ELSEVIER

Food Chemistry 83 (2003) 13–26

Food Chemistry

www.elsevier.com/locate/foodchem

Kinetic modelling of reactions in heated disaccharide–casein systems

Carline M.J. Brands, Martinus A.J.S. van Boekel*

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

Received 16 May 2002; received in revised form 20 December 2002; accepted 10 January 2003

Abstract

The reactions occurring in disaccharide–casein reaction mixtures during heating at 120 °C and pH 6.8 were studied. The existence of two main degradation routes were established: (1) Isomerisation of the aldose sugars lactose and maltose in their ketose isomers lactulose and maltulose, respectively, and subsequent degradation. Lactulose was degraded into galactose and formic acid, whereas maltulose was degraded into glucose and formic acid among other unidentified reaction compounds. (2) The Maillard reaction, in which the aldose sugars and sugar degradation products react with the protein, eventually leading to the formation of brown compounds. Based on these reactions a kinetic model was built and extensively tested using multiresponse modelling. The iterative process of kinetic modelling—proposing a model, confronting it with experiments and criticising the model—was passed through several times. The final kinetic model was able to describe the observed changes in reactants and products and allowed a quantitative prediction of reactions in heated disaccharide–casein systems.

Keywords: Maillard reaction; Sugar isomerisation; Disaccharides; Lactose; Lactulose; Maltose; Maltulose; Protein; Reaction network model; Kinetic modelling; Multiresponse modelling

1. Introduction

The Maillard reaction, that is the reaction of carbohydrates with amino acids or proteins, has been thoroughly investigated. This reaction, which is of importance in many heated, dried and stored foods, leads to the formation of flavouring ingredients, browning products, the loss of nutritive value (Ledl, 1990), formation of antioxidants (Lignert & Hall, 1986), protein cross-linking (Pellegrino, Van Boekel, Gruppen, Resmini, & Pagani, 1999), while mutagenic but also antimutagenic compounds might be formed (Brands, Alink, Van Boekel, & Jongen, 2000).

Several investigations have shown that the reaction mechanisms of monosaccharides and disaccharides differ and that reaction products obtained from monosaccharides are different from those obtained from disaccharides. Kato and co-workers (Kato, Matsuda, Kato, & Nakamura, 1988) compared glucose and several disaccharides (having glucose at the reducing end) in their amino-carbonyl reaction behaviour in the presence of ovalbumin at reaction accelerating storage conditions. Although they did not find a major difference in the decrease of the free amino group, browning differed strongly and was dependent on sugar structure. It appeared that sugars with a glycosidic bond at the C4 hydroxyl group of the glucopyranose (like lactose, cellobiose and maltose) were difficult to cleave and browned therefore slowly. When sugars were bonded at the C6 hydroxyl group (like in melibiose and isomaltose) further degradation was not blocked and the rate of browning was therefore comparable to that of glucose. Other investigations have shown the difference in reaction products. The reaction products β-pyranone and 3-furanone that were obtained from the 1,4-glycosidic linked disaccharides lactose and maltose (Kramhöller, Pischetsrieder, & Severin, 1993; Pischetsrieder & Severin, 1996), have never been detected in glucose reaction mixtures. Troyano and co-workers (Troyano, Olano, Jimeno, Sanz, & Martinez Castro, 1992) showed that 3-deoxypentulose was formed as a reaction product of lactose during heating of milk, whereas it was not formed from glucose or galactose. Apparently, the glycosidic linked sugar gives rise to typical disaccharide degradation products, while typical monosaccharide degradation products were prevented to be formed.

* Corresponding author. Tel.: +31-317-482520; fax: +31-317-483669.

E-mail address: tiny.vanboekel@wur.nl (M.A.J.S. van Boekel).

0308-8146/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00031-1

To be able to control the Maillard reaction, the reaction steps of interest need to be studied in a quantitative way. With knowledge of kinetics it becomes possible to describe the changes in a quantitative way and to predict changes from certain time–temperature combinations. Kinetics is also a tool for understanding reaction mechanisms. Due to the complexity of the Maillard reaction mechanism, changes of a compound in time can not be described with simple kinetics (like a zero-, first- or second-order reaction). The observed reaction rate constant then reflects a mixture of many elementary rate constants. This simple kinetics approach is only a mathematical fit procedure and does not give any mechanistic insight. For kinetic modelling of complex reactions, a new approach was therefore introduced, called multiresponse modelling. The basic idea is to take into account as many responses as possible at once, as opposed to only one response. By applying multiresponse modelling, more realistic models and more accurate parameter estimates will be obtained (Van Boekel, 2001). The multiresponse modelling approach has been successfully used for modelling the Maillard reaction in monosaccharide–casein systems (Brands & Van Boekel, 2002).

The purpose of the present paper was to study the kinetics of disaccharide–casein reactions at temperatures corresponding to sterilisation conditions in the food industry, using multiresponse modelling. First, the main reaction products in heated disaccharide–casein systems were identified and quantified, and the main reaction pathways were established. The 1,4-glycosidic linked disaccharides lactose and maltose were studied. These sugars are of considerable relevance for foods. Maltose is an important degradation product of starch and occurs as such particularly in malted food, whereas the milk sugar lactose is found in dairy products. Maltose consists of two glucose units. Lactose consists of a glucose and a galactose unit, with the reactive carbonyl group on the glucose unit. The disaccharides lactulose and maltulose, the ketose isomers of lactose and maltose, respectively, were also studied. Maltulose consists of a fructose and a glucose unit and lactulose of a fructose and a galactose unit, both having fructose at the reducing end. To show the power of multiresponse modelling, the iterative process of kinetic modelling—proposing a model, confronting it with experiments, criticising the model, adjusting the model and confronting the adapted model with experiments again—will be gone through until a satisfactory model is obtained.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Lactose and maltose were supplied by Merck. Lactulose was supplied by Fluka Chemie and maltulose by Aldrich Chemie. Sodium caseinate, a spray-dried powder containing 90% protein, was obtained from DMV (Veghel, The Netherlands).

2.2. Preparation of reaction mixtures

A disaccharide sugar (150 mM lactose, lactulose, maltose or maltulose) and sodium caseinate (3% w/w) were 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 or 5:1. Reaction mixtures without casein were also prepared. The samples were heated for various times (0–60 min) at 120 °C in an oil bath in screw-capped glass tubes (Schott, 16 × 160 mm). The reported heating times include the heating-up period of about 2–3 min. After a given heating time, samples were immediately cooled in ice water.

2.3. Analyses of sugars and organic acids

Sugars and organic acids were separated from the protein via Sephadex G25 disposable columns (NAP-25, Pharmacia), as described previously (Brands & Van Boekel, 2001). The protein-free fraction was analysed by HPLC using an ion-exchange column. The ION-300 column (Interaction Chromatography Inc.) was used to separate galactose and tagatose or glucose and fructose, and organic acids. The eluent consisted of 0.0025 M sulphuric acid in water and the flow rate was 0.4 ml/min. The Benson Carbohydrate Lead column (Alltech) was used to separate lactose and lactulose or maltose and maltulose. The eluent was water with a flow rate of 0.5 ml/min. The HPLC columns were kept at 85 °C. The sugars were detected by monitoring the refractive index. The organic acids were separated with the ION-300 column and detected by their UV absorbance at 210 nm.

2.4. Analyses of total acids

Titrations were performed to determine the total acid concentration in heat-treated samples. Samples of 15 ml reaction mixture were titrated with 0.1 N NaOH to pH 8.3. From the difference in added NaOH between the heated and unheated sample the total amount of acid formed was calculated.

2.5. Analyses of available lysine residues

Available lysine residues were determined as described previously (Brands & Van Boekel, 2001).

2.6. Analyses of Amadori compound

The Amadori compound was determined as described previously (Brands & Van Boekel, 2001).

2.7. Analyses of brown compounds

The browning intensity of the heated reaction mixtures and the protein-free fraction was determined by measuring the absorbance at 420 nm with a spectrophotometer (Pharmacia Biotech) as described previously (Brands & Van Boekel, 2001). The browning of the protein fraction was calculated by subtracting the browning of the sugar fraction from the browning of the total mixture. The absorbance can be re-calculated to the concentration of melanoidins by using the equation of Lambert-Beer. The extinction coefficient of protein-bound melanoidins formed in disaccharide–casein systems is not known. In glucose–casein and fructose–casein systems the effective extinction coefficient was estimated to be 500 $mol^{-1}\,l\,cm^{-1}$ (Brands, Wedzicha, & Van Boekel, 2002). Since the extinction coefficient is expected to depend more on the type of amino group than on the type of sugar, it was assumed that the same value could be used for disaccharide systems. The concentration of melanoidins is thus expressed as sugar units (C12) incorporated in the brown products.

2.8. Kinetic modelling

Simulation was 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 non-linear regression using the determinant criterion (Stewart, Caracotsios, & Sørensen, 1992). The software package Gregpak/Athena Visual Workbench was used for numerical integration and to minimise the determinant (http://www.athenavisual.com). To discriminate between various models, the posterior probability was calculated (Stewart, Shon, & Box, 1998). The model with the highest posterior probability was defined as the most likely one.

3. Results and discussion

3.1. Identification and quantification of reactants and products

In the lactose–casein and lactulose–casein system, the detected reaction products were the corresponding isomer (lactulose and lactose, respectively), galactose and its isomer tagatose, and formic acid. In the maltose–casein and maltulose–casein system the monosaccharides glucose and its isomer fructose were formed instead of galactose and tagatose, as well as the corresponding isomers (maltulose and maltose, respectively) and formic acid. In contrast with the monosaccharide–casein systems (Brands & Van Boekel, 2001) no acetic acid was formed. In the lactose–casein and maltose–casein system also the protein-bound Amadori compounds lactulosyllysine and maltulosyllysine, respectively, were detected. In all systems brown compounds were formed.

In Fig. 1 the decrease of reactants and formation of reaction products is shown as averages of duplicates. No significant differences were observed between the lactose–casein and maltose–casein system (top graphs A_1–D_1). When comparing the lactulose–casein system with the maltulose–casein system the same conclusion could be drawn (bottom graphs A_2–D_2). The differences between the aldoses and the ketoses, on the other hand, were much more apparent. Although the decrease of initial sugar was about the same (compare graph A_1 and A_2), the decrease of lysine was much faster in the ketose systems (C_2) than in the aldose systems (C_1). Also the increase of all reaction products, including browning, was much faster in the ketose systems than in the aldose systems. The shape of degradation of reactants and formation of reaction products also differed strongly. After 20 min of heating, the formation of reactions products in the ketose–casein systems (except browning) started to flatten off, whereas the reaction products of the aldose–casein systems tended to increase linearly. Most likely, this was caused by a faster decrease in pH in the ketose–casein systems (see Fig. 2). The rate of the Maillard reaction, sugar isomerisation and subsequent degradation decreases with decreasing pH (Brands & Van Boekel, 2002). The drop in pH was partially (about half) caused by the formation of formic acid (compare Figs. 1B and 2). Other acids such as lactic, levulinic, propionic, butyric, pyruvic, citric and saccharinic acids might have been formed (Berg, 1993), but they were rather difficult to identify. Compared to monosaccharide systems, about four times more formic acid was formed (Brands & Van Boekel, 2001).

A mass balance was calculated for the lactose–casein and lactulose–casein system to see whether the main reaction compounds were identified (Fig. 3). The amounts of sugar degradation products have been expressed as percentage mol-C12 from the starting C12-disaccharide according to the following definition:

$$\text{mol Cn} = \frac{n}{12} \times \text{mol C12}$$

where Cn represents a sugar degradation product with n carbon atoms. The melanoidin concentration was calculated from the browning of the protein fraction as described in the Materials and Methods section. Less than 10% of the initial amount of sugar was not identified (see Fig. 3).

In Fig. 4, the results of lactose and lactulose heated in the absence of casein are shown. Comparing with the graphs from Fig. 1, these graphs show that the formation of sugars and formic acid (A and B) did not depend on the presence of casein. Browning (D), on the contrary, did depend on the presence of casein and was about three times lower when no casein was present.

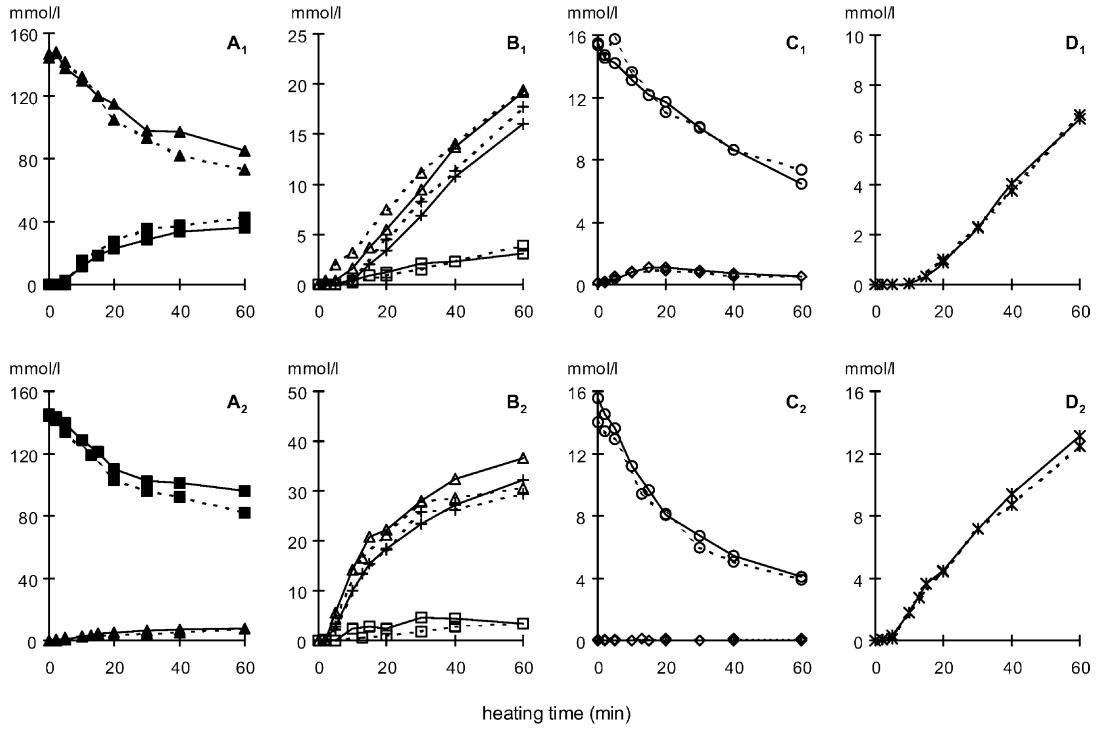


Fig. 1. Lactose–casein (solid lines) and maltose–casein (dotted lines) systems (top) and lactulose–casein (solid lines) and maltulose–casein (dotted lines) systems (bottom) heated at 120 °C. Lactose/maltose (▲), lactulose/maltulose (■), galactose/glucose (Δ), tagatose/fructose (□), formic acid (+), lysine residues (○), Amadori compound (◇), melanoidins (*).

Furthermore, it can be observed that the amounts of galactose and formic acid were much higher in the lactulose systems than in the lactose systems.

The results of the present study were compared with literature data. Berg and Van Boekel (1994; Van Boekel, 1998) studied the reactions of lactose in heated milk. They identified the same reaction compounds as in the present study in the lactose–casein system, except that they did not find tagatose. The presence of tagatose in heated milk was however described earlier by Troyano and co-workers (Troyano, Martinez Castro, & Olano, 1992). In milk, lower concentrations of all identified reaction compounds were formed and the decrease of reactants was also slower than in the lactose–casein model system that was studied in the present paper. This might be due to the phosphate buffer used in the present study. Except for a buffering effect, phosphate is reported to have a catalytic effect on the sugar-protein reactions (Bell, 1997).

3.2. Reaction network model

The reactions that will be described in the present section were put together into a model for lactose and lactulose reactions (see Fig. 5). Lactose and maltose can undergo two types of transformation: (1) the Lobry de Bruyn-Alberda van Ekenstein (LA) rearrangement and (2) the Maillard reaction (Troyano, Olano et al., 1992). Via the LA transformation lactose can isomerise into

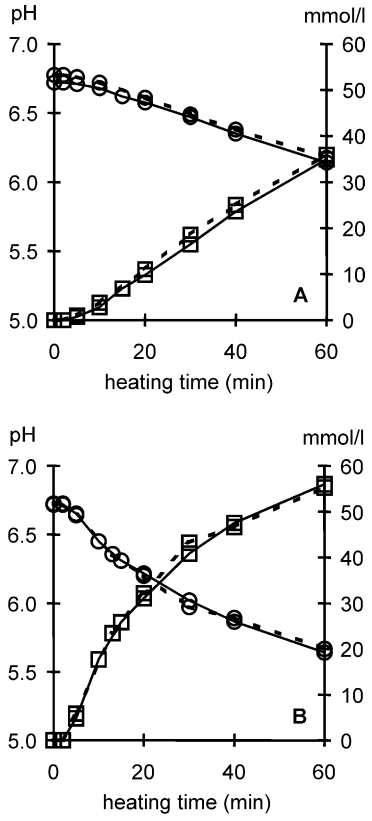


Fig. 2. pH (○) and total amount of acids as found by titration (□) in heated lactose–casein (solid line) and maltose–casein (dotted line) systems (A) and lactulose–casein (dotted lines) and maltulose–casein (solid lines) systems (B).

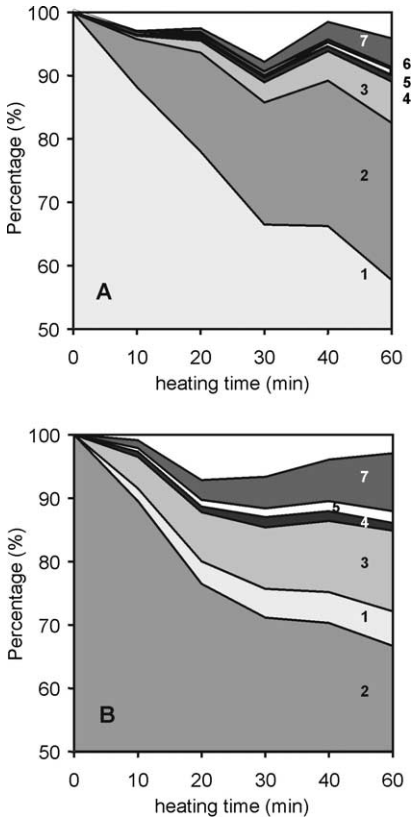


Fig. 3. Mass balance of reactants and reaction products in heated lactose–casein (A) and lactulose–casein (B) systems. Lactose (1), lactulose (2), galactose (3), tagatose (4), formic acid (5), Amadori compound (6) and melanoidins (7).

lactulose and maltose into maltulose. A 1,2-enediol anion is the key intermediate in this reaction, and is also considered to be the starting intermediate in degradation reactions. The 1,2-enediol anion can undergo β-elimination to yield 3-deoxyaldoketose. Neither the formation of this reactive dicarbonyl compound nor the further decomposition is affected by the presence of the glycosidic linked sugar (Pischetsrieder & Severin, 1996), which is easily split off. The 3-deoxyaldoketose can undergo α-dicarbonyl cleavage to yield formic acid and 2-deoxyribose, or it can undergo a retroaldolisation reaction to form glyoxal, or it can cyclise to form HMF. This 3-deoxyaldoketose reaction route is favoured under slightly acidic conditions. Under the conditions used in this study formation of HMF was therefore very low (0–10 μM).

In the Maillard reaction lactose and maltose can react with available amino groups of the protein to form an N-substituted glycosyl amine, which is in equilibrium with its 1,2-enaminol. This 1,2-enaminol can undergo the same reactions as the 1,2-enediol as described above. Furthermore, the 1,2-eneaminol can react to an aminoketose, the Amadori product, which is subject to further degradation via its 2,3-eneaminol. Hollnagel and Kroh (2000) proposed a peeling off mechanism for the formation of 1,4-deoxyglucosone from oligosaccharides, whereby the glycosidic residue at C4 and the amino residue at C1 are eliminated. 1,4-Deoxyglucosone can degrade into formic acid and 3-deoxypentulose (Holl-

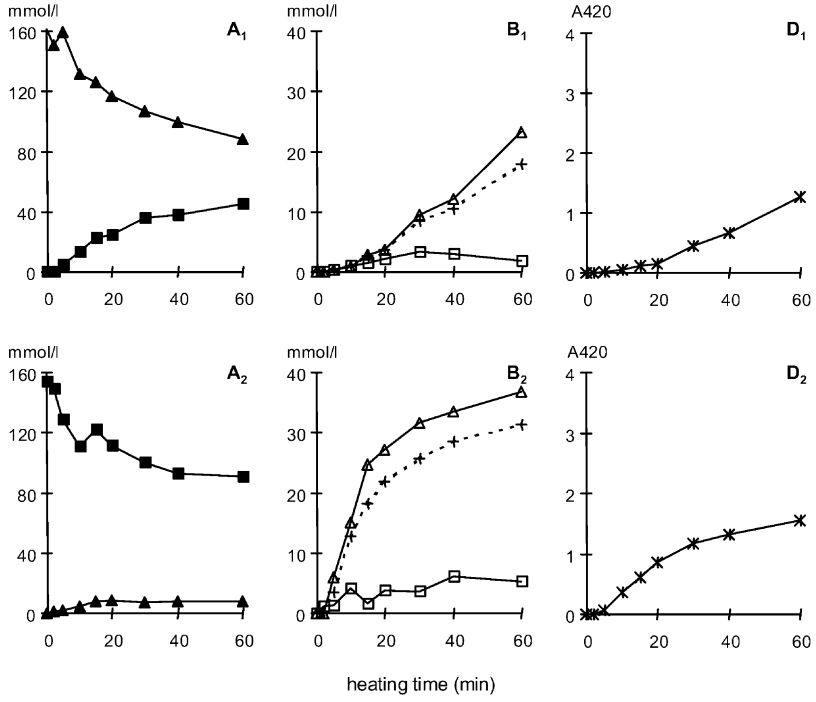


Fig. 4. Lactose solutions (top) and lactulose solutions (bottom) heated without casein at 120 °C. Lactose (▲), lactulose (■), galactose (Δ), tagatose (□), formic acid (+), absorbance at 420 nm (*).

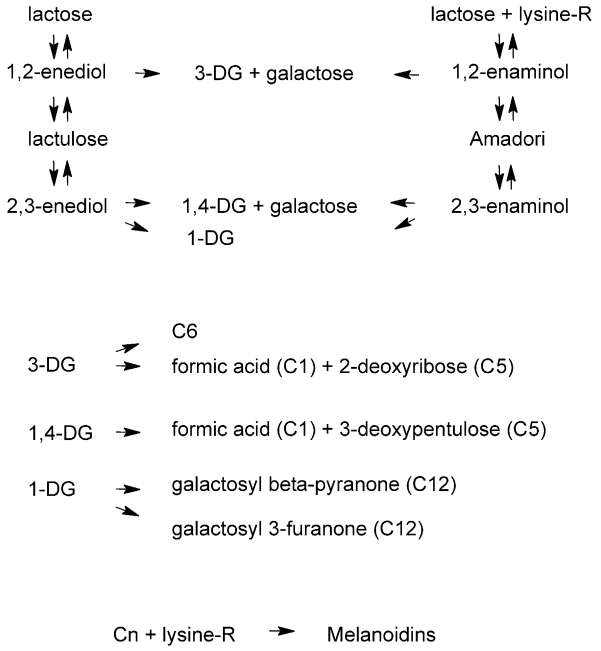


Fig. 5. Reaction network model for disaccharide–casein reactions (lysine-R: protein bound lysine residues; Cn: unidentified sugar degradation compound with n carbon atoms).

nagel & Kroh, 2000; Troyano, Olano et al., 1992). This reaction pathway is specific for di- and oligosaccharide sugars and therefore 3-deoxypentulose was never found in monosaccharide systems. The 2,3-eneaminol can also lead to the formation of 1-deoxyglucosones. Cyclisation and enolisation of 1-deoxyglucosone result in 5- or 6-membered rings. Starting from these intermediates, the degradation of lactose and maltose differs from that of glucose. For the case of glucose, the pyranoid product eliminates water to give a γ-pyranone and the furanoid dehydrates to a 4-furanone. This reaction is very difficult if a sugar is bound to position 4. Therefore, lactose and maltose favour pathways toward compounds that still possess the glycosidic substituent, such as glycosyl β-pyranone [4-(glycopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one] and glycosyl 3-furanone [4-(glycopyranosyloxy)-5-hydroxymethyl-2-methyl-3(2)-furanone] (Pellegrino, Noni, & Cattaneo, 2000; Pischetsrieder & Severin, 1996).

Lactulose and maltulose, both ketose sugars, can be transformed via the 1,2-enolisation route, but they can also degrade via their 2,3-enediol anion in the same way as described for the Amadori compound. From previous research it is known that ketoses themselves are not reactive in the Maillard reaction and that they can degrade into fragments without any involvement of an amino group (Brands & Van Boekel, 2001).

Galactose and glucose are formed via the 3-deoxyglucosone and 1,4-deoxyglucosone pathways starting from lactose or lactulose and maltose or maltulose, respectively, as the initial sugar. Galactose and glucose can react in the LA transformation and Maillard reaction as described previously (Brands & Van Boekel, 2002). The sugars tagatose and fructose were identified as the isomer sugars of galactose and glucose, respectively.

The sugar degradation products β-pyranone and 3-furanone (Pischetsrieder & Severin, 1996), 3-deoxypentulose (Troyano, Olano et al., 1992), 2-deoxyribose (Berg & Van Boekel, 1994) and fragmentation products with dicarbonyl structure (Hofmann, 1999) are not very stable. Sugar degradation products might easily condense with available amino groups and form brown-coloured melanoidins.

The reaction network model of Fig. 5 is quite complex but can be simplified for modelling purposes (see next section).

3.3. Kinetic modelling of lactose and lactulose reactions

The reaction network model of Fig. 5 was simplified to obtain a more workable kinetic model with less parameters. The degradation reactions via the 1,2-enediol or 1,2-eneaminol of the disaccharides were neglected. This reaction step is favoured under acidic conditions, whereas the initial pH of the systems used in this study is near neutral. The 3-deoxyglucosone route is not essential to explain the formation of certain reaction compounds, since, for instance, formic acid can also be formed via the 1,4-deoxyglucosone route. Furthermore, it can be shown that, assuming steady state behaviour of the enediols, the concentration of enediols is directly proportional to the sugar concentration (De Bruijn, 1986). Neglecting the reactions via galactose (except its isomerisation into tagatose) simplifies the model further (Scheme 1). The mechanism was translated into a mathematical model by setting up the following differential equations, based on the general rules and principles of chemical kinetics:

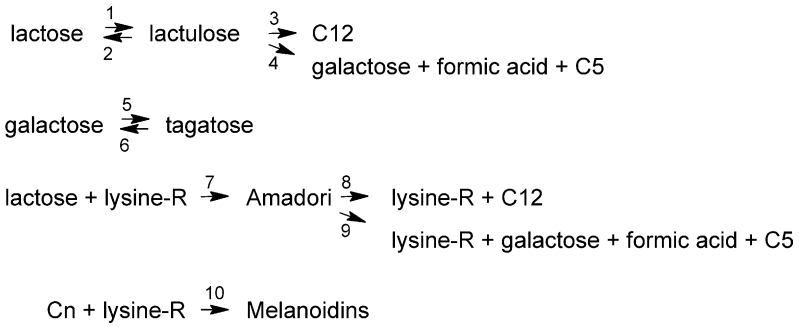
$$\frac{\mathrm{d[lac]}}{\mathrm{d}t} = -k_1[\mathrm{lac}] + k_2[\mathrm{lu}] - k_7[\mathrm{lac}][\mathrm{lys}]$$

$$\frac{\mathrm{d[lu]}}{\mathrm{d}t} = k_1[\mathrm{lac}] - k_2[\mathrm{lu}] - k_3[\mathrm{lu}] - k_4[\mathrm{lu}]$$

$$\frac{\mathrm{d[gal]}}{\mathrm{d}t} = k_4[\mathrm{lu}] - k_5[\mathrm{gal}] + k_6[\mathrm{tag}] + k_9[\mathrm{Ama}]$$

$$\frac{\mathrm{d[tag]}}{\mathrm{d}t} = k_5[\mathrm{gal}] - k_6[\mathrm{tag}]$$

$$\frac{\mathrm{d[formic]}}{\mathrm{d}t} = k_4[\mathrm{lu}] + k_9[\mathrm{Ama}]$$



Scheme 1. Kinetic model no. 1 for disaccharide–casein reactions.

$$\frac{d[lys]}{dt} = -k_7[lac][lys] + k_8[Ama] + k_9[Ama] - k_{10}[C_{12}] \times [lys] - k_{10}[C_5][lys]$$

$$\frac{d[Ama]}{dt} = k_7[lac][lys] - k_8[Ama] - k_9[Ama]$$

$$\frac{d[M]}{dt} = k_{10}[C_{12}][lys] + \frac{5}{12}k_{10}[C_5][lys]$$

$$\frac{d[C_{12}]}{dt} = k_3[lu] + k_8[Ama] - k_{10}[C_{12}][lys]$$

$$\frac{d[C_5]}{dt} = k_4[lu] + k_9[Ama] - k_{10}[C_5][lys]$$

These differential equations were solved by numeric integration and fitted to the data. Because the data of the lactose–casein and lactulose–casein system were fitted simultaneously, the number of responses and therefore also the number of differential equations had to be doubled. The results of the fits are shown in Fig. 6. For the clarity of the graphs the averages of the experimental data for every response are shown, but the actual modelling was done using all experimental data.

The model of Scheme 1 did not describe all reactions in the sugar–casein system very well (Fig. 6). In the lactose–casein system the decrease of lactose and its isomerisation into lactulose were described quite well (A_1). Also the formation of formic acid was well described (B_1). The formation of galactose, and therefore also that of tagatose, was underestimated by the model (B_1).

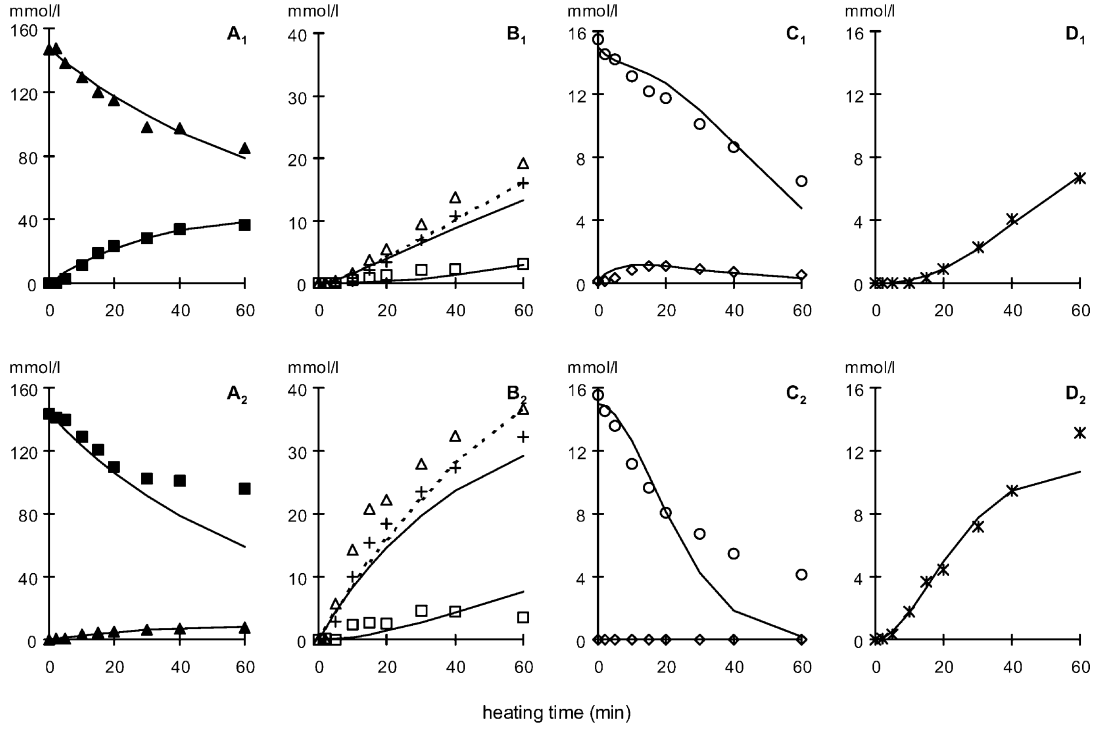


Fig. 6. Simulations (drawn lines) based on the kinetic model of Scheme 1 for lactose–casein and lactulose–casein systems heated at 120 °C. Lactose (▲), lactulose (γ), galactose (Δ), tagatose (□), formic acid (+), lysine-residues (○), Amadori compound (◇), melanoidins (*).

Whereas the Amadori compound (C_1) and the melanoidins (D_1) were described very well, the model underestimated the loss of lysine (C_1) at the beginning of heating and overestimated it at the end. In the lactulose–casein system, the isomerisation of lactulose into lactose was predicted very well, but the decrease of lactulose was strongly overestimated (A_2). Galactose was clearly underestimated and formic acid was initially underestimated, but overestimated at the end of heating (B_2). Like in the lactose system, loss of lysine was somewhat overestimated at the beginning of heating but strongly overestimated at the end (C_2). Since the predicted lysine concentration approaches zero, browning flattens off after about 40 min of heating (D_2). The lack of fit in the lactulose–casein system might partially be due to the very rapid pH drop, which is much faster than in the lactose–casein system (see Fig. 2). Since the pH has a big effect on the reaction rates and because the reaction rate constants were estimated using both the data of the lactose–casein system and the data of the lactulose–casein system simultaneously, it was aimed for to reduce the difference in pH decrease between the two systems.

To reduce the effect of difference in pH, a system with half the amount of lactulose (75 mM) was heated. It was observed that the decrease in pH in this system was very similar to the pH decrease in the lactose–casein system (only slightly higher). The data of these two model systems were modelled simultaneously (except for tagatose data which was not quantified). The fit for the lactose–casein system did not change (results not shown). The fits for lactulose and formic acid in the lactulose–casein system were indeed improved, but galactose formation was still underestimated (Fig. 7). The fit for lysine loss was not improved. The logarithm of the posterior probability was −11.550.

In a previous paper (Brands & Van Boekel, 2002), the effect of pH drop on the reactivity of lysine residues was described. During heating, the pH of the sugar–casein systems drops and due to protonation of the lysine residues, the reactivity of the protein decreases. The concentration of reactive lysine residues will decrease 10^1 times when the pH drops 1 unit. To improve the fit of the model to the data, the effect of pH on reactivity of lysine residues was taken into account in the differential equations. This was done by multiplying the lysine concentration with the factor $10^{-\Delta pH}$. The results are shown in Fig. 8. The logarithm of the posterior probability was increased to −9.587, which means that the overall fit of the model was noticeable improved. Although the loss of lysine was overestimated in the lactulose–casein system (C_2) and underestimated in the lactose–casein system (C_1), the estimation of the loss of lysine was improved. Since galactose (B_1) was still underestimated and lactulose loss (A_2) was overestimated by the model, the fit of the sugars will first be focused on.

Berg and Van Boekel (1994) developed a model in which lactulose and the Amadori compound react towards a C6 compound (splitting off galactose) in stead of a C12 compound (as depicted in Scheme 1). In an adapted scheme (Scheme 2), it was assumed that in stead of galactosyl 3-furanone and galactosyl β-pyranone unglycosylated furanones and pyranones were formed. This model was tested (results shown in Fig. 9) and it improved the fit for lactulose degradation in the lactulose–casein system (A_2) and the galactose fit in the lactose–casein system (B_1), but galactose formation in the lactulose system (B_2) was now strongly overestimated. The logarithm of the posterior probability was –11.273.

To improve the fit for galactose, the reactions of galactose were taken into account (Scheme 3). The reaction rate constants for the galactose reactions were estimated in a former paper (Brands & Van Boekel, 2002) and were used here as fixed values. The Maillard reaction of lactose was neglected in the lactulose–casein system and replaced by the reaction of galactose with

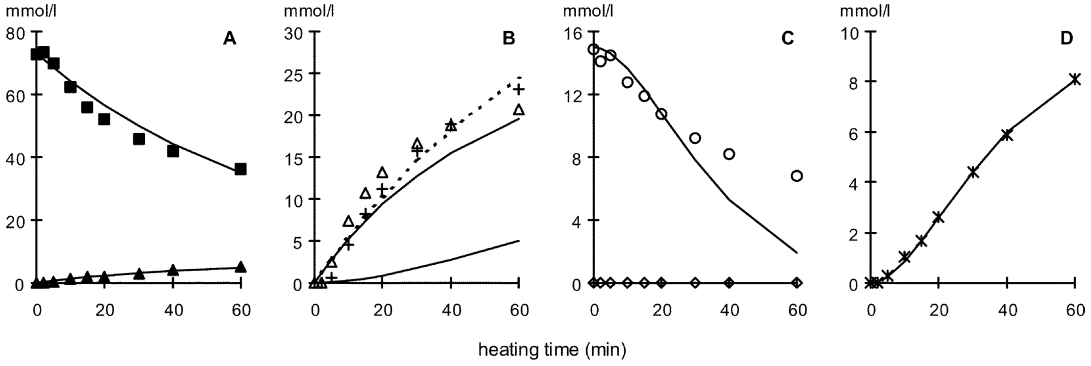


Fig. 7. Simulations (drawn lines) based on the kinetic model of Scheme 1 for lactulose–casein systems heated at 120 °C with an initial sugar concentration of 75 mM. Lactulose (γ), lactose (▲),galactose (Δ), tagatose (□), formic acid (+), lysine-residues (○), Amadori compound (◇), melanoidins (*).

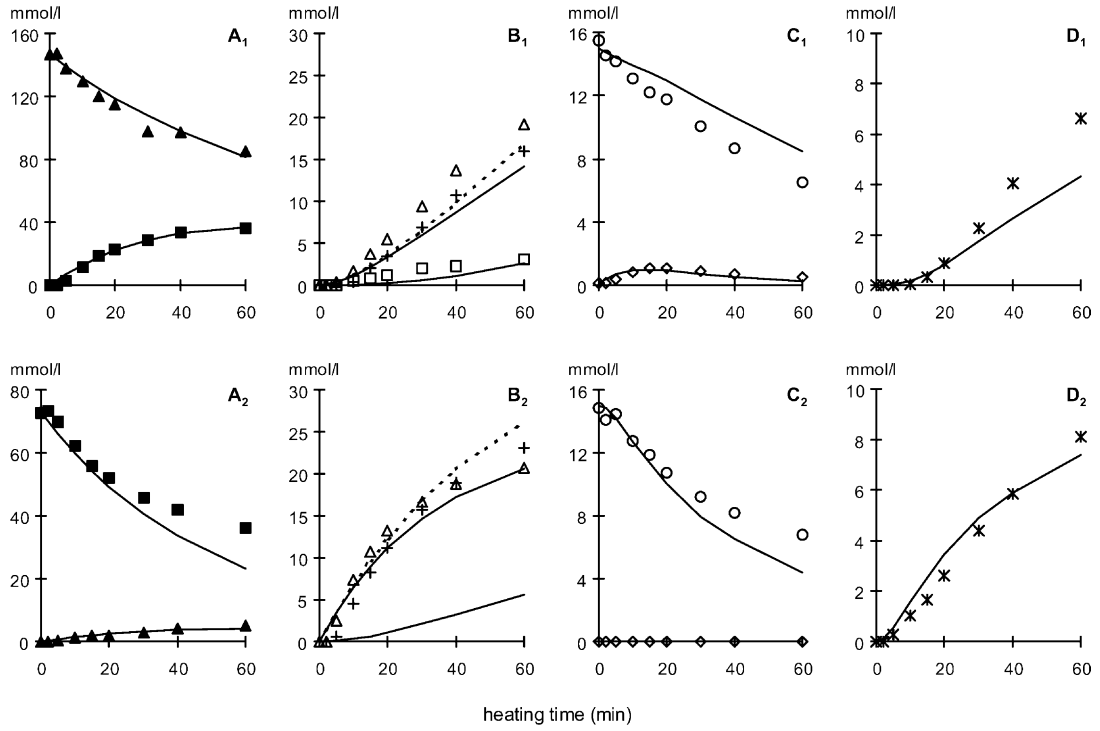
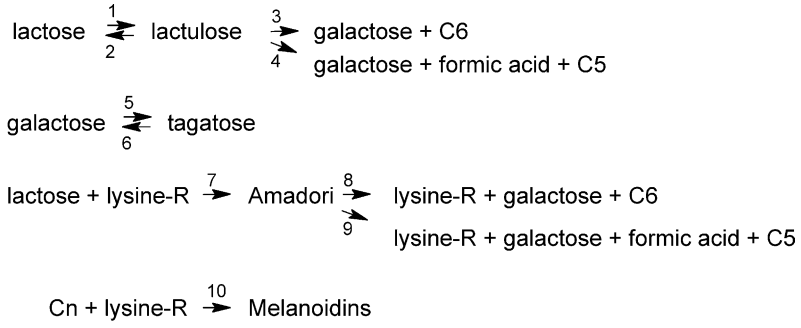


Fig. 8. Simulations (drawn lines) based on the kinetic model of Scheme 1, taking into account the effect of pH-decrease on the activity of lysine, for lactose–casein and lactulose–casein systems heated at 120 °C. Lactose (▲), lactulose (■), galactose (Δ), tagatose (□), formic acid (+), lysine-residues (○), Amadori compound (◇), melanoidins (*).



Scheme 2. Kinetic model no. 2 for disaccharide–casein reactions.

the lysine-residues of the protein. This adaptation improved the fit slightly (the logarithm of the posterior probability was −11.014): the formation of galactose tended to flatten off when heating proceeded, but was still estimated too high (results not shown). Apparently, the galactose concentration is estimated too high in the lactulose–casein system in order to predict enough galactose in the lactose–casein system. Therefore, the model was adapted once again.

The reaction via the 3-DG route was taken into account (Scheme 4). It is assumed that this route does not occur at neutral pH (Pischetsrieder & Severin, 1996), but since the pH decreases during heating it may become important. It was assumed that via this route galactose is split off and that another C6 component is formed. The results of the fit are shown in Fig. 10. The fit of the model to the data was indeed improved (the logarithm of the posterior probability was increased to −6.342). The rate of the reaction from Amadori to C6 compounds (k_8) approached zero. Still the fit of lysine (C) and browning (D) is not correct. Since the loss of lysine flattens off whereas browning keeps increasing, a reaction of some importance is probably neglected.

In a previous paper on monosaccharides (Brands & Van Boekel, 2002), this ongoing browning was explained by reaction of sugar fragments with guanidine groups of the arginine residues. Taking the reaction with arginine residues into account (Scheme 5) improves

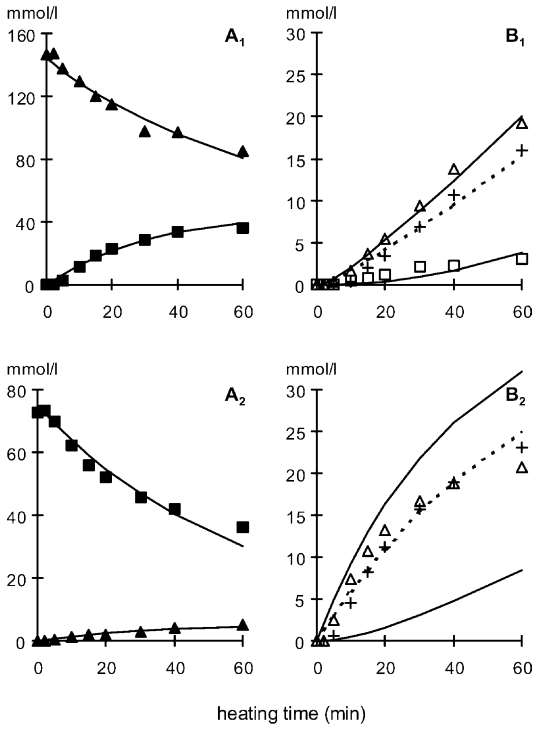


Fig. 9. Simulations (drawn lines) based on the kinetic model of Scheme 2 for lactose–casein and lactulose–casein systems heated at 120 °C. Lactose (▲), lactulose (■), galactose (Δ), tagatose (□), formic acid (+).

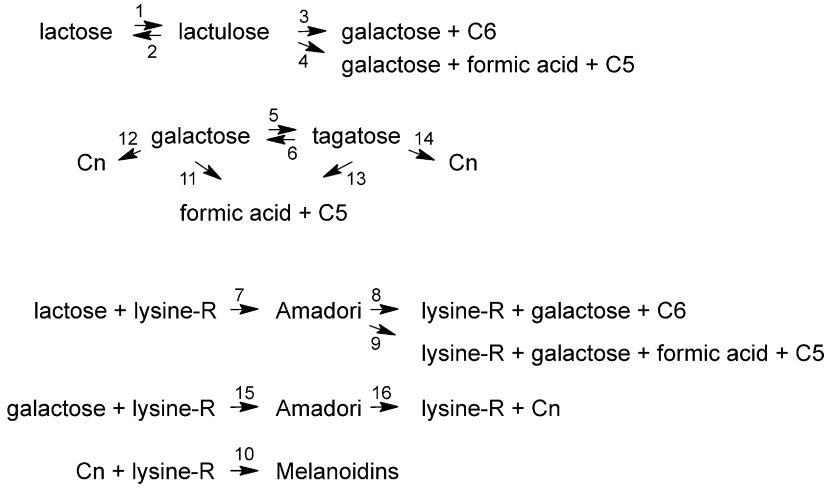
Table 1
Rate constants and their 95% highest posterior density intervals as found by kinetic modelling for the model displayed in Scheme 5 to the data of lactose–casein and lactulose–casein systems (120 °C; initial pH 6.8)

Rate constant	
k_1 (min^{-1})	0.00947±0.00054
k_2 (min^{-1})	0.00207±0.00012
k_3 (min^{-1})	0.00219±0.00079
k_4 (min^{-1})	0.00850±0.00062
k_5 (min^{-1})[a]	0.00992
k_6 (min^{-1})[a]	0.01559
k_7 (l $mmol^{-1}$ min^{-1})	0.00013±0.00003
k_8 (min^{-1})	0.00000
k_9 (min^{-1})	0.10478±0.02812
k_{10} (l $mmol^{-1}$ min^{-1})	0.00517±0.00051
k_{11} (min^{-1})[a]	0.00070
k_{12} (min^{-1})[a]	0.00322
k_{13} (min^{-1})[a]	0.00224
k_{14} (min^{-1})[a]	0.01517
k_{15} (l $mmol^{-1}$ min^{-1})[a]	0.00018
k_{16} (min^{-1})[a]	0.37200
k_{17} (min^{-1})	0.00123±0.00022
k_{18} (l $mmol^{-1}$ min^{-1})	0.00143±0.00017

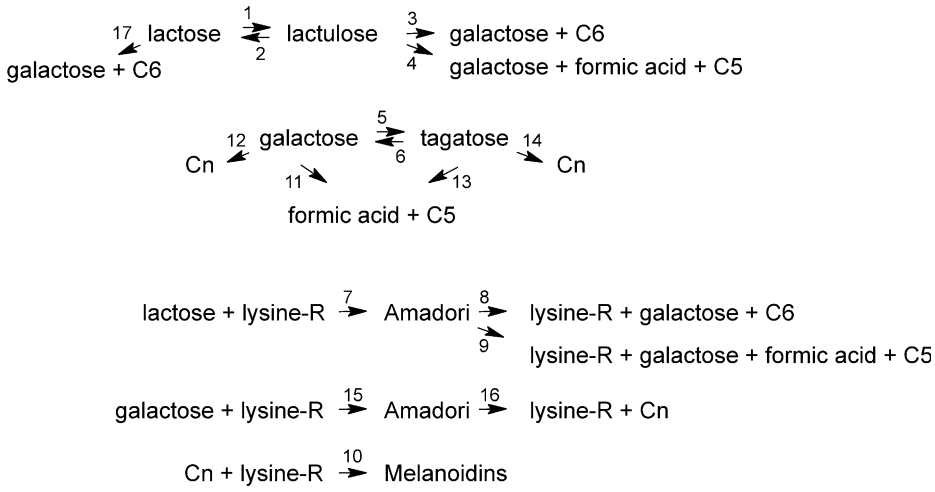
[a] Rate constants of the galactose reactions (Brands & Van Boekel, 2002) were used as fixed values.

the fit enormously, at least, visually (Fig. 11). According to goodness of fit criterion of Athena the fit is not improved: log posterior probability is −7.078. This is apparently due to the loss of degrees of freedom because more parameters are taken into account. The reaction rate constants (±95% highest posterior density intervals) are shown in Table 1.

If a kinetic model is consistent, the reaction rate constants should be independent of the concentration of the reactants. Therefore, the change in reactant concentration of lactose–casein systems that were heated with only half the initial sugar concentration was predicted using the reaction rate constants from Table 1. In Fig. 12, the simulations are shown. This model fits the data extremely well, thus supporting the validity of the model depicted in Scheme 5.



Scheme 3. Kinetic model no. 3 for disaccharide–casein reactions.



Scheme 4. Kinetic model no. 4 for disaccharide–casein reactions.

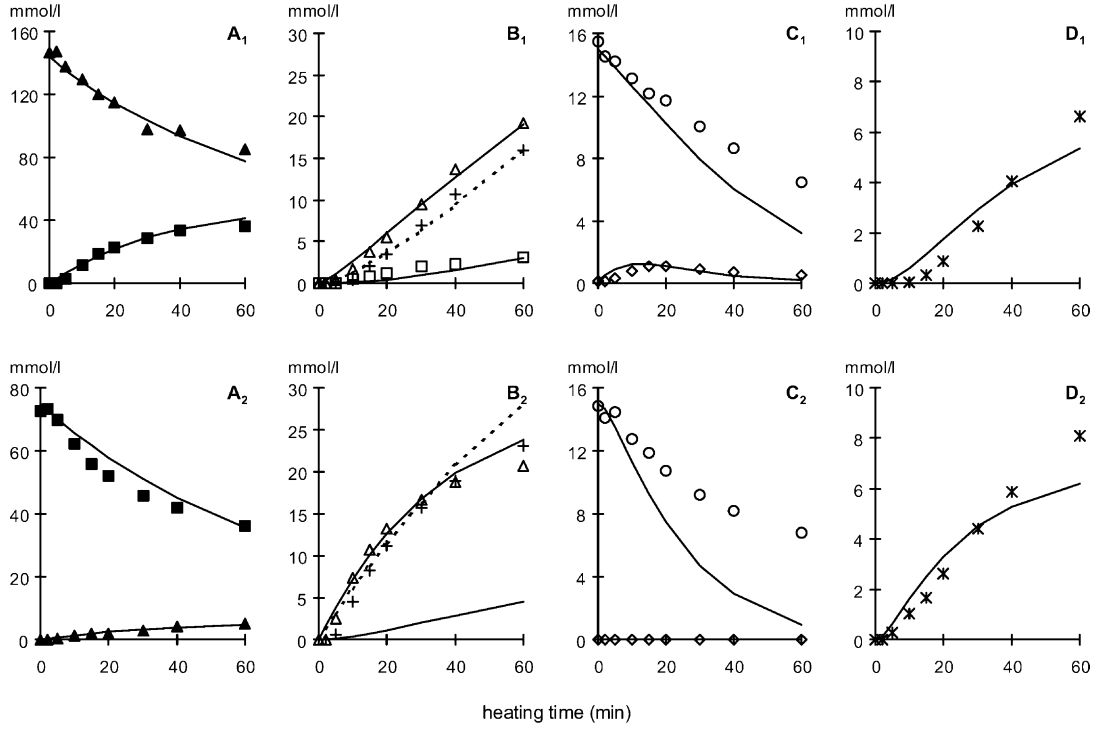
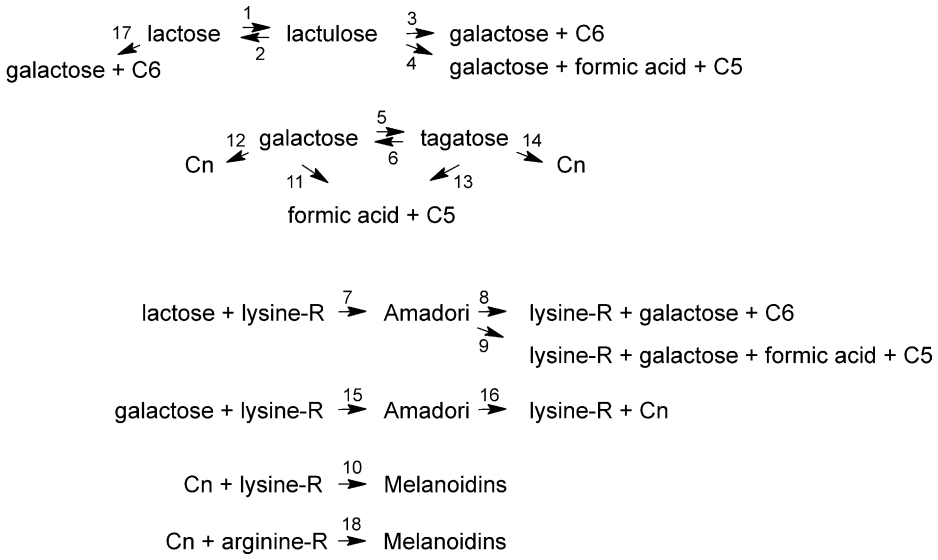


Fig. 10. Simulations (drawn lines) based on the kinetic model of Scheme 4 for lactose–casein and lactulose–casein systems heated at 120 °C. Lactose (▲), lactulose (■), galactose (Δ), tagatose (□), formic acid (+), lysine-residues (○), Amadori compound (◇), melanoidins (*).

3.4. Maltose and maltulose reactions

The reactions occurring in a maltose–casein and maltulose–casein system were simulated using the reaction rate constants of Table 1. In stead of the reaction rate constants of galactose the reactions rate constants of glucose, obtained from previous work (Brands & Van Boekel, 2002), were used to describe the reactions of the breakdown products of maltose. The results of the prediction are shown in Fig. 13. The model describes the reactions very good. Apparently it was not important which sugar, either glucose (like in maltose) or galactose (as in lactose), was glycosylated at the C4 position of the reducing part of the disaccharide sugar. The reactions in the maltulose reaction mixture were not very well described (results not



Scheme 5. Kinetic model no. 5 for disaccharide–casein reactions.

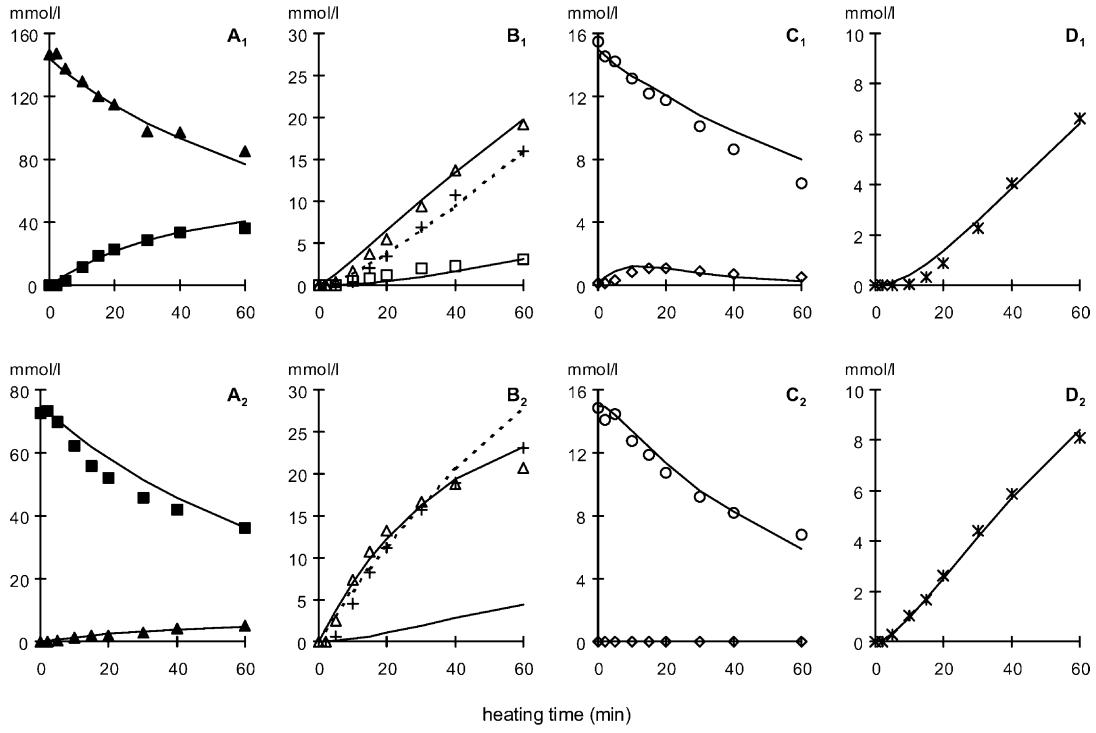


Fig. 11. Simulations (drawn lines) based on the kinetic model of Scheme 5 for lactose–casein and lactulose–casein systems heated at 120 °C. Lactose (▲), lactulose (■), galactose (Δ), tagatose (□), formic acid (+), lysine-residues (○), Amadori compound (◇), melanoidins (*).

shown) which was most likely due to the much faster pH decrease in that system.

4. Concluding remarks

In this paper, a reaction model was proposed and optimised for the reactions of the disaccharide sugars lactose and maltose and their ketose isomers in the presence of the protein casein at initial neutral pH and 120 °C. Comparing the kinetic model for disaccharide sugars with the one developed for monosaccharide reactions previously (Brands & Van Boekel, 2002), it can be concluded that some degradation reactions are hindered by the glycosidic linked sugar in disaccharides (formation of acetic acid since apparently no triose intermediates can be formed and formation of formic acid via the 3-DG route) but that other routes are favoured (formation of formic acid via 1,4-DG route). Kinetic modelling now approves the

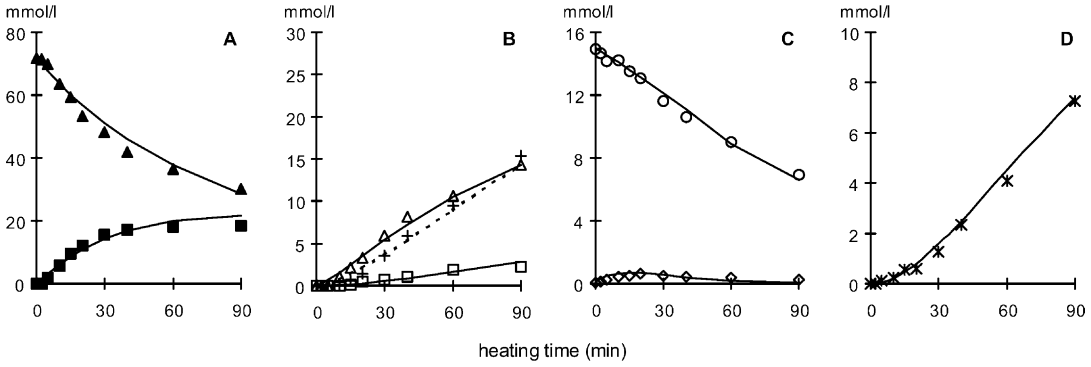


Fig. 12. Predictions (drawn lines) based on the reaction rate constants of Table 1 for lactose–casein systems heated at 120 °C with an initial sugar concentration of 75 mM. Lactose (▲), lactulose (■), galactose (Δ), tagatose (□), formic acid (+), lysine-residues (□), Amadori compound (◇), melanoidins (*).

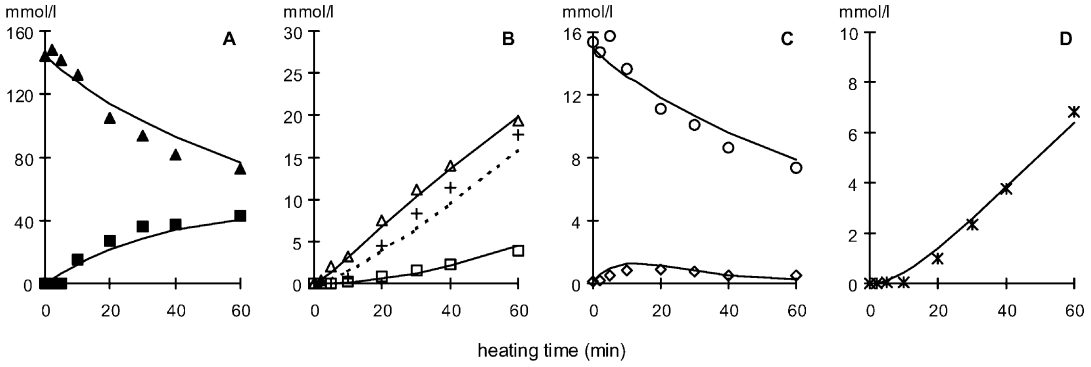


Fig. 13. Predictions (drawn lines) based on the reaction rate constants of Table 1 for maltose–casein systems heated at 120 °C. Maltose (▲), maltulose (■), glucose (Δ), fructose (□), formic acid (+), lysine-residues (○), Amadori compound (◇), melanoidins (*).

observation which was described in literature before (Pischetsrieder & Severin, 1996). The multiresponse modelling approach as used in this study appears to be a very powerful tool to unravel complicated reaction routes. The kinetic model derived in this study can be used to predict and optimise the quality of foods containing the disaccharides sugars maltose or lactose. For the reaction mechanism of the disaccharides it was not important whether galactose or glucose was glycosylated to the reducing end of the sugar.

Acknowledgements

We are grateful to Korina Pieters for performing some of the experimental work. The results reported in this paper are 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.

References

Bell, L. N. (1997). Maillard reaction as influenced by buffer type and concentration. *Food Chemistry*, *59*, 143–147.

Berg, H. E. (1993). *Reactions of lactose during heat treatment of milk. A quantitative study*. PhD dissertation, Wageningen Agricultural University, Wageningen, The Netherlands.

Berg, H. E., & Van Boekel, M. A. J. S. (1994). Degradation of lactose during heating of milk. I. Reaction pathways. *Netherlands Milk and Dairy Journal*, *48*, 157–175.

Brands, C. M. J., Alink, G. M., Van Boekel, M. A. J. S., & Jongen, W. M. F. (2000). Mutagenicity of heated sugar–casein systems: effect of the Maillard reaction. *Journal of Agricultural and Food Chemistry*, *48*, 2271–2275.

Brands, C. M. J., & Van Boekel, M. A. J. S. (2001). Reactions of monosaccharides during heating of sugar–casein systems: building of a reaction network model. *Journal of Agricultural and Food Chemistry*, *49*, 4667–4675.

Brands, C. M. J., & Van Boekel, M. A. J. S. (2002). Kinetic modeling of reactions in heated monosaccharide–casein systems. *Journal of Agricultural and Food Chemistry*, *50*, 6725–6739.

Brands, C. M. J., Wedzicha, B. L., & Van Boekel, M. A. J. S. (2002).

Quantification of melanoidin concentration in sugar–casein systems. *Journal of Agricultural and Food Chemistry*, *50*, 1178–1183.

De Bruijn, J. M. (1986). *Monosaccharides in alkaline medium: isomerisation, degradation, oligomerization*. PhD dissertation, University of Technology, Delft, The Netherlands.

Hofmann, T. (1999). Quantitative studies on the role of browning precursors in the Maillard reaction of pentoses and hexoses with L-alanine. *European Food Research and Technology*, *209*, 113–121.

Hollnagel, A., & Kroh, L. W. (2000). Degradation of oligosaccharides in nonenzymatic browning by formation of alpha-dicarbonyl compounds via a 'peeling off' mechanism. *Journal of Agricultural and Food Chemistry*, *48*, 6219–6226.

Kato, Y., Matsuda, T., Kato, N., & Nakamura, R. (1988). Browning and protein polymerization induced by amino-carbonyl reaction of ovalbumin with glucose and lactose. *Journal of Agricultural and Food Chemistry*, *36*, 806–809.

Kramhöller, B., Pischetsrieder, M., & Severin, T. (1993). Maillard reactions of lactose and maltose. *Journal of Agricultural and Food Chemistry*, *41*, 347–351.

Ledl, F. (1990). Chemical pathways of the Maillard reaction. In P. A. Finot, H. U. Aeschbacher, R. F. Hurrell, & R. Liardon (Eds.), *The Maillard reaction in food processing, human nutrition and physiology*. Basel, Switzerland: Birkhäuser Verlag.

Lignert, H., & Hall, G. (1986). Formation of antioxidative Maillard reaction products during food processing. In M. Fujimaki, M. Namiki, & E. Kato (Eds.), *Amino-carbonyl reactions in food and biological systems*. Tokyo, Japan: Elsevier.

Pellegrino, L., Noni, I. D., & Cattaneo, S. (2000). Formation of protein bound lysine-derived galactosyl and glucosyl pyrroles in heated model systems. *Nahrung*, *44*, 193–200.

Pellegrino, L., Van Boekel, M. A. J. S., Gruppen, H., Resmini, P., & Pagani, M. A. (1999). Heat-induced aggregation and covalent linkages in beta-casein model systems. *International Dairy Journal*, *9*, 255–260.

Pischetsrieder, M., & Severin, T. (1996). Advanced Maillard products of disaccharides: analysis and relation to reaction conditions. In T. C. Lee, & H. J. Kim (Eds.), *Chemical markers for processed and stored foods*. Washington, USA: American Chemical Society.

Stewart, W. E., Caracotsios, M., & Sørensen, J. P. (1992). Parameter estimation from multiresponse data. *AIChE Journal*, *38*, 641–650.

Stewart, W. E., Shon, Y., & Box, G. E. P. (1998). Discrimination and goodness of fit of multiresponse mechanistic models. *AIChE Journal*, *44*, 1404–1412.

Troyano, E., Martinez Castro, I., & Olano, A. (1992). Kinetics of galactose and tagatose formation during heat-treatment of milk. *Food Chemistry*, *45*, 41–43.

Troyano, E., Olano, A., Jimeno, M. L., Sanz, J., & Martinez Castro, I. (1992). Isolation and characterization of 3-deoxypentulose and its determination in heated milk. *Journal of Dairy Research*, *59*, 507–515.

Van Boekel, M. A. J. S. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry*, *62*, 403–414.

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