JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 6725−6739 **6725**

Kinetic Modeling of Reactions in Heated Monosaccharide−Casein Systems

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In the present study, a kinetic model of the Maillard reaction occurring in heated monosaccharide−casein systems was proposed. Its parameters, the reaction rate constants, were estimated via multiresponse modeling. The determinant criterion was used as the statistical fit criterion instead of the familiar least squares to avoid statistical problems. The kinetic model was extensively tested by varying the reaction conditions. Different sugars (glucose, fructose, galactose, and tagatose) were studied regarding their effect on the reaction kinetics. This study has shown the power of multiresponse modeling for the unraveling of complicated reaction routes as occur in the Maillard reaction. The iterative process of proposing a model, confronting it with experiments, and criticizing the model was passed through four times to arrive at a model that was largely consistent with all results obtained. A striking difference was found between aldose and ketose sugars as suggested by the modeling results: not the ketoses themselves but only their reaction products were found to be reactive in the Maillard reaction.

KEYWORDS: Maillard reaction; sugar isomerization; monosaccharides; glucose; fructose; galactose; tagatose; casein; kinetic modeling; multiresponse modeling

INTRODUCTION

To be able to control chemical reactions in foods, the reactions of interest need to be studied in a quantitative way. The reactions occurring in a monosaccharide−protein system were the subject of study in a previous paper (*1*). Three main reaction routes were found: (a) isomerization of the sugar, (b) degradation of the sugar, and (c) the Maillard reaction in which the sugar (and/or its breakdown products) reacts with the ϵ-amino group of lysine residues of the protein. The second and third reaction routes have reaction products in common. In **Figure 1**, the proposed reaction network model is shown, which summarizes the main findings. The chemistry behind the reaction scheme was extensively discussed in the previous paper (*1*). The present paper will deal with the kinetic analysis of the reaction network model. With knowledge of kinetics, it becomes possible to describe the changes in a quantitative way and to predict changes from certain time−temperature combinations. In addition, kinetics is a tool for understanding reaction mechanisms.

Some useful steps to be considered in elucidating complex reaction networks, such as the one in **Figure 1**, were listed in the literature (*2*−*4*):

1. *Identify all stable reaction products and determine the mass balance.* A mass balance should give insight into the question of whether indeed the main products have been identified or that perhaps reaction products are missing. This has been done as described in the previous paper (*1*). The main reaction products were the sugar isomer, the organic acids formic and acetic acid as stable sugar breakdown products, the protein-bound Amadori compounds, and the brown melanoidins. Unidentified products were characterized as advanced Maillard reaction products (AMPs) and sugar degradation products, including unidentified organic acids (C_n with $1 \leq n \leq 6$).

2. *Identify primary and secondary reaction routes.* The primary reaction routes in the sugar−casein systems were sugar isomerization, sugar degradation, and the reaction of the protein with the sugar and/or its breakdown products in the Maillard reaction, in the end leading to the formation of protein-bound brown compounds. The Heyns compound, 5-hydroxymethylfurfural (HMF), and other heterocyclic compounds such as HHMF and DDMP were the products of secondary reaction routes.

3. *Propose a mechanism for the reaction network.* This was the subject of the previous paper and is summarized in **Figure 1**. In this article, the reaction network model will be further refined by the modeling exercise.

4. *Determine the effect of temperature.* The reactions described here are of a chemical nature. It should therefore be possible to model the effect of temperature quantitatively by the Arrhenius equation or the Eyring equation. This will also be addressed in this paper.

5. *Determine the effect of pH.* This will be a subject of study in the present paper. During heating, the pH decreases due to the formation of organic acids, and therefore we need to study

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10.1021/jf011164h CCC: $22.00
Published on Web 10/11/2002

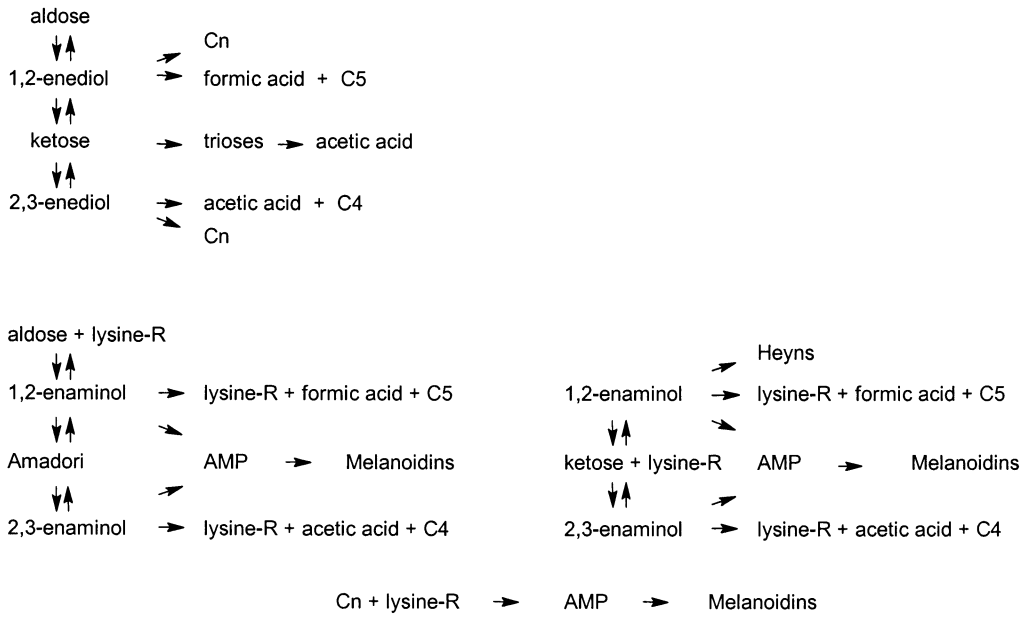


Figure 1. Proposed reaction network model for monosaccharide sugars in the presence of casein (C_n, unidentified sugar reaction compounds with n carbon atoms ($1 \leq n \leq 6$); AMP, advanced Maillard reaction products; lysine-R, protein-bound lysine residues).

the effect of pH. The pH can have an effect on both the reaction rate and the reaction mechanism.

6. *Determine the influence of reactant concentration.* The kinetic model of **Figure 1** should not be dependent on the initial concentration of the reactants. This can be tested by varying the concentrations of the reacting sugars and protein.

7. *Test whether the model is able to quantitatively fit the experimental data.* This step, together with the three preceding ones, will be the subject of the present paper and needs some further consideration.

To fit the model to the experimental data, the reaction network (**Figure 1**) needs to be translated into a mathematical model. This can be done by setting up differential equations for each reaction step. These nonlinear coupled differential equations are difficult to solve analytically, if it is possible at all, but they can be solved by numerical integration. The mathematical model then needs to be fitted to the experimental data. The question of how well the proposed model describes the experimental data must be addressed from a statistical point of view. If reactants and products involved in a reaction are measured at the same time, it is possible to take all such responses into account, which is called multiresponse modeling. The advantage of multiresponse modeling is the use of the information in various responses so that more precise parameter estimates and more realistic models can be determined (*5*). It gives at the same time more insight into the reaction mechanism. However, such an approach requires a special statistical treatment. The most simple (but mostly incorrect) approach to fit mathematical models to data and to estimate rate constants is to minimize the overall residual sum of squares (RSS) from all the responses. However, Hunter (*6*) showed that this criterion is valid only under the restrictions that (a) each of the responses has a normally distributed uncertainty, (b) the data on each response have the same variance, and (c) there is no correlation between the variances of the individual measurements of the responses. These restrictions are mostly not met when dealing with multiresponse modeling. For instance, samples will be analyzed simultaneously for different compounds (restriction c is not met) and some responses will be measured more precisely than others (restriction b is not met). Box and Draper (*7*) provided a solution for this problem following a Bayesian approach, assuming normally distributed errors. It is possible to form the so-called dispersion matrix from the responses. If the determinant of the dispersion matrix is minimized, the most probable estimates of the parameters will be found. Software is now available to use this approach (www.athenavisual.com).

The purpose of the present paper was to study the kinetics of the reactions occurring during heating of sugar−casein systems, starting with the kinetic model developed in our previous paper (*1*) and using the above-mentioned multiresponse approach. Kinetic modeling is an iterative process: proposing a model, confronting it with experiments, criticizing the model, adjusting the model, and confronting the adapted model with experiments again. In the present study, we will go through this iterative process several times. New results, obtained when studying the effect of heating temperature, pH, reactant concentration, and type of sugar, were used to test the kinetic model very rigorously.

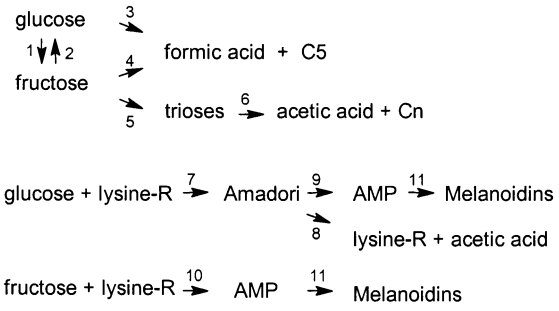
MATERIALS AND METHODS

Chemicals. All chemicals were of analytical grade. Glucose, fructose, and galactose were supplied by Merck (Darmstadt, Germany). Fluka Chemie (Buchs, Switzerland) supplied tagatose. Sodium caseinate (a spray-dried powder) was obtained from DMV (Veghel, The Netherlands) and contained 90% protein.

Preparation of Reaction Model Systems. The standard model system consisted of a reducing sugar (150 mM glucose, fructose, galactose, or tagatose) and sodium caseinate (3% w/w) dissolved in a phosphate buffer (0.1 M, pH 6.8) to give a molar ratio of sugar to lysine residues of about 10:1. The samples were heated at 120 °C in an oil bath in screw-capped glass tubes (Schott, 16 × 160 mm). When the concentration, heating temperature, or pH deviated from the standard, this will be mentioned. The reported heating times include the heating up period of about 2−3 min. After a given heating time, samples were cooled in ice water, prior to analysis. The reaction mixtures were heat-treated at least in duplicate.

Analyses of Reactants and Products. The reaction mixtures were analyzed as described previously (*1*). Available lysine residues were determined after derivatization with *ortho*-phthaldialdehyde (*8*) and subsequent fluorescence detection (emission wavelength, 430 nm; excitation wavelength, 340 nm). The samples were therefore diluted four times in sodium dodecyl sulfate (SDS, 16%). These samples were

Scheme 1. Kinetic Model for Monosaccharide–Casein Reactions



also used to determine the browning intensity by measuring the absorbance at 420 nm spectrophotometrically. The low molecular weight (sugars and sugar breakdown products) fraction was separated from the high molecular weight (protein) fraction via Sephadex G25 disposable columns and analyzed for sugars and organic acids by high-performance liquid chromatography (HPLC) (*1*) and by spectrophotometer for browning. The browning of the protein fraction was calculated by subtracting the browning of the sugar fraction from the browning of the total mixture. This absorbance was recalculated to the concentration of protein-bound melanoidins by dividing by the extinction coefficient according to Lambert−Beer's law. The extinction coefficient of protein-bound melanoidins formed in glucose−casein and fructose−casein systems was measured to be 500 [mol^{-1} L cm^{-1}] (*9*). The concentration of melanoidins is thus expressed as sugar units incorporated in the brown products. The Amadori compound was determined by means of furosine, using HPLC (*10*). Furosine concentration was converted to that of the Amadori compound using a conversion factor of 3.1 (*1*).

Kinetic Modeling. Computer simulations of reactions were done by numerical integration of differential equations that were set up for a particular reaction scheme. The parameters of the model, the rate constants, were estimated by nonlinear regression using the determinant criterion (*11*). The software package Gregpak/Athena Visual Workbench was used for numerical integration and to minimize the determinant (www.athenavisual.com). To discriminate between various models, the posterior probability was calculated (*12*). The model with the highest posterior probability was defined as the most likely one. In the example in which we used the least-squares fitting, the software package DynaFit (*13*) was used.

RESULTS AND DISCUSSION

The reaction network model of **Figure 1** is quite complex but can be simplified for modeling purposes. On the assumption of steady-state behavior of the enediols, it can be shown that the concentration of enediols is directly proportional to the monosaccharide concentration (*14*). This kinetic model still has many parameters, namely, a rate constant for every reaction step. Estimation of all these parameters at once requires a large number of data points. For multiresponse modeling with the determinant criterion, the following constraints are in order: the number of responses r cannot exceed the number of runs n and the number of parameters p must be less than the number of runs n (*5*). Therefore, the kinetic model was further simplified. As we mentioned in the previous paper (*1*), acid formation in the ketose system was about equal in the absence and in the presence of casein, and therefore acid formation via the Maillard reaction will be neglected. The Heyns compound could not be detected, and therefore this reaction step will be omitted. It is assumed that the ketose reacts directly with lysine residues to AMPs. The formation of formic acid in the aldose systems was only slightly catalyzed by amino groups, and therefore it was assumed that formic acid was formed only via sugar degradation. Acetic acid can be formed via the Amadori compound and via the degradation of ketoses in triose intermediates. The formation of acetic acid via the ketoses directly was neglected. Furthermore, the degradation of the sugars in unidentified reaction products (C_n) and the reaction of the sugar degradation compounds with the lysine residues of the protein will first be neglected. This all leads to the simplified kinetic model of **Scheme 1**, including 11 parameters. This reaction scheme gives the following differential equations for the concentrations:

$$\frac{d[glu]}{dt} = -k_1[glu] + k_2[fru] - k_3[glu] - k_7[glu][lys] \quad (1.1)$$

$$\frac{d[fru]}{dt} = k_1[glu] - k_2[fru] - k_4[fru] - k_5[fru] - k_{10}[fru][lys] \quad (1.2)$$

$$\frac{d[formic]}{dt} = k_3[glu] + k_4[fru] \quad (1.3)$$

$$\frac{d[acetic]}{dt} = k_6[triose] + k_8[Amadori] \quad (1.4)$$

$$\frac{d[triose]}{dt} = 2k_5[fru] - k_6[triose] \quad (1.5)$$

$$\frac{d[lys]}{dt} = -k_7[glu][lys] + k_8[Amadori] - k_{10}[fru][lys] \quad (1.6)$$

$$\frac{d[Amadori]}{dt} = k_7[glu][lys] - k_8[Amadori] - k_9[Amadori] \quad (1.7)$$

$$\frac{d[AMP]}{dt} = k_9[Amadori] + k_{10}[fru][lys] - k_{11}[AMP] \quad (1.8)$$

$$\frac{d[M]}{dt} = k_{11}[AMP] \quad (1.9)$$

In the previous paper (*1*), we studied isolated reactions (for instance, the reaction of glucose or fructose in the absence of casein and the reaction of the isolated protein-bound Amadori compound fructosyllysine) to make it possible to construct a reaction network model that describes the reactions in a chemically justified way. These isolated reactions could also be used to make an independent estimate of the reaction rate constants for the reactions concerned. Sugar isomerization and degradation reactions could be studied independently of the Maillard reaction by omitting protein. The reactions of glucose could, however, not be studied independently from the reactions of fructose since considerable amounts of fructose were formed when heating glucose, and vice versa (although less). Therefore, the reaction rate constants were estimated by modeling the data obtained from experiments with glucose and fructose simultaneously, thus using the data of both sugar−casein systems. This means that the number of responses increased by a factor of 2. Another way to estimate the reaction rate constants is to fix the rate constants of the reactions in the fructose system while estimating the rate constants in the glucose system, and vice versa. This method is very time consuming since it is an iterative process. Comparable results were obtained, but much more easily, by jointly modeling the reactions of glucose and fructose. The double number of differential equations were numerically integrated and fitted to the data. In **Figure 2**, the results are shown of the fit of the kinetic model of **Scheme 1** to the experimental data. For the clarity of the graphs, the averages of the experimental data for every response are shown, but the

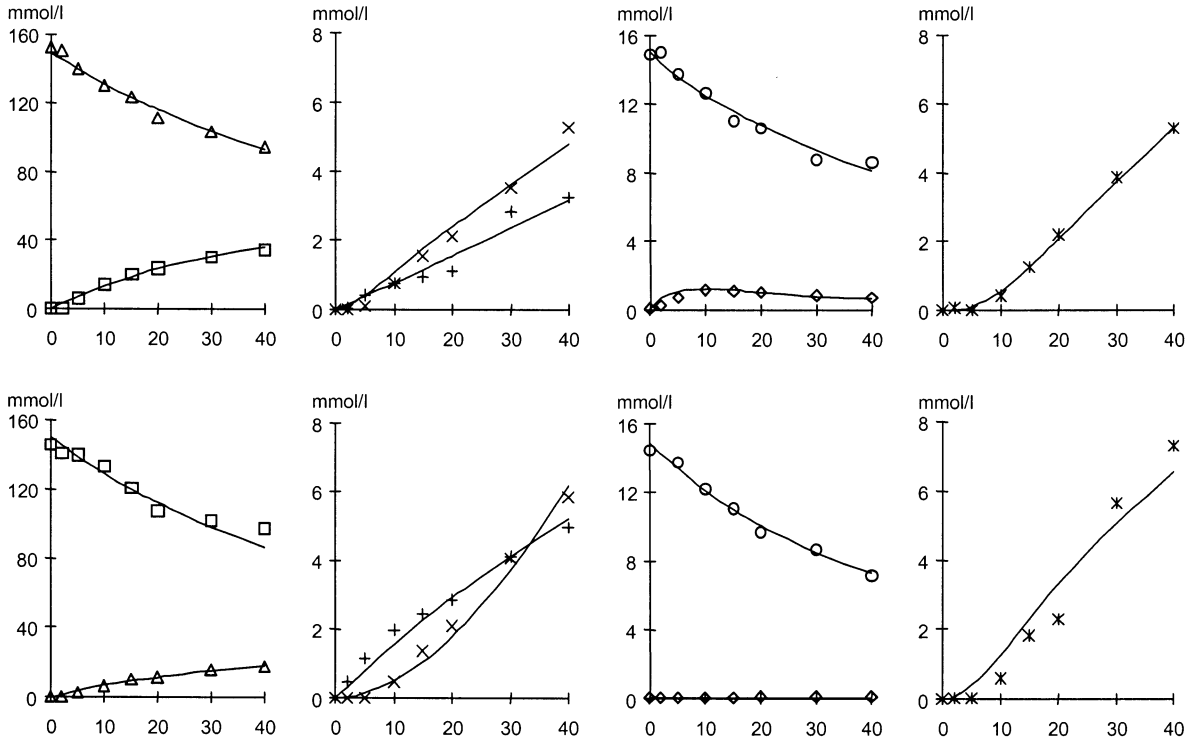


Figure 2. Simulation (drawn lines) based on the kinetic model of **Scheme 1** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

Table 1. Rate Constants and Their 95% Confidence Intervals as Found by Kinetic Modeling for the Model Displayed in **Scheme 1** to the Data of Glucose–Casein and Fructose–Casein Systems (120 °C, Initial pH 6.8), Using the Determinant Criterion and the Least-Squares Criterion

rate constant	determinant	least-squares
k_1 (min^{-1})	0.010 28 ± 0.000 46	0.010 20 ± 0.000 21
k_2 (min^{-1})	0.005 09 ± 0.000 23	0.005 08 ± 0.000 22
k_3 (min^{-1})	0.000 47 ± 0.000 04	0.000 66 ± 0.000 17
k_4 (min^{-1})	0.001 10 ± 0.000 06	0.001 05 ± 0.000 18
k_5 (min^{-1})	0.007 12 ± 0.002 14	0.004 61 ± 0.000 46
k_6 (min^{-1})	0.004 39 ± 0.001 43	0.006 73 ± 0.001 52
k_7 (L $mmol^{-1}$ min^{-1})	0.000 18 ± 0.000 01	0.000 24 ± 0.000 02
k_8 (min^{-1})	0.111 34 ± 0.011 46	0.112 24 ± 0.046 83
k_9 (min^{-1})	0.143 59 ± 0.017 10	0.168 31 ± 0.075 95
k_{10} (L $mmol^{-1}$ min^{-1})	0.000 15 ± 0.000 01	0.000 17 ± 0.000 03
k_{11} (min^{-1})	0.125 14 ± 0.030 48	0.073 02 ± 0.026 21

actual modeling was done using all experimental data. In general, the fits were deemed very good as judged from the residuals. Only a minor lack of fit could be observed in the fructose−casein system: browning was overestimated at the beginning and underestimated at the end of heating. Overall, it seemed that the model was able to describe the reactions occurring in heated sugar−casein systems very well. The logarithm of the posterior probability was −31.992. The 95% confidence intervals of the rate constants for reaction steps 5 and 6 (degradation of ketoses into triose intermediates and subsequent reaction to acetic acid) and reaction step 11 (formation of melanoidins) were somewhat wide (**Table 1**). This means that these reaction steps were not very important or, more likely, that the data obtained do not contain enough information to estimate them. From a mechanistic point of view, these reaction steps cannot be omitted.

It was mentioned in the Introduction that it could be incorrect to use the least-squares criterion for estimating the reaction rate constants in multiresponse modeling and that therefore the determinant criterion was used. To show that it indeed matters which fit criterion is used, the model was also fitted to the experimental data by minimizing the residual sum of squares. Although the fit of the model to the data did not differ that much, much larger 95% confidence intervals were obtained than when the determinant was minimized (**Table 1**). Taking into account differences in variances and covariances, as is implicitly done when using the determinant criterion, clearly leads to a much more precise estimation of the kinetic parameters. Obviously, precision of parameter estimates is an important issue, and therefore it is essential to use the determinant criterion.

Under the above-mentioned conditions (heating temperature, 120 °C; pH, 6.8; sugar concentration, 150 mM; protein concentration, 3%), the proposed kinetic model fitted the experimental data very well. To strain the model, the reaction conditions were altered.

Effect of Heating Temperature. Generally, the rate of chemical reactions increases with increasing temperature. Since the Maillard reaction consists of several reaction steps, each with a possibly different temperature sensitivity, it strongly depends on temperature which reaction route prevails and what pattern of intermediates and end products is formed. Furthermore, temperature affects the activities of the reactants. The active form of the sugar is considered to be the open chain, the concentration of which increases with temperature. The percentage of fructose in its acyclic form at neutral pH is about 0.7% at room temperature and 13.1% at 80 °C (*15*). The concentration of the acyclic form of glucose is much lower and therefore more difficult to detect. In the literature, the reported percentage of glucose in its acyclic form varies from 0.002% (*16*) to 0.022% (*17*) at neutral pH and room temperature. The percentage of the acyclic form at higher temperatures is not known.

To be able to predict the reactions at various temperatures, the temperature dependence has to be determined. A consistent temperature dependence is an additional indication that a model is acceptable. The relationship between the rate constant (k) and

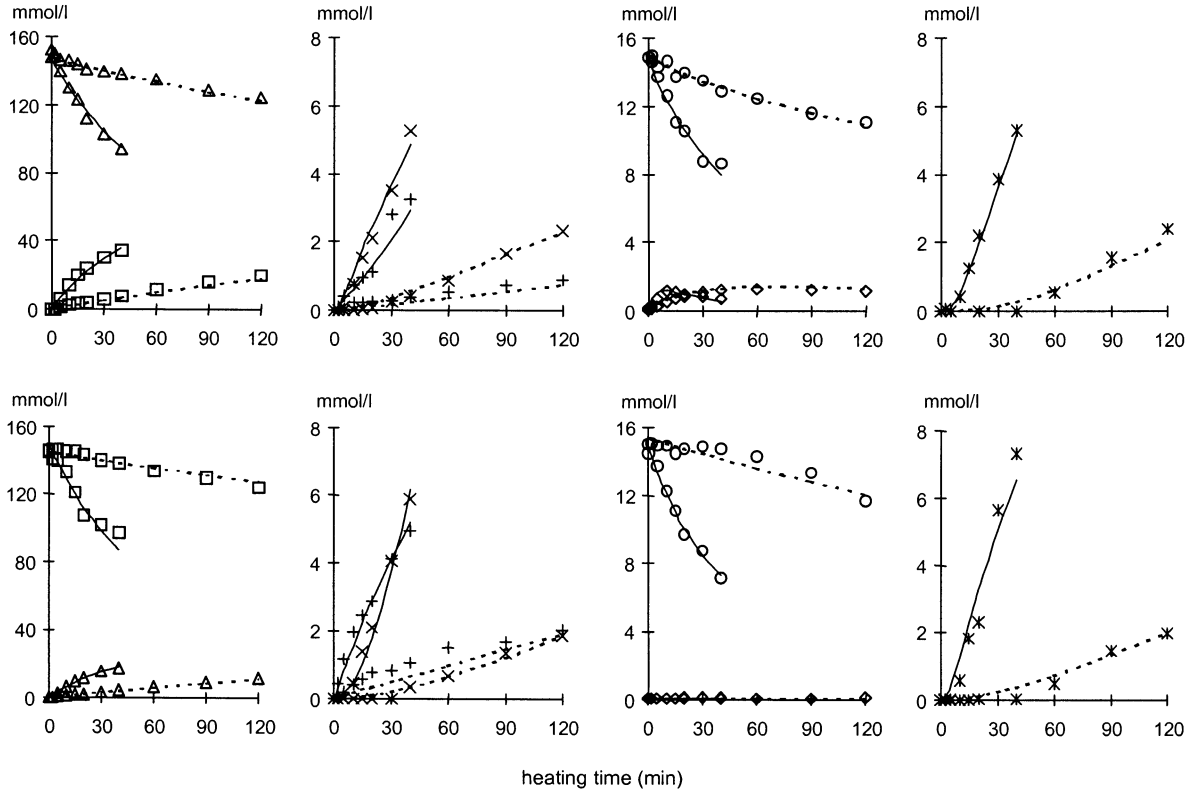


Figure 3. Simulation (drawn lines) based on the kinetic model of **Scheme 1** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 100 °C (dotted line) and 120 °C (solid line). Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

temperature (T) is frequently indicated by the well-known Arrhenius equation:

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

k_0 is the so-called pre-exponential factor, R is the gas constant (8.31 J mol^{-1} K^{-1}), and E_a is the activation energy, the kinetic energy of reactant molecules. E_a is usually determined experimentally from the plot of ln k versus $1/T$. The Arrhenius equation is an empirical equation and appears to fit many reactions and therefore is frequently used. It is, however, an oversimplification. Eyring developed a theoretical basis for the relation between k and T in the so-called transition-state theory:

$$k = \left(\frac{k_B T}{h}\right) \exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \exp\left(-\frac{\Delta H^{\ddagger}}{RT}\right) \tag{3}$$

where k_B is Boltzmann's constant (1.38×10^{-23} J K^{-1}) and h is Planck's constant (6.62×10^{-34} J s^{-1}). $\Delta S^{\ddagger}$ is the activation entropy, and $\Delta H^{\ddagger}$ is the activation enthalpy. Direct estimation of the kinetic parameters k_0 and E_a or $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ from these equations is to be preferred over the usual stepwise estimation, where reaction rate constants at constant temperature are determined first and subsequently E_a or $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are estimated from the rate constants so obtained. The stepwise procedure generally results in a relatively large confidence interval of the kinetic parameters due to a large standard deviation resulting from propagation of errors and a small number of degrees of freedom (*5*).

Generally, when estimating activation energies or activation enthalpies and entropies a high correlation is found between the parameters, because the experimental range of temperatures studied is narrow compared to the absolute temperature range over which the Arrhenius or Eyring equation would apply. Therefore the equations should be reparametrized. The reparametrized Arrhenius equation was used to model the reactions at various heating temperatures simultaneously and appears as follows (*18*):

$$k = X \exp(-YE_a) \tag{4.1}$$

$$X = k_0 \exp\left(-\frac{E_a}{RT_{av}}\right) \tag{4.2}$$

$$T_{av} = \frac{\Sigma T}{n} \tag{4.3}$$

$$Y = \frac{1}{R}\left(\frac{1}{T} - \frac{1}{T_{av}}\right) \tag{4.4}$$

Glucose−casein and fructose−casein systems were heated at 90, 100, 110, 120, and 130 °C. The model of **Scheme 1** was fitted to the data. In **Figure 3**, the results of the fit for the sugar−casein systems heated at 100 and 120 °C are given as an example. An increase of temperature leads to a higher loss of the reactants and an increased formation of the reaction products. Formation of the Amadori compound was faster, but its degradation was also faster, which resulted in the same maximum concentration for all heating temperatures. The estimates of the activation energies and their 95% confidence intervals are shown in **Table 2**. The activation energies of most reactions were in the order of 120 kJ/mol, which was as expected for chemical reactions (*19*). This consistent temperature dependence is an indication that the model is acceptable. How well the model fits to the data is another indication for the acceptability of the model (see **Figure 3**). The kinetic model

Table 2. Rate Constants and Activation Energies of Glucose–Casein and Fructose–Casein Systems Heated in the Range from 90 to 130 °C as Found by Kinetic Modeling of the Model Displayed in **Scheme 1**

	heating temperature					
rate constants	90 °C	100 °C	110 °C	120 °C	130 °C	activation energy (kJ/mol)
k_1 (min^{-1})	0.000 39	0.001 18	0.003 42	0.009 34	0.024 31	126 ± 2
k_2 (min^{-1})	0.000 26	0.000 72	0.001 93	0.004 91	0.011 94	117 ± 2
k_3 (min^{-1})	0.000 01	0.000 04	0.000 12	0.000 36	0.001 03	137 ± 7
k_4 (min^{-1})	0.000 03	0.000 11	0.000 42	0.001 49	0.004 98	159 ± 5
k_5 (min^{-1})	0.000 12	0.000 37	0.001 09	0.003 06	0.008 16	129 ± 17
k_6 (min^{-1})	0.000 71	0.001 87	0.004 66	0.011 10	0.025 31	109 ± 19
k_7 (L $mmol^{-1}$ min^{-1})	0.000 01	0.000 03	0.000 08	0.000 20	0.000 46	114 ± 2
k_8 (min^{-1})	0.005 10	0.015 28	0.043 23	0.116 01	0.296 47	124 ± 4
k_9 (min^{-1})	0.006 16	0.019 14	0.056 07	0.155 54	0.410 17	128 ± 4
k_{10} (L $mmol^{-1}$ min^{-1})	0.000 00	0.000 01	0.000 04	0.000 12	0.000 34	138 ± 4
k_{11} (min^{-1})	0.016 31	0.031 72	0.059 58	0.108 38	0.191 38	75 ± 11

fitted the data of the glucose−casein systems heated at various temperatures very well. The fit for the heated fructose−casein systems was not as good, especially for the formation of formic acid, loss of lysine, and browning. At 90, 100, and 110 °C, formic acid formation was underestimated by the model in the beginning of heating, while at 120 and 130 °C it was estimated correctly at the beginning of heating but overestimated at the end. This might be due to a pH effect (see the next section). Browning in the fructose−casein system was underestimated at the end of heating at 110, 120, and 130 °C and overestimated at the beginning of heating at 90, 100, and 110 °C. Another lack of fit was the loss of lysine residues in fructose−casein systems heated at 90, 100, or 110 °C. At 100 °C, for instance, the lysine residues did not decrease until 60 min, whereas the model predicts lysine to decrease immediately. Obviously, there is something wrong in the model for the fructose−casein system.

Therefore, a new model (**Scheme 2**) was proposed in which not fructose but unidentified reaction compounds (sugar degradation products (C_n) formed via reaction steps 3, 4, 5, 6, and 8 and the introduced reaction step 12) react with lysine residues to form AMPs (reaction step 13). The differential equations for the concentration of C_n (including the trioses) and AMP were then as follows:

$$\frac{d[\text{triose}]}{dt} = 2k_5[\text{fru}] - k_6[\text{triose}] - k_{13}[\text{triose}][\text{lys}] \quad (5.1)$$

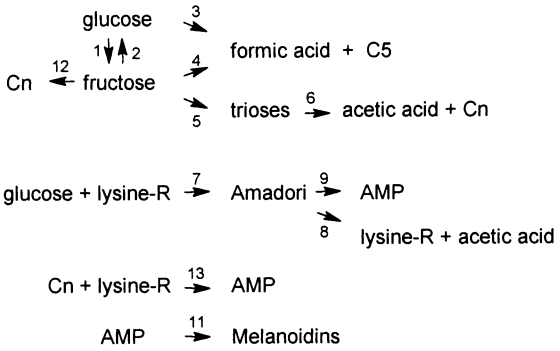
$$\frac{d[C_n]}{dt} = k_3[\text{glu}] + k_4[\text{fru}] + k_{12}[\text{fru}] + k_6[\text{trioses}] + k_8[\text{Amadori}] - k_{13}[C_n][\text{lys}] \quad (5.2)$$

$$\frac{d[\text{AMP}]}{dt} = k_9[\text{Amadori}] + \frac{3}{6}k_{13}[\text{triose}][\text{lys}] + \frac{n}{6}k_{13}[C_n][\text{lys}] - k_{11}[\text{AMP}] \quad (5.3)$$

In the differential equation of AMP, the parameter n is introduced. AMPs are the precursors of the melanoidins. Melanoidins have been defined as the number of C_6-equivalents incorporated in the brown high molecular weight compounds. A C_n compound reacts in a 1:1 ratio with the lysine residues of the protein. If 1 mmol of C_n reacts with 1 mmol of lysine, $n/6$ × 1 mmol AMP and subsequently melanoidins are formed. Parameter n is expected to be smaller than or equal to 6, because C_n is defined as a sugar fragment with $1 \leq n \leq 6$ carbon atoms.

The adapted model was able to fit the data, although an inconsistent temperature dependence was observed for reaction step 11 since the activation energy approaches zero (not shown).

Scheme 2. Kinetic Model for Monosaccharide–Casein Reactions



The parameter n was estimated to be 5.8 (±0.4). The adapted model did not show an obvious change in fit for the glucose−casein system. In the fructose−casein system, the fit for the loss of lysine was improved at lower temperatures and that for browning at higher temperatures, but the fit for lysine loss at higher temperatures and for browning at lower temperatures was worsened (**Figure 4**). We assumed that the rate constant of the reaction between the intermediate compounds and lysine residues was the same for the various C_n compounds. Furthermore, we assumed that the average number of carbon atoms (n) per C_n compound did not depend on the reaction temperature. These assumptions might have led to the improper fit of the model to the lysine and melanoidin data. Another reason for the imperfect fit is the drop of pH during heating. This will be the topic of study in the next section.

Effect of pH. During heating of sugar−casein systems, the pH decreases due to the formation of organic acids, among which are formic acid and acetic acid. To study the effect of pH on the reaction kinetics, a glucose−casein and a fructose−casein system with an initial pH of 5.9 were heated at 120 °C and kinetic modeling was carried out, using the kinetic model of **Scheme 1**. The results are shown in **Figure 5**, and these were compared with the results of systems with an initial pH of 6.8 (**Figure 2**). In both sugar−casein systems, the loss of reactants was less and the formation of reaction products was lower when the initial pH was decreased. Whereas the loss of lysine was about equal in both sugar−casein systems heated at pH 6.8, it was less in the fructose−casein system than in the glucose−casein system at pH 5.9. This difference was also observed for the degree of browning. From the reaction rate constants (compare **Tables 3** and **1**), it can be observed that the rate of all reactions decreased with decreasing pH. Obviously, the pH has an effect both on the sugar reactions (isomerization and degradation) and on the Maillard reaction.

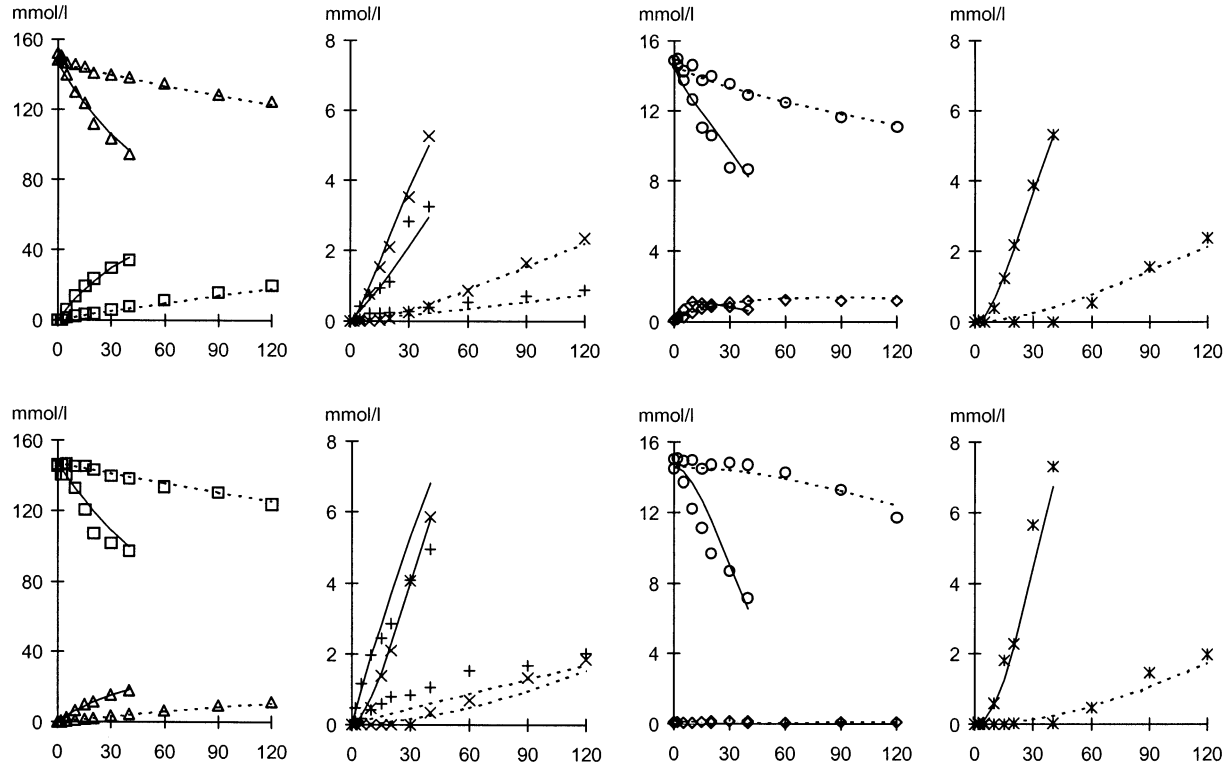


Figure 4. Simulation (drawn lines) based on the kinetic model of **Scheme 2** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 100 °C (dotted line) and 120 °C (solid line). Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

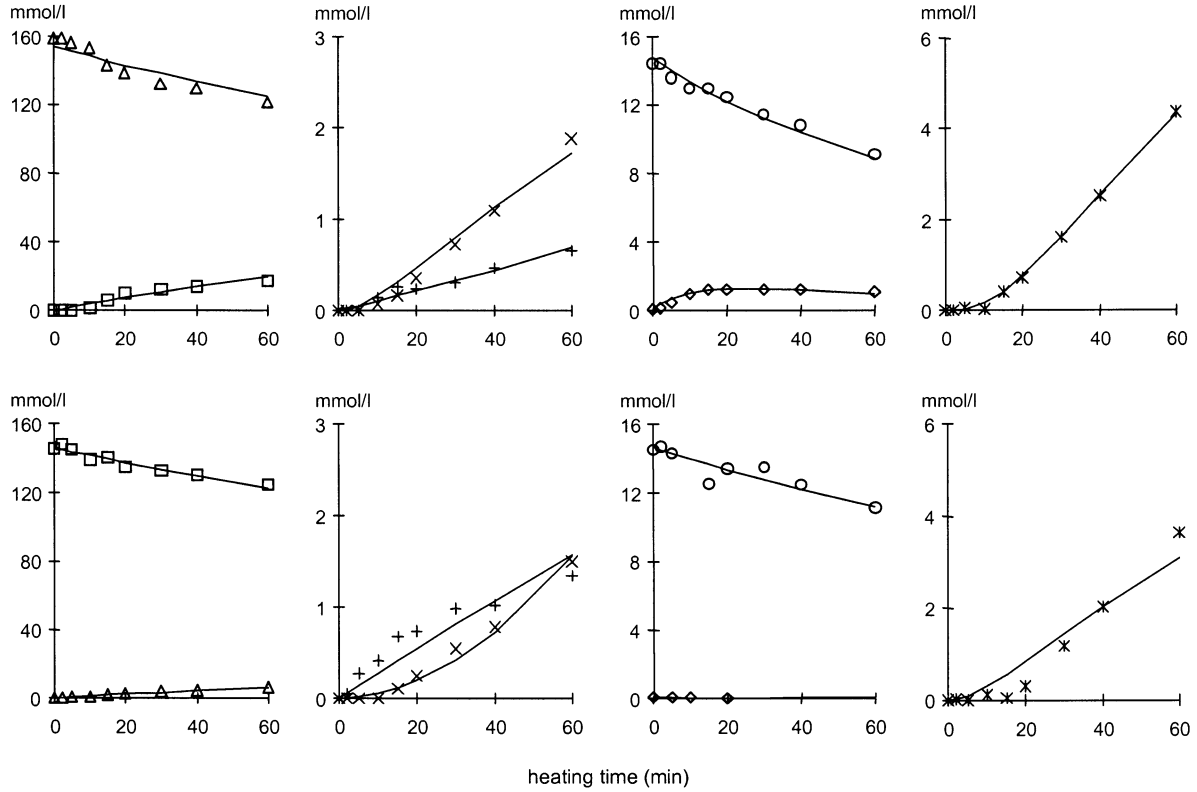


Figure 5. Simulation (drawn lines) based on the kinetic model of **Scheme 1** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C, initial pH 5.9. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

Except for the fact that the pH has an influence on the rates of the reactions, the reaction mechanism could also be influenced by the pH. It is claimed that certain reaction pathways are favored at lower pH and others at higher pH. For instance, HMF formation increased with decreasing pH (**Figure 6**), but still its amounts are an order of magnitude lower than those of other reaction products. This change in reaction mechanism might be an explanation for the poor fit of the formic acid data in the

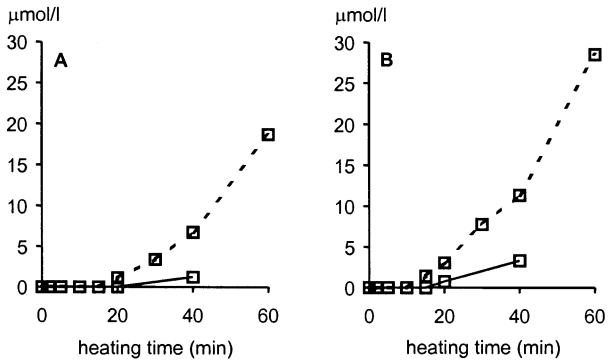


Figure 6. Formation of HMF in glucose–casein (A) and fructose–casein (B) systems heated at 120 °C. Dotted line, initial pH 5.9; solid line, initial pH 6.8.

Table 3. Rate Constants and Their 95% Confidence Intervals as Found by Kinetic Modeling for the Model Displayed in **Scheme 1** to the Data of Glucose–Casein and Fructose–Casein Systems (120 °C, Initial pH 5.9)

rate constant	
k_1 (min^{-1})	0.002 57 ± 0.000 31
k_2 (min^{-1})	0.000 87 ± 0.000 03
k_3 (min^{-1})	0.000 07 ± 0.000 01
k_4 (min^{-1})	0.000 19 ± 0.000 02
k_5 (min^{-1})	0.001 49 ± 0.000 69
k_6 (min^{-1})	0.002 14 ± 0.001 05
k_7 (L $mmol^{-1}$ min^{-1})	0.000 08 ± 0.000 00
k_8 (min^{-1})	0.025 28 ± 0.002 25
k_9 (min^{-1})	0.073 34 ± 0.006 09
k_{10} (L $mmol^{-1}$ min^{-1})	0.000 03 ± 0.000 00
k_{11} (min^{-1})	0.120 67 ± 0.030 18

fructose−casein system. Formic acid and HMF are both considered to be formed via the 1,2-enolization route. As the pH lowers during heating, HMF starts to be formed at the expense of formic acid and therefore the formation of formic acid levels off.

Generally, it has been stated that the rate of browning and substrate loss increases with increasing pH, up to a pH of about 10 (*20*−*22*). An explanation for this pH effect is that protonation of the amino group of the lysine residues of the protein increases with decreasing pH. Due to this protonation, the nucleophilicity and therefore the reactivity of the amino group are lost. The higher the pH, the larger the percentage of amino groups in the unprotonated form, and therefore more lysine residues can react with reducing sugars. The pK_a (the pH where 50% of the amino groups are protonated) of the ϵ-amino group of lysine residues is 10.53 at room temperature (*21*). It can be calculated that at 25 °C at pH 6.8, 0.0186% of the lysine residues are in the unprotonated form, whereas the value is 8 times less (0.0023%) at pH 5.9. Since some subsequent steps in the Maillard reaction are proton catalyzed, both effects will result in an optimum pH for the reaction rate of the Maillard reaction browning. Another factor causing pH rate dependence is related to the amount of the acyclic form of the reducing sugars. The acyclic form is the reactive form, and it is considered to decrease with decreasing pH (*21*). A pH decrease will therefore slow both the Maillard reaction and the isomerization and degradation reaction of the sugars. The amount of the acyclic form of glucose was found to increase about 3-fold in the pH range from 6.5 to 7.5 at 25 °C (*17*). The acyclic form of fructose, however, was not found to increase as a function of pH at 25 °C, while it increased only by a factor of 1.1 in the pH range from 2 to 9 at 80 °C (*15*). The observed difference between glucose and fructose in Maillard reaction reactivity due to lowering of the pH can therefore not be explained by a relatively higher concentration decrease of the acyclic form of fructose. According to Hayashi and Namiki (*23*), formation of C_2 and C_3 sugar fragments increased with increasing pH. These fragments are much more reactive toward amino groups than the original sugars. This can explain why browning and loss of lysine are less in the fructose−casein system compared to the glucose−casein system at pH 5.9, while at pH 6.8 the opposite pattern was observed. If fructose itself is not reactive in the Maillard reaction but only its degradation products are reactive (as proposed in the previous section), less lysine will react and less browning will occur when the pH is lowered. This is another indication that reaction step 13 (**Scheme 2**) cannot be neglected.

In an additional experiment, we heated glucose−casein and fructose−casein systems at 100 °C with an initial pH of 6.8 and the pH was kept constant during heating, using a pH Stat Controller. The reaction rate constants of **Table 2** for the reaction at 100 °C were used to predict the loss of reactants and formation of reaction products. The results are shown in **Figure 7**. In the glucose−casein system, the decrease of glucose and formation of fructose were predicted very well but the formation of acids and browning and the loss of lysine were somewhat underestimated. Apparently, the drop in pH during heating slows the Maillard reaction mainly and the effect on isomerization is less. In the fructose−casein system, the prediction of loss of lysine and formation of melanoidins is even worse. The loss of fructose is also underestimated, which is an indication that fructose is decreased via an additional reaction route (reaction step 12) and that these degradation products react in the Maillard reaction with lysine residues (reaction step 13).

In this part of our study, the kinetic model will be optimized for pH effects. The conversion between the protonated and the unprotonated lysine residues is very fast, just as is the reaction between the cyclic and acyclic forms of the sugars. The available reactant concentration is therefore in direct proportion to the total concentration, at least, when the pH is constant during heating. A difference in pH is then taken into account in the reaction rate constants. If the pH is not constant during heating, the reaction rate constants change during heating as a function of pH. The model of **Scheme 1** was fitted to the data of the sugar−casein systems heated at 120 °C (initial pH 6.8), taking the effect of the pH drop on the loss of lysine into account in the differential equations. This was done by multiplying the lysine concentration by the factor $10^{-\Delta pH}$. When the pH is decreased by 1 unit, the concentration of unprotonated lysine residues, the reactive form, decreases 10^1 times. The effect of pH was less for the sugars and was therefore neglected. In **Figure 8**, the results of the fit are shown. As can be observed, a maximum loss of lysine residues was predicted for the fructose−casein system, while, according to the data, lysine should further decrease. This can be effectuated by taking into account the reaction of lysine residues with sugar degradation products (reaction step 13). Furthermore, the fit of the model to the acetic acid data of the glucose−casein system flattened off, while it had to increase according to the data. In the fructose system (a ketose), it was assumed that acetic acid is formed via triose intermediates. Logically, it can be expected that formation of acetic acid via the Amadori compound (an aminoketose) also arises from these triose intermediates. All these changes are shown in the adapted kinetic model of **Scheme 3**. The model was fitted to the data of the glucose−casein and fructose−casein systems heated at 120 °C. **Scheme 3** gave a very good fit (see **Figure 9**) as judged from the residuals. The formation of acetic

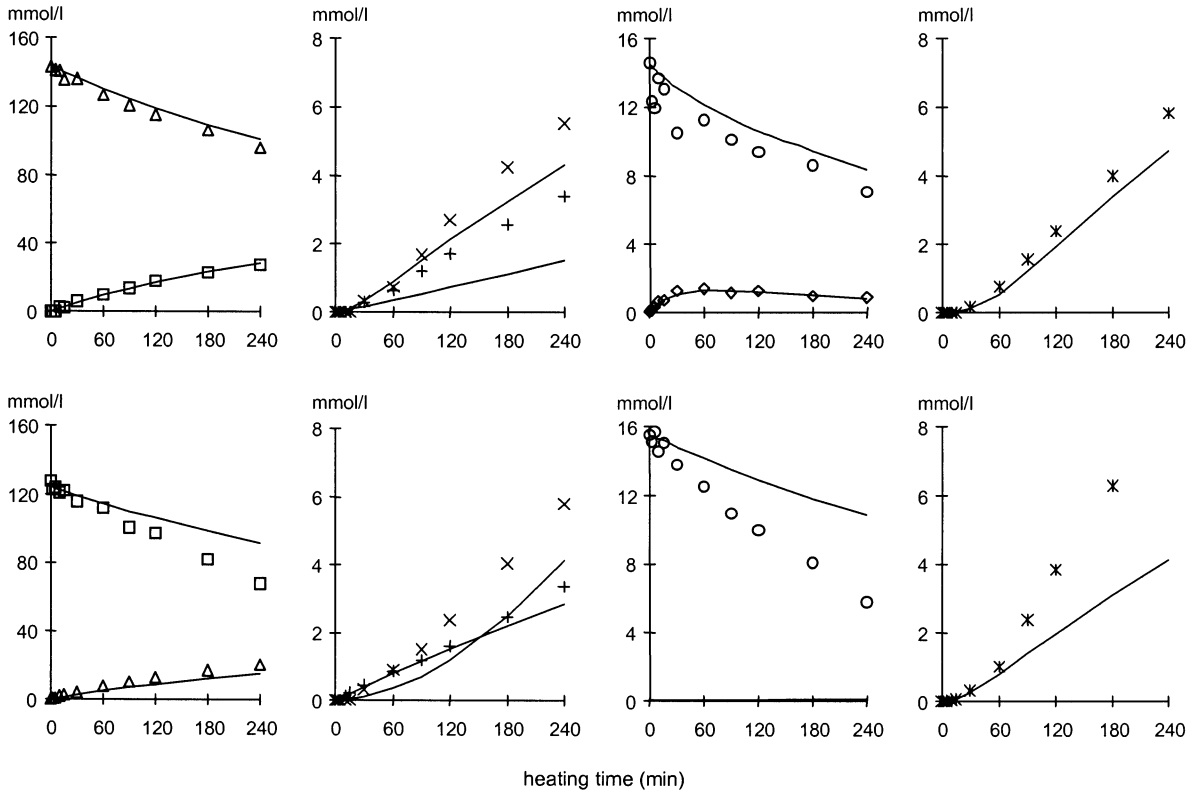


Figure 7. Predictions (drawn lines) based on the reaction rate constants of **Table 1** for glucose–casein (upper part) and fructose–casein (lower part) reactions at 100 °C and constant pH 6.8. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

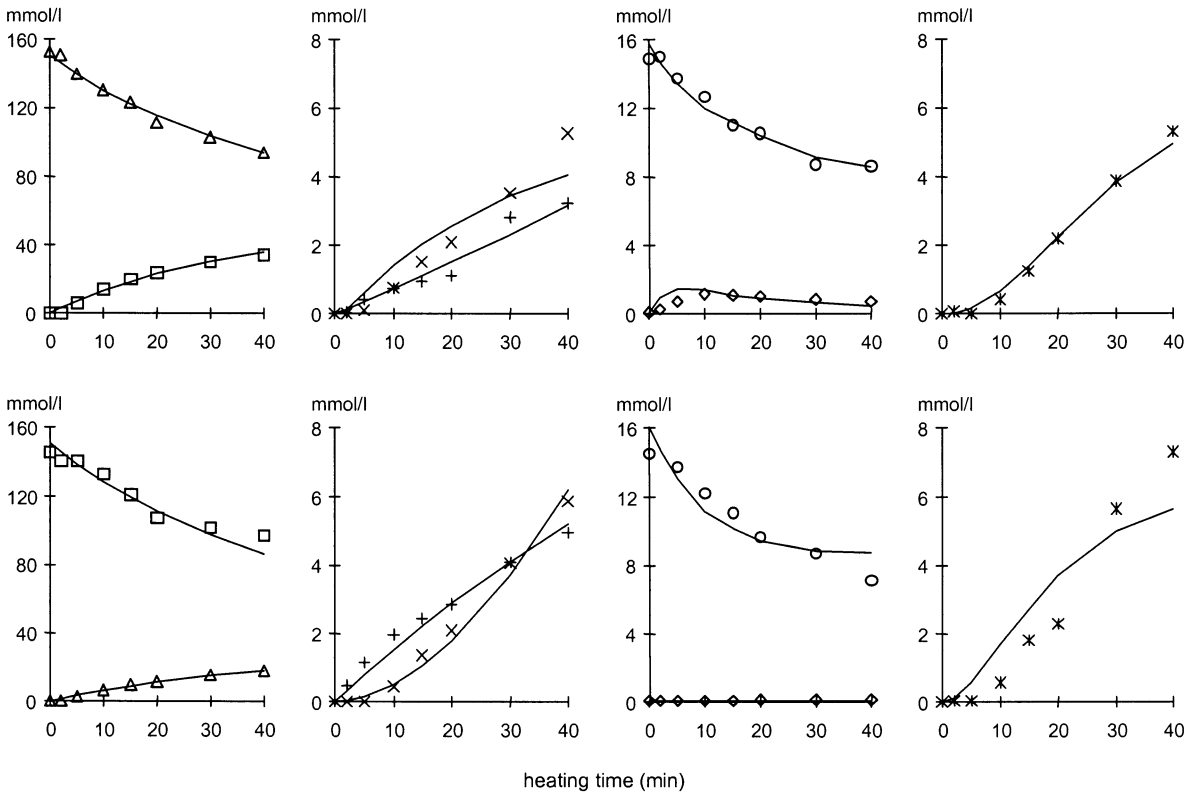


Figure 8. Simulation (drawn lines) based on the kinetic model of **Scheme 1** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C, taking into account the effect of a pH drop on the reactivity of lysine residues. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

acid in the glucose−casein system was better predicted and both the loss of lysine and browning were better described when reaction step 13 was included. A minor lack of fit in the glucose−casein system is the fit for the Amadori compound, which is somewhat overestimated in the beginning of heating and underestimated after 20 min of heating. In **Table 4**, the

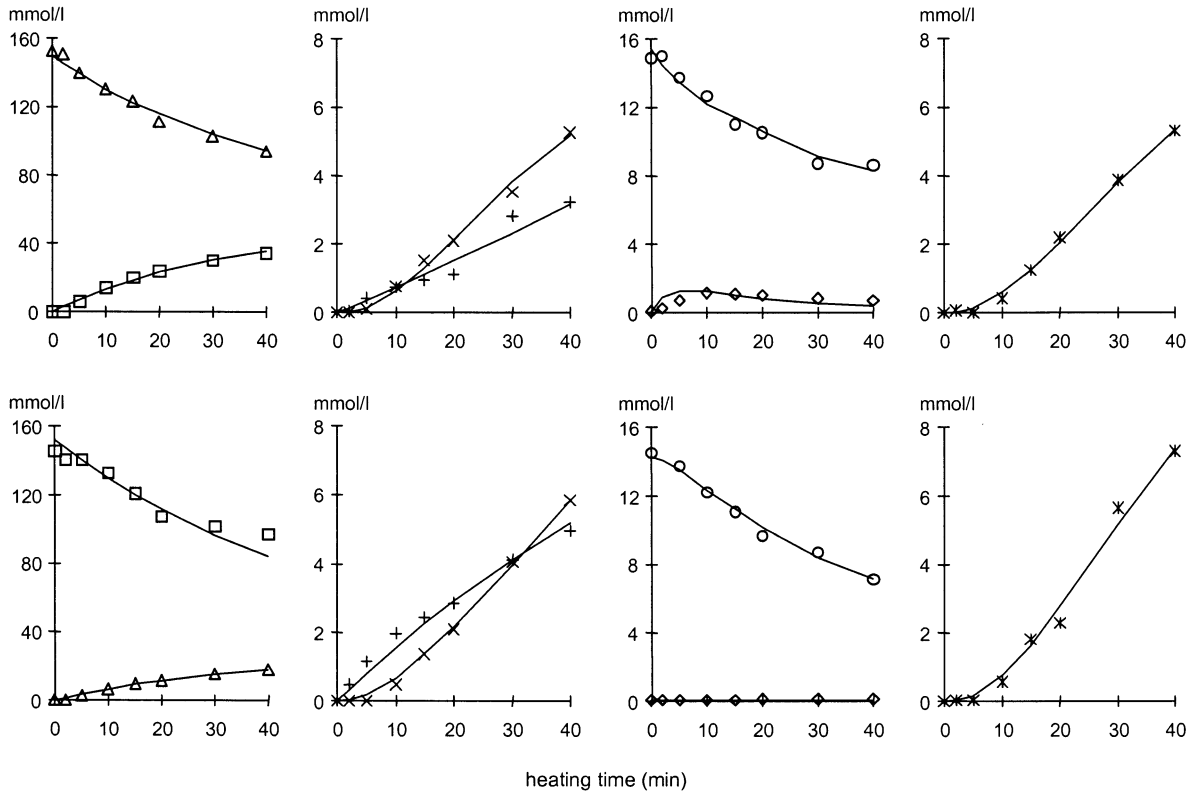
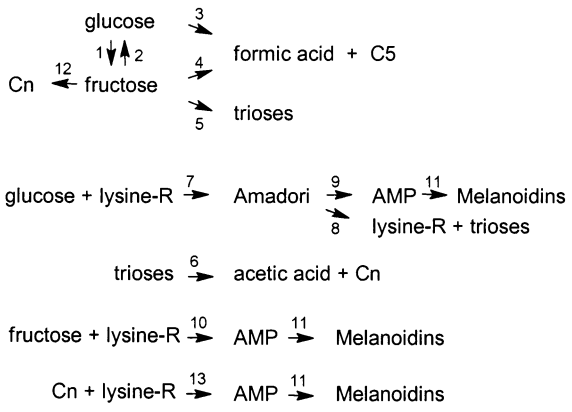


Figure 9. Simulation (drawn lines) based on the kinetic model of **Scheme 3** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C, taking into account the effect of a pH drop on the reactivity of lysine residues. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

Scheme 3. Kinetic Model for Monosaccharide–Casein Reactions

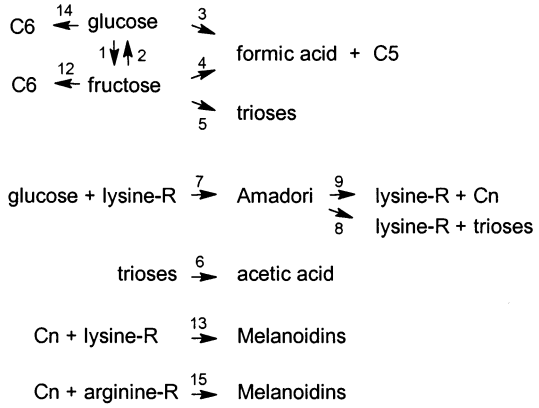


reaction rate constants are given. It was observed that the 95% confidence interval of k_{10} was very wide and that the rate constant itself was very small. The fit of the model to the data did not change when reaction step 10, the reaction of fructose with lysine residues, was neglected. This is yet another indication that not fructose itself but its degradation products are reactive in the Maillard reaction. Furthermore, the 95% confidence interval of k_{11} was very wide. The parameter n (number of carbon atoms per C_n compound) was estimated to be 10.5 ± 2.0. A value higher than 6 was not expected. To predict enough browning, this was apparently necessary. Despite these imperfections, **Scheme 3** described the reactions occurring in a monosaccharide−casein system better than **Scheme 1**, when taking the effect of pH into account. This visual observation was supported by the objective criterion of the software program Athena: the logarithm of the posterior probability increased from −39.871 to −34.882.

Table 4. Rate Constants and Their 95% Confidence Intervals as Found by Kinetic Modeling for the Model Displayed in **Scheme 3** to the Data of Glucose–Casein and Fructose–Casein Systems (120 °C, Initial pH 6.8)

rate constant	
k_1 (min^{-1})	0.010 39 ± 0.000 47
k_2 (min^{-1})	0.005 03 ± 0.000 24
k_3 (min^{-1})	0.000 47 ± 0.000 04
k_4 (min^{-1})	0.001 09 ± 0.000 06
k_5 (min^{-1})	0.001 04 ± 0.000 20
k_6 (min^{-1})	0.053 82 ± 0.018 40
k_7 (L $mmol^{-1}$ min^{-1})	0.000 32 ± 0.000 03
k_8 (min^{-1})	0.154 28 ± 0.037 65
k_9 (min^{-1})	0.161 65 ± 0.035 38
k_{10} (L $mmol^{-1}$ min^{-1})	0.000 06 ± 0.000 04
k_{11} (min^{-1})	0.089 85 ± 0.027 29
k_{12} (min^{-1})	0.008 38 ± 0.002 25
k_{13} (L $mmol^{-1}$ min^{-1})	0.001 75 ± 0.000 44

Scheme 4. Kinetic Model for Monosaccharide–Casein Reactions



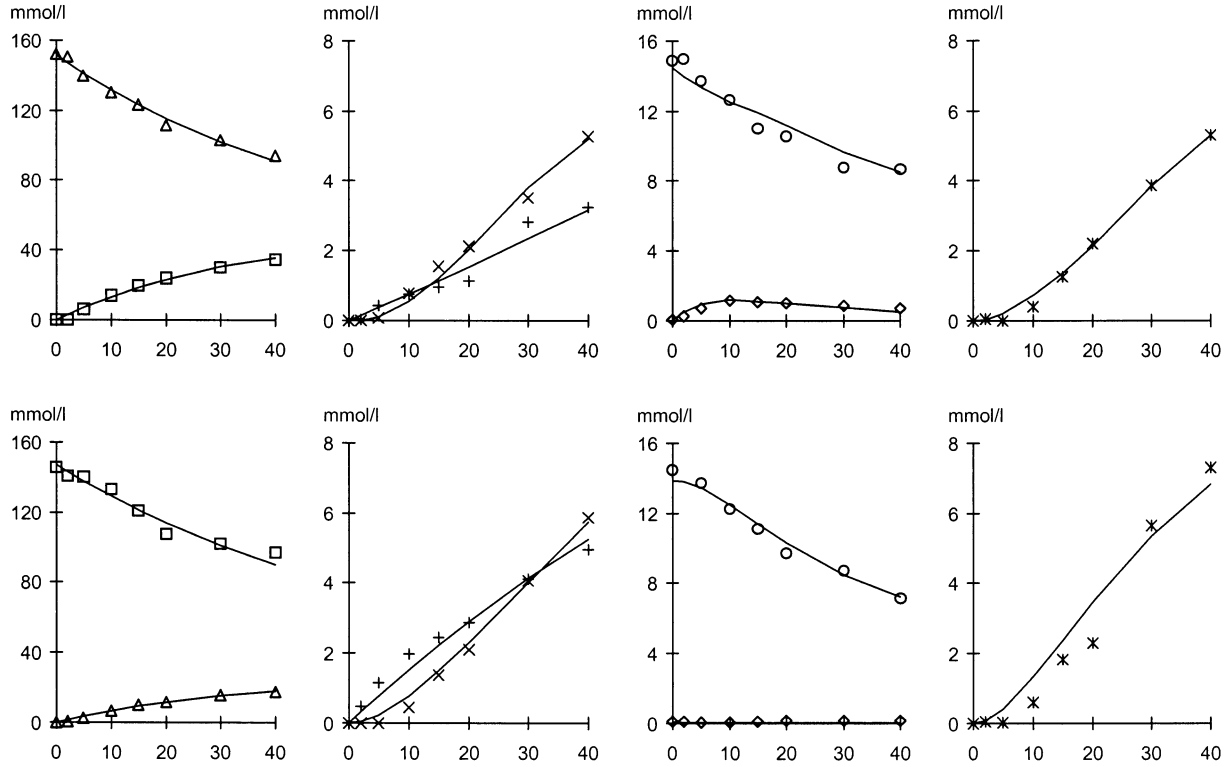


Figure 10. Simulation (drawn lines) based on the kinetic model of **Scheme 4** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

Table 5. Rate Constants and Their 95% Confidence Intervals as Found by Kinetic Modeling for the Model Displayed in **Scheme 4** to the Data of Glucose–Casein and Fructose–Casein Systems (120 °C, Initial pH 6.8)

rate constant	
k_1 (min^{-1})	0.009 93 ± 0.000 38
k_2 (min^{-1})	0.005 12 ± 0.000 23
k_3 (min^{-1})	0.000 47 ± 0.000 04
k_4 (min^{-1})	0.001 09 ± 0.000 06
k_5 (min^{-1})	0.000 86 ± 0.000 16
k_6 (min^{-1})	0.083 80 ± 0.034 49
k_7 (L $mmol^{-1}$ min^{-1})	0.000 15 ± 0.000 02
k_8 (min^{-1})	0.131 15 ± 0.027 08
k_9 (min^{-1})	0
k_{12} (min^{-1})	0.006 23 ± 0.001 33
k_{13} (L $mmol^{-1}$ min^{-1})	0.002 92 ± 0.000 60
k_{14} (min^{-1})	0.002 57 ± 0.000 58
k_{15} (L $mmol^{-1}$ min^{-1})	0.000 18 ± 0.000 24

To eliminate the imperfections of **Scheme 3**, the model was further improved (**Scheme 4**). In this scheme, the reaction of fructose with lysine (reaction step 10) and the reaction via AMP to browning (reaction step 11) were neglected. It was proposed that the Amadori compound reacted toward C_n compounds and that the protein was released (reaction step 9). These C_n compounds were expected to react with lysine toward melanoidins (reaction step 13). Furthermore, it was proposed that browning in the glucose−casein system could also occur without involvement of the Amadori compound. Glucose can degrade into C_n compounds (reaction step 14), which subsequently can react with lysine residues to form brown compounds (reaction step 13). Until now, we neglected the roll of arginine in the Maillard reaction. The guanidine group of arginine is not very reactive toward sugars but can be very reactive in the presence of some sugar degradation products, mainly deoxyosones and other dicarbonyls (*24*). When the lysine concentration becomes limiting or when considerable amounts of degradation products

Table 6. Rate Constants and Activation Energies of Glucose–Casein and Fructose–Casein Systems Heated in the Range from 90 to 130 °C as Found by Kinetic Modeling of the Model Displayed in **Scheme 4**

	heating temperature					
rate constants	90 °C	100 °C	110 °C	120 °C	130 °C	activation energy (kJ/mol)
k_1 (min^{-1})	0.000 39	0.001 17	0.003 36	0.009 22	0.025 28	126 ± 2
k_2 (min^{-1})	0.000 27	0.000 72	0.001 92	0.004 86	0.012 32	116 ± 2
k_3 (min^{-1})	0.000 01	0.000 04	0.000 13	0.000 38	0.001 16	139 ± 7
k_4 (min^{-1})	0.000 03	0.000 10	0.000 39	0.001 38	0.004 94	159 ± 6
k_5 (min^{-1})	0.000 04	0.000 11	0.000 29	0.000 71	0.001 75	113 ± 4
k_6 (min^{-1})	0.003 61	0.012 65	0.043 12	0.138 60	0.445 57	146 ± 11
k_7 (L $mmol^{-1}$ min^{-1})	0.000 01	0.000 03	0.000 06	0.000 13	0.000 28	97 ± 3
k_8 (min^{-1})	0.007 82	0.019 22	0.046 26	0.106 81	0.246 59	105 ± 5
k_9 (min^{-1})	0	0	0	0	0	0
k_{12} (min^{-1})	0.000 10	0.000 34	0.001 09	0.003 37	0.010 37	141 ± 12
k_{13} (L $mmol^{-1}$ min^{-1})	0.000 25	0.000 61	0.001 47	0.003 42	0.007 94	105 ± 10
k_{14} (min^{-1})	0.000 13	0.000 35	0.000 94	0.002 43	0.006 28	119 ± 11
k_{15} (L $mmol^{-1}$ min^{-1})	0.000 02	0.000 04	0.000 08	0.000 14	0.000 25	71 ± 103

are formed, the reaction of arginine can no longer be neglected. The concentration of the arginine residues in our model systems is about 6 mmol/L, and this reaction step (reaction step 15) will be included in the model of **Scheme 4**. The following differential equations were numerically integrated and fitted to the data:

$$\frac{d[glu]}{dt} = -k_1[glu] + k_2[fru] - k_3[glu] - k_7[glu][lys] \times 10^{-\Delta pH} - k_{14}[glu] \quad (6.1)$$

$$\frac{d[fru]}{dt} = k_1[glu] - k_2[fru] - k_4[fru] - k_5[fru] - k_{12}[fru] \quad (6.2)$$

$$\frac{d[formic]}{dt} = k_3[glu] + k_4[fru] \quad (6.3)$$

$$\frac{d[acetic]}{dt} = k_6[triose] \quad (6.4)$$

$$\frac{d[triose]}{dt} = 2k_5[fru] + 2k_8[Amadori] - k_6[triose] - k_{13}[triose][lys] \times 10^{-\Delta pH} - k_{15}[triose][arg] \quad (6.5)$$

$$\frac{d[lys]}{dt} = -k_7[glu][lys] \times 10^{-\Delta pH} + k_8[Amadori] + k_9[Amadori] - k_{13}[triose][lys] \times 10^{-\Delta pH} - k_{13}[C_5][lys] \times 10^{-\Delta pH} - k_{13}[C_6][lys] \times 10^{-\Delta pH} \quad (6.6)$$

$$\frac{d[Amadori]}{dt} = k_7[glu][lys] \times 10^{-\Delta pH} - k_8[Amadori] - k_9[Amadori] \quad (6.7)$$

$$\frac{d[C_5]}{dt} = k_3[glu] + k_4[fru] - k_{13}[C_5][lys] \times 10^{-\Delta pH} \quad (6.8)$$

$$\frac{d[C_6]}{dt} = k_{12}[fru] + k_{14}[glu] + k_9[Amadori] - k_{13}[C_6][lys] \times 10^{-\Delta pH} - k_{15}[C_6][arg] \quad (6.9)$$

$$\frac{d[arg]}{dt} = -k_{15}[triose][arg] - k_{15}[C_6][arg] \quad (6.10)$$

$$\frac{d[M]}{dt} = \frac{3}{6}k_{13}[triose][lys] + \frac{5}{6}k_{13}[C_5][lys] + \frac{6}{6}k_{13}[C_6][lys] + \frac{3}{6}k_{14}[triose][arg] + \frac{6}{6}k_{14}[C_6][arg] \quad (6.11)$$

The fit of this model to the data is shown in **Figure 10**. The reaction rate constants are given in **Table 5**. A reasonable fit for all reactants and products was obtained as judged from the residuals. The logarithm of the posterior probability increased to −31.233. **Scheme 4** was therefore the best model obtained so far. The rate of reaction step 9 approached zero. This means that the Amadori compound is only involved in the browning reaction of the glucose−casein system via the formation of triose intermediates. The model of **Scheme 4** was also fitted to the data of glucose−casein and fructose−casein heated at 90, 100, 110, 120, and 130 °C using the reparametrized Arrhenius equation. Reasonable fits were obtained. The activation energies of the reactions are shown in **Table 6**. The confidence interval of the temperature dependence of the reaction between arginine residues and sugar degradation products C_n (k_{15}) was very high. This might be caused by the fact that we did not have any information on the concentrations of arginine residues and the C_n compounds. It is also possible that the reaction step is not important, since the rate of the reaction is very low.

Effect of Sugar Concentration. To study the effect of sugar concentration, 75 mM instead of 150 mM glucose or fructose was heated at 120 °C in the presence of casein. If a kinetic model is consistent, the reaction rate constants should be independent of the concentration of the reactants. The reaction rate constants of **Table 5** (using **Scheme 4**, including pH effect) were used to predict the reactions. In **Figure 11**, the simulations are shown. The prediction of the sugars and acids was perfect; the prediction of the loss of lysine was somewhat less but reasonable. Amadori and browning in the glucose−casein system were predicted very well. Browning in the fructose−casein system was, however, somewhat overestimated. All in all, it seems that the model performed reasonably well.

Effect of Protein Concentration. Sugar−casein systems with a concentration of 1.5% sodium caseinate instead of the standard 3% were heated at 120 °C with 150 mM sugar. As mentioned before, the reaction rate constants should be independent of the concentration of the reactants if the kinetic model is consistent. The reaction rate constants of **Table 5** were used to predict the reactions. In **Figure 12**, the simulations are shown. The predictions of the reactants and reaction products were quite good. The major lack of fit was the formation of brown components. Since there is a lack of data in formation of C_n and loss of arginine, browning can only be predicted with less certainty. Again, we conclude that the model holds well.

Effect of the Type of Sugar. In this study, big differences were observed between the reaction behavior of glucose and fructose when heated in the presence of a protein. These differences are mainly due to differences in the reaction mechanism between aldose and ketose sugars. In **Scheme 5**, the reactions occurring in monosaccharide−casein systems are given for aldose sugars and ketose sugars in general. The aldose sugar and the ketose sugar can isomerize into each other via the Lobry de Bruin−Alberda van Ekenstein transformation. Both the aldose and the ketose can degrade into formic acid. Consequently, a C_5 compound, possibly 2-deoxyribose, is formed (*1*). The ketose can also react to a 3-deoxyaldoketose via its 1,2-enediol and to a 1-deoxy-2,3-diketose or 4-deoxy-2,3-diketose via its 2,3-enediol, or it can degrade into triose intermediates such as glyceraldehyde and 1,3-dihydroxyaceton, both fast reacting toward methylglyoxal. The aldose can, whether or not catalyzed by the protein, react to a 3-deoxyaldoketose or react with lysine residues of the protein to the Amadori compound. This Amadori compound (an aminoketose) can react further to 1-deoxy-2,3-diketose or 4-deoxy-2,3-diketose or degrade into trioses (like a ketose) whereby the protein is released. The C_5 (2-deoxyribose), C_3 (trioses), and C_6 (deoxyosones) can react with the lysine residues of the protein to form the brown protein-bound melanoidins. The C_3 and C_6 compounds can also react with the arginine residues of the protein to form melanoidins.

For comparison, also the reactions occurring in a galactose−casein and tagatose−casein system heated at 120 °C were studied. The reaction mechanisms of glucose and galactose (both aldoses) and fructose and tagatose (both ketoses) were assumed to be comparable (*1*). The kinetic model of **Scheme 5**, which is basically the same as that in **Scheme 4**, was used to fit the data. In **Figure 13**, the fit of the model to the galactose and tagatose data is shown, and in **Table 7** the reaction rate constants

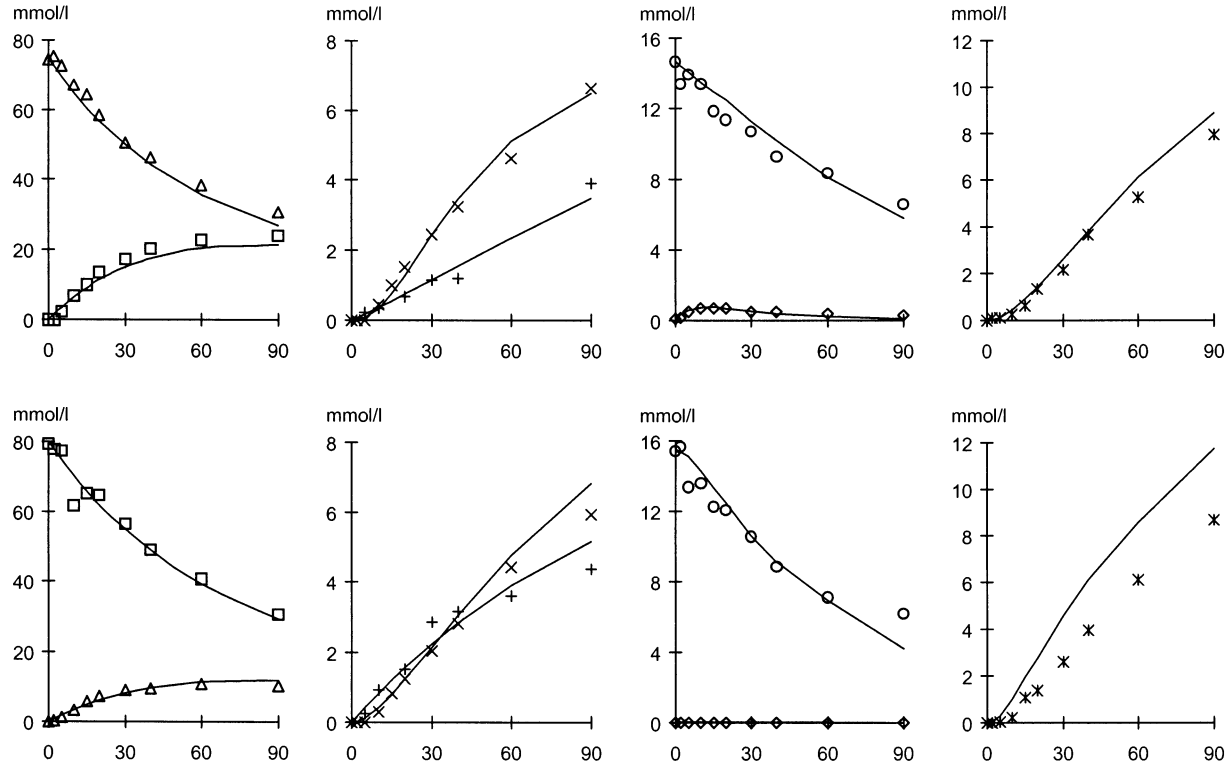


Figure 11. Effect of sugar concentration: prediction (drawn lines) based on the reaction rate constants of **Table 5** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C with an initial sugar concentration of 75 mM. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

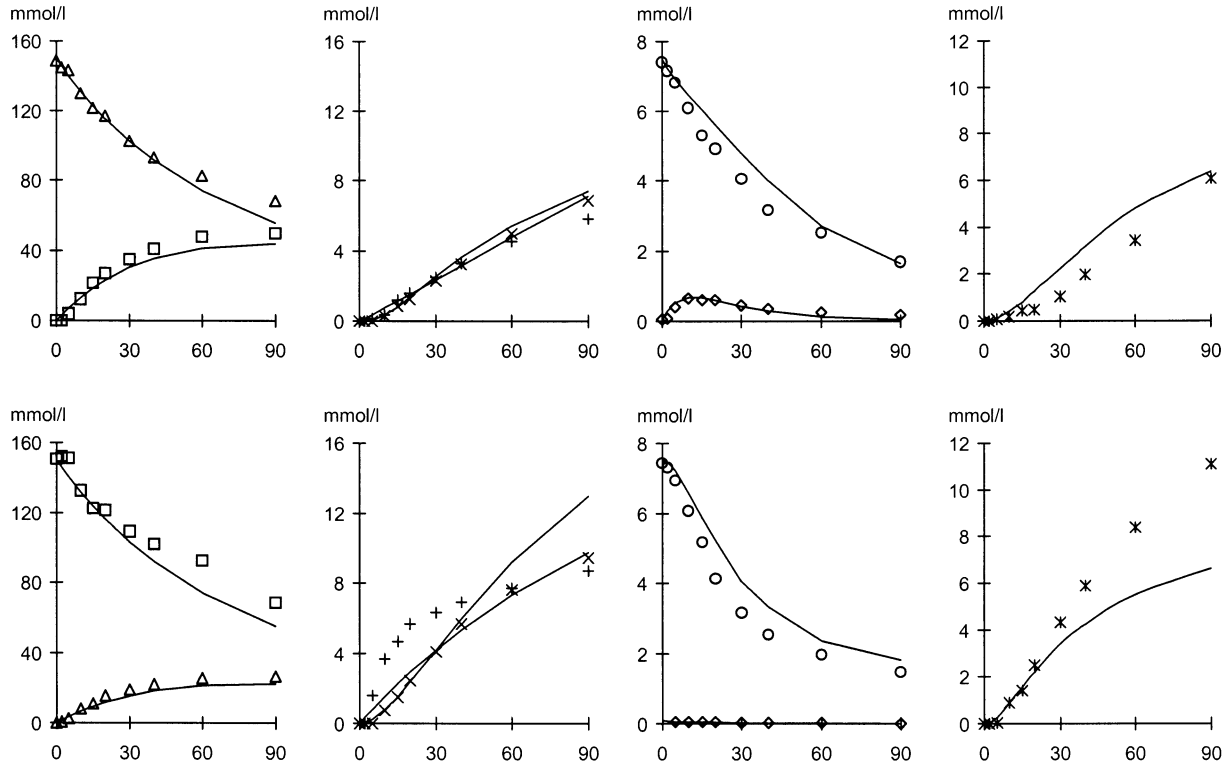


Figure 12. Effect of protein concentration: prediction (drawn lines) based on the reaction rate constants of **Table 5** for glucose–casein (upper part) and fructose–casein (lower part) systems heated at 120 °C with an initial sugar concentration of 75 mM. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

are given. The percentage of galactose in its acyclic form is about 10 times higher than that of glucose at neutral pH at room temperature (*16*), which is generally believed to be the reason for a higher reactivity of galactose than glucose. The isomerization rate of galactose into tagatose (k_1) was not influenced by the higher percentage of the acyclic form. Apart from that, the formation of acids (via k_3 and k_8), the loss of lysine (k_7), and the degradation of the Amadori compound (k_8) were indeed

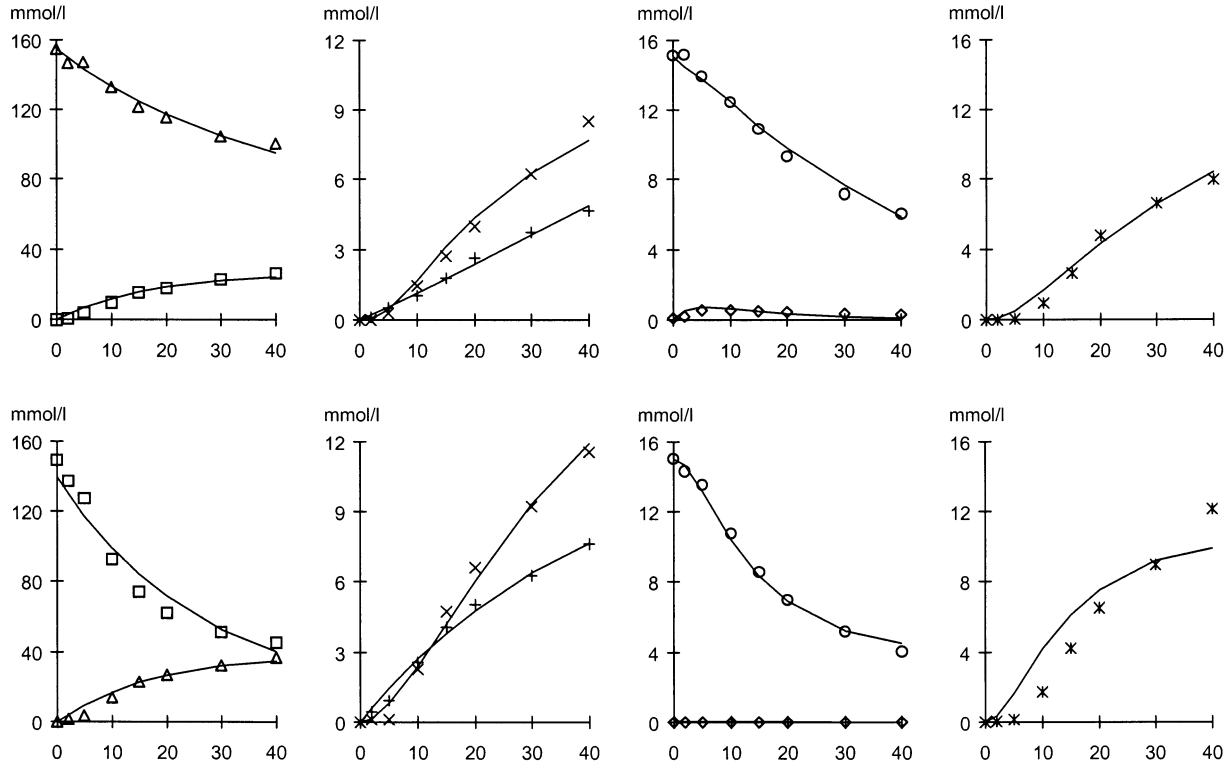
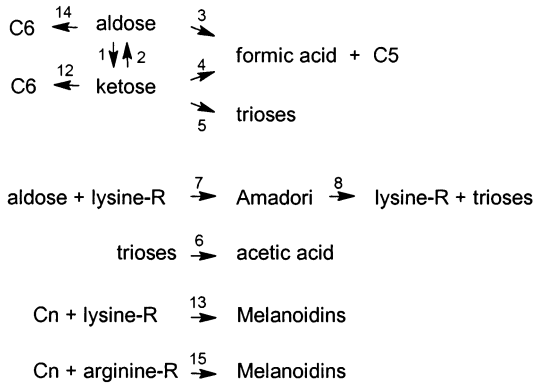


Figure 13. Simulation (drawn lines) based on the kinetic model of **Scheme 5** for galactose–casein (upper part) and tagatose–casein (lower part) systems heated at 120 °C, taking into account the effect of a pH drop. Glucose (△), fructose (□), formic acid (+), acetic acid (×), lysine residues (○), Amadori compound (◇), melanoidins (∗).

Scheme 5. Kinetic Model for Monosaccharide–Casein Reactions



faster in the galactose−casein system compared to the glucose−casein system. Furthermore, more sugar degradation products were formed (k_3, k_8, k_{14}), and therefore browning was also faster. The percentage of acyclic tagatose is 15% lower than the percentage of fructose in its open-chain form at neutral pH at room temperature (*25*). This did not automatically lead to a lower reactivity of tagatose compared to fructose when heated at 120 °C. Also, the isomerization reaction of the ketose into the aldose (k_2) and the degradation (k_4, k_5, and k_{12}) were significantly higher in the tagatose−casein system than in the fructose−casein system. Since more reaction products were formed, browning was also higher.

Except for the formation of melanoidins in the tagatose−casein system, the fit of the model to the data was very good. This lack of fit for the melanoidins was also observed when fitting the model to the fructose−casein data with different sugar or protein concentrations. Apparently, the model needs some minor adjustments. The determination of the concentrations of the reaction intermediates (C_n) will certainly contribute to a better understanding of the reactions.

In a subsequent paper, the effects of disaccharide sugars will be studied.

Table 7. Rate Constants and Their 95% Confidence Intervals as Found by Kinetic Modeling for the Model Displayed in **Scheme 5** to the Data of Galactose–Casein and Tagatose–Casein Systems (120 °C, Initial pH 6.8)

rate constant	
k_1 (min^{-1})	0.009 92 ± 0.001 15
k_2 (min^{-1})	0.015 59 ± 0.001 45
k_3 (min^{-1})	0.000 70 ± 0.000 08
k_4 (min^{-1})	0.002 24 ± 0.000 19
k_5 (min^{-1})	0.002 19 ± 0.000 28
k_6 (min^{-1})	0.141 47 ± 0.055 28
k_7 (L $mmol^{-1}$ min^{-1})	0.000 18 ± 0.000 03
k_8 (min^{-1})	0.375 25 ± 0.060 70
k_{12} (min^{-1})	0.015 17 ± 0.003 85
k_{13} (L $mmol^{-1}$ min^{-1})	0.005 09 ± 0.001 30
k_{14} (min^{-1})	0.003 22 ± 0.000 86
k_{15} (L $mmol^{-1}$ min^{-1})	0

CONCLUSIONS

The main conclusion from this work is that it is possible to model a complex reaction like the Maillard reaction. The multiresponse modeling approach as used in this study appears to be a very powerful tool to unravel complicated reaction routes. This is so because use is made of all the information in the observed responses. In the present study, the iterative process of modeling, proposing a model, confronting it with experiments, and criticizing the model, was passed through four times to arrive at a model that was able to describe the reactions in monosaccharide−casein systems under different reaction condi-

tions rather well. The results obtained in this study show remarkable differences in reaction mechanism between ketose and aldose sugars. The differences found may offer possibilities to optimize food quality with respect to the Maillard reaction.

ACKNOWLEDGMENT

We are grateful to Ruud van Gorp and Anton de Bruin for performing some of the experimental work.

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Received for review August 28, 2001. Revised manuscript received July 18, 2002. Accepted August 1, 2002. This paper reports results from research carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD program CT96-1080, "Optimization of the Maillard Reaction. A Way to Improve Quality and Safety of Thermally Processed Foods". It does not necessarily reflect the Commission's views and in no way anticipates its future policy in this area.

JF011164H