HPD) as found by kinetic modelling for hypothesis B of Model 2 (M_2)

Rate constant (min^{-1})	A (100 °C, pH 5.5)	B (120 °C, pH 5.5)	C (100 °C, pH 6.8)	D (120 °C, pH 6.8)
k_1	0.19 ± 0.02	1.11 ± 0.07	0.57 ± 0.03	8.89 ± 0.83
k_2	0.10 ± 0.04	0.86 ± 0.40	1.56 ± 0.09	6.29 ± 0.66
k_3	0.18 ± 0.01	0.88 ± 0.28	1.55 ± 0.11	8.62 ± 0.36
k_4	20.14 ± 3.37	31.13 ± 9.97	7.94 ± 1.51	215.79 ± 50.08
k_5	1.38 ± 0.39	2.23 ± 1.17	9.07 ± 3.45	506.69 ± 62.87
k_6	0.19 ± 0.04	4.30 ± 0.69	2.74 ± 2.82	30.41 ± 2.17
k_7	60.17 ± 9.11	76.79 ± 10.65	21.25 ± 9.18	55.93 ± 2.14
k_8	5.90 ± 4.06	15.41 ± 1.71	0.00 ± 0.00	0.00 ± 0.00
k_9	3.93 ± 0.21	22.50 ± 1.81	190.85 ± 22.85	653.55 ± 94.08
k_{10}	11.31 ± 1.94	19.42 ± 6.84	7.07 ± 1.11	25.07 ± 5.28
k_{11}	6.42 ± 1.07	10.96 ± 4.25	11.31 ± 1.81	50.55 ± 10.63
k_{12}	0.39 ± 0.13	1.27 ± 0.24	0.08 ± 0.05	1.06 ± 0.17
k_{13}	0.73 ± 0.28	1.41 ± 0.26	0.22 ± 0.05	2.03 ± 0.23
k_{14}	0.12 ± 0.03	70.68 ± 3.93	0.34 ± 0.06	2.47 ± 0.99
k_{15}	1.45 ± 0.42	5.15 ± 0.99	1.59 ± 0.22	16.82 ± 5.91
k_{16}			1.34 ± 0.59	4.51 ± 1.72

not only Fru was formed but also Glc was formed in higher amounts than Man. Besides enolization, DFG was also assumed to degrade through retro-aldolization, where the rate determining step leads to C_n, the fragment without the amino group. In a previous multi-step kinetic analysis study[5] 1-morpholino-1-deoxy-D-fructose degradation was also assumed to undergo retro-aldolization reaction but in that case the resulting product kept the amino group. No attempt was made however in identifying and quantifying any reaction product that might be formed from it. The same assumption was tested in the present study and it showed a lack-of-fit in particular in the MG as well as in the organic acids formation. These results support the hypothesis that from DFG retro-aldolization the rate-determining step leads to the fragment without the amino group.

In Scheme 3 both deoxyosones are presented as essential steps in organic acids formation. In fact, steps 6 and 9 are the most significant in formic and acetic acid formation, respectively. The same result was observed in a previous study with different sugars and casein.[24] In Part I,[8] the obtained results suggested that 1-DG was more reactive than 3-DG. Also, acetic acid was identified as one of the main end products of DFG decomposition. From the kinetic analysis these results are supported. Not only the rate constant for the degradation of 1-DG into acetic acid (step 9) prevailed to the degradation of 1-DG into carbohydrate fragments (C_n) (step 8), in particular at pH 6.8, but also under these conditions the rate constant of step 9 is the highest of the system. However, in terms of colour formation the influence of 1-DG seems to be very low compared with 3-DG. As the pH increased 1-DG is no longer involved in carbohydrate fragments responsible for colour formation, but mainly in acetic acid formation. Under these conditions 3-DG becomes the main precursor in colour formation, through step 5. In a previous study[6] the significance of DFG in colour formation in Maillard reaction has been questioned. This result in combination with the fact that 3-DG is the main precursor of carbohydrate fragments involved in colour formation raises an important question about the importance of ARP reversibility in Maillard reaction. This question will be addressed in a following paper.

4. Conclusions

The multiresponse kinetic analysis was shown to be both helpful in deriving relevant kinetic parameters as well as in obtaining insight into the reaction mechanism. It becomes more fundamental than simple kinetics. It is important to realize that multiresponse kinetic analysis, contrary to uniresponse kinetic analysis is based on the rate-determining steps of the reaction, regarding both the reaction mechanism and the number of steps involved. The multiresponse modelling approach as used in this study is a helpful tool to unravel complicated reaction routes. Acetic acid, identified as a main end product in DFG thermal degradation, is according to the kinetic analysis mainly formed through 1-DG degradation. Also 3-DG was determined as a main precursor in carbohydrate fragments responsible for colour formation. As the reaction proceeded other compounds besides DFG are suggested to become reactants themselves with the formation among others, of methylglyoxal. Kinetic modelling is an iterative process: propose a model, confront it with experimental data, criticise the model, adjust the model and confront the adapted model with experiments again, until an acceptable model results. If more models are possible from a scientific point of view, then a statistical treatment may help to choose. We would like to stress that scientific insight should be the first and the foremost discrimination tool in discussing model discrimination. Model discrimination is not about finding out whether or not the model is right or wrong, but rather to find the best performing model, from a scientific and statistical point of view.

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References

1. Van Boekel, M. A. J. S. *J. Food Sci.* **1996**, *61*, 477–485.
2. Van Boekel, M. A. J. S. *Nahrung/Food* **2001**, *45*, 150–159.
3. Yaylayan, V.; Forage, N. G. *J. Agric. Food Sci.* **1991**, *39*, 364–369.
4. Debrauwer, L.; Vernin, G.; Metzger, J.; Siouffi, A. M.; Larice, J. L. *Bull Soc. Chim. Fr.* **1991**, *128*, 244–254.
5. Huyghues-Despointes, A.; Yaylayan, V. A. *J. Agric. Food Chem.* **1996**, *44*, 1464–1469.
6. Wedzicha, B. L.; Molero-Vilchez, M. D. *Food Chem.* **1997**, *58*, 249–254.
7. Martins, S. I. F. S.; Jongen, W. M. F.; Van Boekel, M. A. J. S. *Trends Food Sci. Technol.* **2001**, *11*, 364–373.
8. Martins, S.I.F.S.; Van Boekel, M.A.J.S. Kinetic modelling of the Amadori *N*-(1-deoxy-D-fructos-1-yl)glycine degradation pathways, Part I—Reaction mechanism, *Carbohydr. Res.* **2003** (in press).
9. http://www.athenavisual.com.
10. Stewart, W. E.; Caracotsis, M.; Sørensen, J. P. *AIChe J.* **1992**, *38*, 641–650.

11. Stewart, W. E.; Shon, Y.; Box, G. E. P. *AIChe J.* **1998**, *44*, 1404–1412.
12. Burham, K. P.; Anderson, D. R. *Model Selection and Inference. A Practical Information and Theoretic Approach*; Springer Verlag: New York, 1998.
13. Hodge, J. E.; Mills, F. D. *Carbohydr. Res.* **1976**, *51*, 9–21.
14. Yaylayan, V.; Sporns, P. *Food Chem.* **1987**, *26*, 283–305.
15. Huyghues-Despointes, A.; Yaylayan, V. A. *J. Agric. Food Chem.* **1996**, *44*, 672–681.
16. Keyhani, A.; Yaylayan, V. A. *J. Agric. Food Chem.* **1996**, *44*, 2511–2516.
17. Ginz, M.; Balzer, H. H.; Bradbury, A. G. W.; Maier, H. G. *Eur. Food Res. Technol.* **2000**, *211*, 404–410.
18. Anet, E. F. L. J. *Adv. Carbohydr. Chem.* **1964**, *19*, 181–218.
19. Tressl, R.; Nittka, C.; Kersten, E. *J. Agric. Food Chem.* **1995**, *43*, 1163–1169.
20. De Bruin, J. M. Ph.D.Thesis, University of Technology, Delft, The Netherlands, 1986.
21. MacLaurin, D. J.; Green, J. W. *Can. J. Chem.* **1996**, *47*, 3947.
22. Isbell, H. S.; Linek, K.; Hepner, K. E., Jr. *Carbohydr. Res.* **1971**, *19*, 319–327.
23. Box, G. E. P.; Draper, N. R. *Biometrika* **1965**, *52*, 355–365.
24. Brands, C. M. J. Ph.D. Thesis, Wageningen University, The Netherlands, 2002.