

A review of Maillard reaction in food and implications to kinetic modelling

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This paper reviews some of the research designed to lead to an increased understanding of the chemistry of the Maillard reaction, based on recent developments, and its influence on food properties like colour, flavour and nutritional value. A critical analysis is given on how quality attributes associated with Maillard reaction can be predicted and controlled by kinetic modelling. Multiresponse modelling (taking more than one reactant and product into consideration in the modelling process) is a powerful tool to model complicated consecutive and parallel reactions, like the Maillard reaction. Such a multiresponse approach provides a major guidance in understanding the reaction mechanism. An illustrative example is given. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

For as long as food has been cooked, the Maillard reaction has played an important role in improving the

appearance and taste of foods. It has been a central and major challenge in food industry, since the Maillard reaction is related to aroma, taste and colour, in particular in traditional processes such as the roasting of coffee and cocoa beans, the baking of bread and cakes, the toasting of cereals and the cooking of meat. Moreover, during the Maillard reaction a wide range of reaction products is formed with significant importance for the nutritional value of foods. This can be reduced by decrease of digestibility and possibly formation of toxic and mutagenic compounds, but can also be improved by the formation of antioxidative products.

The chemistry underlying the Maillard reaction is very complex. It encompasses not one reaction pathway but a whole network of various reactions. The original comprehensive reaction scheme of Hodge [1] has been developed and elaborated by food technologists ever since, so the understanding of the reaction is advancing steadily. Nevertheless the Maillard reaction is notoriously difficult to control. Various factors involved in food processing influence it and they can be considered as food processing variables. The kinetic approach tends to present a complementing view of this mechanism, because it considers the rate-determining steps of the reaction. It is powerful because rate-determining steps provide control points.

This paper discusses a research approach designed to increase the understanding of (a) the chemistry of the reaction and its influence on food properties like colour, flavour and nutritional value, and (b) how quality attributes associated with Maillard reaction, can be predicted and controlled by kinetic modelling.

Chemistry of the reaction

The Maillard reaction has been named after the French chemist Louis Maillard [2] who first described it but it was only in 1953 that the first coherent scheme was put forward by Hodge [1] (Fig. 1). In essence, it states that in an early stage, a reducing sugar, like glucose, condenses with a compound possessing a free amino group (of an amino acid or in proteins mainly the ϵ -amino group of lysine, but also the α -amino groups of terminal amino acids) to give a condensation product N-substituted glycosilamine, which rearranges to form the Amadori rearrangement product (ARP). The subsequent degradation of the Amadori product is dependent on the pH of the system. At pH 7 or below, it

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undergoes mainly 1,2-enolisation with the formation of furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved). At $\text{pH} > 7$ the degradation of the Amadori compound is thought to involve mainly 2,3 enolisation, where reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one (HMF^{one}), and a variety of fission products, including acetol, pyruvaldehyde and diacetyl are formed. All these compounds are highly reactive and take part in further reactions. Carbonyl groups can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. Dicarbonyl compounds will react with amino acids with the formation of aldehydes and α -aminoketones. This reaction is known as the Strecker degradation. Subsequently, in an advanced stage, a range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations, which ultimately, in a final stage, lead to the formation of brown nitrogenous polymers and co-polymers, known as melanoidins.

The complexity and the variety of the Maillard reaction products has, throughout the years, raised the interest of scientists in different fields of research [3–9]. New important pathways, not accounted for by Hodge [1], have been established. McWeeny *et al.* [10] reported that the most important intermediates in colour formation are 3-deoxyosuloses and 3,4-dideoxyosulos-3-enes, which in the case of glucose is 3-deoxyhexosulose (DH) and 3,4-dideoxyhexosuloses-3-ene (DDH). Later, Ghiron *et al.* [11] stated that 3-deoxy-2-hexosuloses, 1-deoxy-2,3-hexodiuloses and other α -dicarbonyl intermediates can undergo nucleophilic addition reactions with amino acids with subsequent decarboxylation to produce the “so-called” Strecker aldehyde ($\text{RHC}=\text{O}$). In 1990, Huber and Ledl [12] isolated and characterised 1-deoxy- and 3-deoxyglucosones from heated Amadori products. More recently, in agreement with the previous reports, Tressl *et al.* [13] using ^{13}C -labeled sugars, have given a new perspective to the reaction mechanism (Fig. 2). It involves different reaction pathways, in which the key intermediates are the 1-, 3- and 4-deoxyhexosuloses.

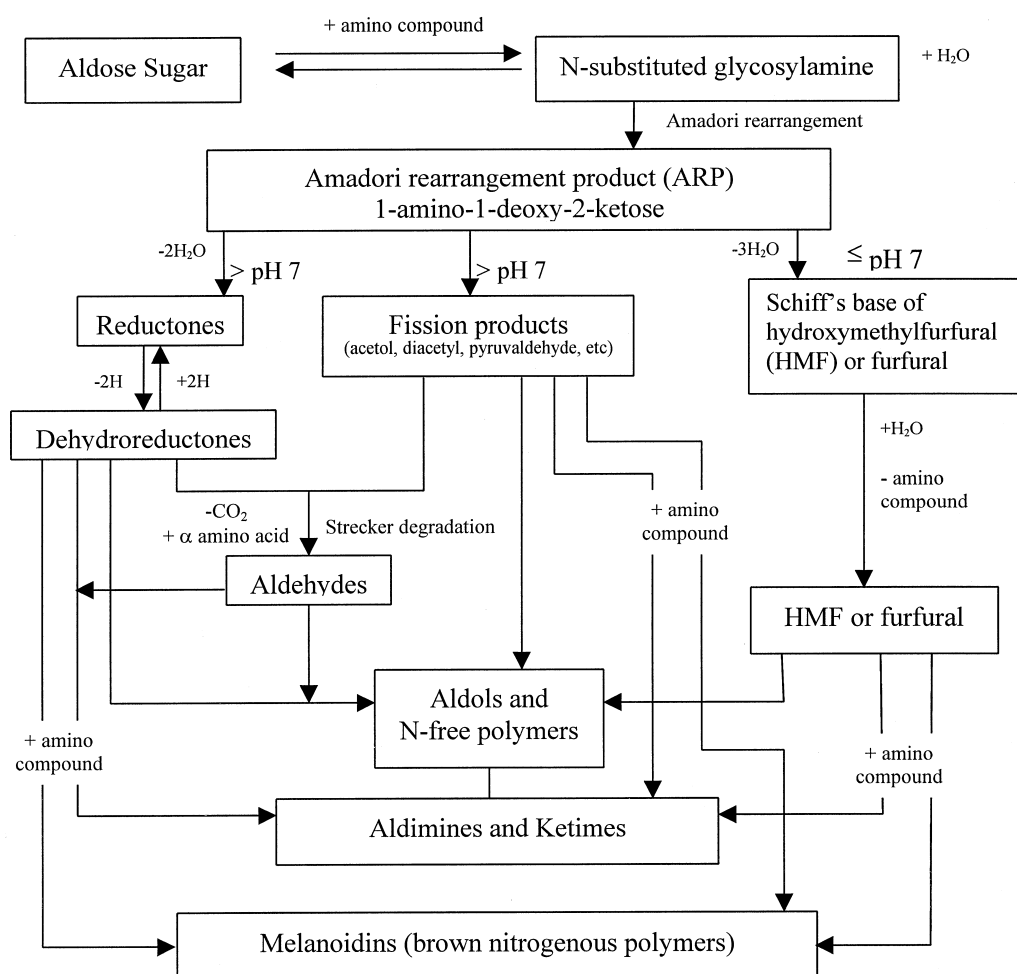


Figure 1. Maillard reaction scheme adapted from Hodge [1].

Moreover, a major influence of the pH is expressed. Along with enolization reactions, the Amadori product and its dicarbonyl derivatives can undergo concurrently retro-aldol reactions producing more reactive C₂, C₃, C₄ and C₅ sugar fragments, such as hydroxyacetone derivatives, glyceraldehyde and diketones. Retro-aldol reactions become more important at higher pH values. Also, Yaylayan and Huyghues-Despointes [14] stated that under basic conditions ARP could generate acetic acid and pyruvaldehyde and other lower sugars in addition to free amino acid. As a result, high pH is suggested to be the main pathway to flavour formation. In addition to retro-aldol reactions, three redox mechanisms have been identified, in which α -hydroxy carbonyls, α -dicarbonyls and formic acid are involved. Berg and Van Boekel [15] reported formic acid as a main degradation reaction product for the Maillard reaction of lactose. Also, Van Boekel and Brands [16] reported formic acid and acetic acid as two main degradation products for the Maillard reaction of glucose and fructose. In line with Tressl's perspective, Yaylayan [17] proposed a classification for the Maillard reaction named the 'chemical pools'. This approach is based on the observation that during the Maillard reaction the amino acids and the sugars also undergo independent degradation, in addition to the conventional degradation where Amadori product is formed.

Berg [18] concluded that isomerization and degradation reactions of the sugar are, from a quantitative point of view, more important than the Maillard reaction, for conditions as in heated milk. Finally, the central importance of the Amadori product, formerly supposed to be the main intermediate of the reaction, has been questioned in both food [19] and medical fields [20]. In spite of all the work that has been done, the mechanism of the Maillard reaction is still a controversial issue.

Influence of Maillard reaction products on food properties

The origin of volatile compounds responsible for flavour is still relatively difficult to determine, due to their multiple origin. The interest shown by food industry stems from a desire to produce and control the characteristic aromas and colours obtained on cooking, baking and roasting. Once the analytical technique of combined gas-chromatography-mass spectrometry was developed for the separation and identification of relatively volatile substances, the search for compounds with specific odours was greatly intensified. The results have been summarised in a series of review articles [21–24]. Like in brown colour formation, it is clear that both quantity and quality depend on the precursors, thermal processing parameters, pH, and quantitative ratio of amino nitrogen to reducing sugar. For example, Lane

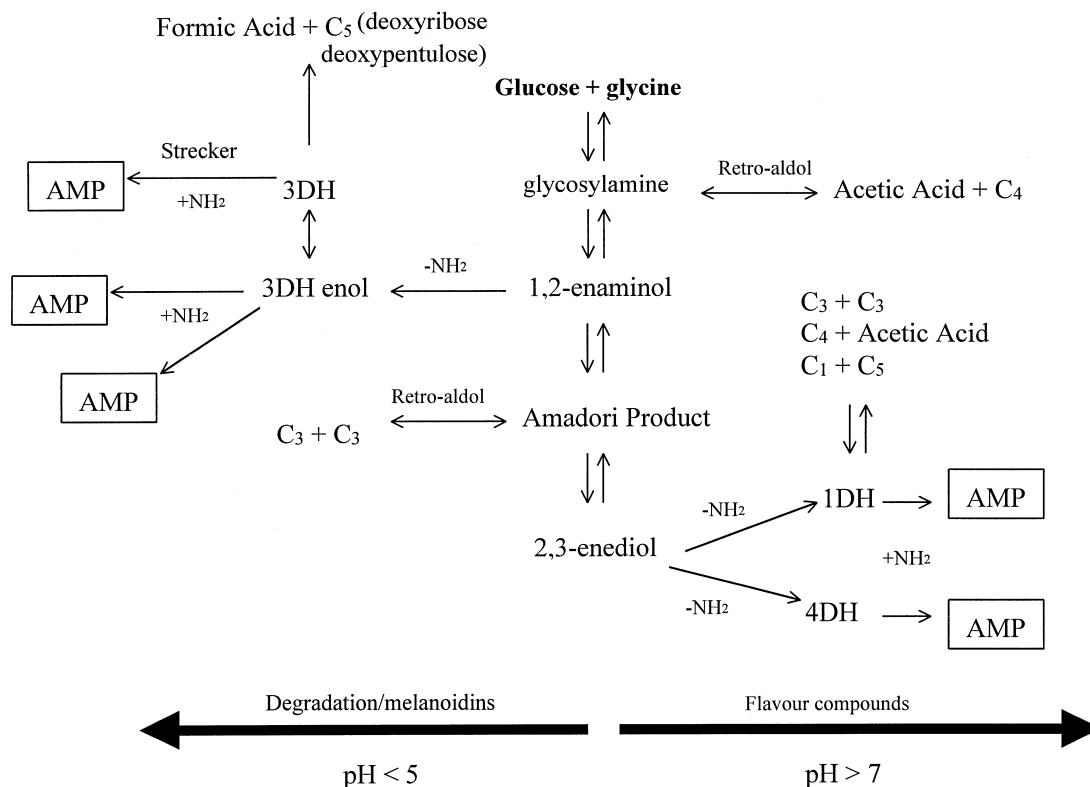


Figure 2. Scheme glucose/glycine Maillard reaction adapted from Tressl *et al.* [13]. AMP (Advanced Maillard Products); 1-DH (1-deoxy-2,3-diketose); 3-DH (3-deoxyaldoketose); 4-DH (4-deoxy-2,3-diketose).

and Nursten [25] reported a thorough study on odours produced in Maillard reaction systems. They identified 12 amino acids, five to seven of which they thought to produce bread, crusty biscuits, cake or toast aroma at each of the four temperatures studied, using single amino acid/glucose combinations at different temperatures. Also, Fors [26] published a literature review of the sensory properties of volatile Maillard products and related compounds. It includes qualitative aroma and flavour descriptions and sensory threshold values for various compounds, classified according to the chemical structure. More recently Teranishi *et al.* [27] have reviewed the thermal generation of Maillard aroma.

From the fact that 'we also eat with our eyes', the significance of Maillard browning in processed foods, in consumer acceptance is obvious. The degree of browning (usually measured via absorbance at 420 nm) is often used analytically to assess the extent to which the Maillard reaction has taken place in foods. Nevertheless, it has been stated that fluorescent compounds are formed prior to brown compounds [28]. In the final stage of the reaction, coloured intermediates and other reactive precursors (enaminol products, low-molecular-weight sugar analogues, unsaturated carbonyl products) condense and polymerise to form brown polymers, under acceleration by an amine catalyst. Some of their known properties are brown, high molecular weight, furan ring-containing and nitrogen-containing polymers; they may contain carbonyl, carboxyl, amine, amide, pyrrole, indole, azomethine, ester, anhydride, ether, methyl and/or hydroxyl groups [29–31]. Studies on melanoidins formation have been summarized in different review articles [32–35]. The isolation and identification of coloured Maillard products has so far been achieved only with model systems, mostly for low molecular weight (<500 Da) products. Hashiba [36] concluded that browning was directly proportional to the reducing power of the sugar and to the amounts of glycine consumed, by comparing different sugars with one single amino acid. However, more recently, Rizzi [35] stated that many coloured products appear to be (retro)aldolization/dehydration products of sugars which may or may not be attached to proteins or other sources of amino nitrogen. Also Hofmann [37] using dosage/activity relationship combined with chemical/instrumental techniques and visual/sensory measurements, identified carbohydrate degradation products as browning precursors (deoxyosones, glyoxal, methylglyoxal, hydroxy-2-propanone, 3-hydroxy-2-butanone and glycoaldehyde), which is in agreement with Tressl *et al.*'s [13] scheme and demonstrated that their activity in producing browning substances changes during thermal treatment. So far, only partial structures of melanoidins have been elucidated. The origin and actual chemical species responsible for it remain largely undefined. Further investigations are needed.

One of the most obvious negative consequences of the Maillard reaction in food is the loss of nutritive value of proteins involved, with a loss of quality and a possible decrease of food safety. In various studies [3,33,34] this loss was attributed to decrease of digestibility, destruction and/or biological inactivation of amino acids, including essential amino acids like lysine and tryptophan, inhibition of proteolytic and glycolytic enzymes, and interaction with metal ions. Also, protein molecules can be crosslinked by Maillard reaction products [38,39]. Moreover, the loss of nutritive value has also been associated with the formation of mutagenic compounds. Nagao *et al.* [40] identified mutagenic compounds in instant and caffeine-free coffee. They consisted of dicarbonyl compounds, methylglyoxal, diacetyl and glyoxal, from which the methylglyoxal presented highest mutagenic activity; however, no quantitative correlation with carcinogenic properties was found. Also in both fried and grilled meat and fish, mutagenic compounds were identified, mainly stemming from heterocyclic amines [41]. However, flavones and flavonoids have been reported as inhibitors of the heterocyclic amine-type mutagens [42] being defined as desmutagens. In the Maillard reaction, desmutagenic effects have also been reported [43,44]. The reaction mechanism seems to have a major influence in the mutagenicity of the reaction products. For instance, ketose sugars showed a higher mutagenic activity than the corresponding aldose sugars [45]. With respect to food safety, the involvement of the Maillard reaction in the formation and elimination of mutagens, is a matter that still needs to be elucidated. Up to date no reports correlated these compounds with human cancer.

Finally, the Maillard reaction has been shown to produce antioxidative components as well. One of the first observations was reported by Griffith and Johnson [46], who demonstrated that the addition of 5% glucose to sugar cookies produced a marked browning in the cookies and resulted in a greater stability to oxidative rancidity. Since then, reaction products from various amino compounds and sugars were studied with regard to antioxidative properties, able to protect food against lipid oxidation [47].

Accordingly, many food quality aspects of the Maillard reaction, some desirable, others undesirable, should be taken into consideration in a food processing operation. The task of the food technologist is then to optimise by finding the best balance between the favourable and unfavourable effects of the reaction in a given process. It could be a question of minimising the nutritional losses while obtaining an optimal flavour production when roasting cereals; of maximising the antioxidant production while minimising flavour and colour production in milk drying, etc. Understanding the Maillard reaction is therefore an added value not only for the traditional processes of roasting, baking and cooking

but also for the development of new technologies, like microwave and high pressure technology [48,49].

Role of kinetic modelling

Quality is a very elusive concept, which depends on many factors. The production management view is to maintain quality during production. For each stage of the production process, specific quality criteria are used to monitor and control that production stage [50]. In a similar way, in a food technologist concept, quality is the result of the ability to control chemical, physical and microbiological changes during processing and storage. For this, kinetic modelling is gaining increasing interest in different fields of research [51]. It has been applied to food microbiology, with the development of mathematical models that describe how microorganisms behave in foods, referred to as predictive food microbiology [52]. It can also be applied to chemical changes in food, for example colour as a function of time and temperature. If the rate and temperature dependence of a reaction is known, its occurrence can, in principle, be predicted and therefore controlled. Moreover, it can also help in understanding the chemistry and mechanism of the reaction.

Basic principles

Van Boekel and Walstra [53] have given a detailed explanation on the use of kinetics in food applications. Based on the general rate law, the disappearance of a compound (in a closed system with only one compound reacting) is:

$$-\frac{d[A]}{dt} = k[A]^n \quad (1)$$

in which the decrease in concentration of component A over time t is related to the concentration of that component, where k is the reaction rate constant and n (usually $0 \leq n \leq 2$) the reaction order. By integration of the differential equation to a chosen order, with respect to time, a zero-order reaction would be:

$$[A] = [A]_0 - kt \quad (2)$$

a first-order reaction would be:

$$[A] = [A]_0 \exp(-kt) \quad (3)$$

and a second-order reaction would be:

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt \quad (4)$$

In literature, most works consider a first order, though zero and second order are also common. This approach, however, is only valid for simple reactions.

The order of a reaction is a parameter that gives a mathematical description of time- or concentration-dependence, it does not necessarily give information on the reaction mechanism. This approach is quite suitable for modelling shelf-life or for instance enzyme inactivation during the process, but less useful for understanding chemical changes and mechanisms. In a complex reaction like Maillard reaction, one should be aware that an observed rate constant reflects a combination of elementary rate constants. If we attempt to unravel and explain a particular reaction in more detail, we should propose a reaction mechanism. This is another approach to kinetic modelling. In the case that we are able to analyse and model more than one component simultaneously, this approach is called Multi-response Modelling [54]. The following steps should be taken into account:

- Identify the most important reactants and products and calculate the mass balance
- Identify species which are co-products of the same reaction pathway
- Differentiate between primary and secondary reaction routes
- Identify the influence of critical process parameters (pH, temperature, etc.)
- Determine the effect of reactant concentrations
- Propose a model mechanism for the reaction network based on elementary reaction-steps
- Test the hypothesised mechanism

Kinetic modelling in Maillard reaction, an illustrative example

As mentioned in Chemistry of the reaction, above, in the last years many analytical techniques have been developed in order to identify important intermediates in the Maillard reaction. The formation and degradation pathways are of major importance for kinetic modelling.

Reaction routes

In our research group, we attempt to follow the various reaction steps as closely as possible, to take into account as many responses as possible at once, by analysing the reactants degradation, sugar isomers, the Amadori product and its degradation products and then apply the multiresponse modelling technique, as opposed to one response. From the Maillard reaction of a glucose/glycine model system, heated at pH 7, we identified as primary reactions:

1. Isomerization of glucose via 1,2-enolization. Isomerisation occurs via the Lobry de Bruyn–Alberda van Ekenstein transformation [55,56]. Depending on the temperature, this step becomes more evident (Fig. 3).

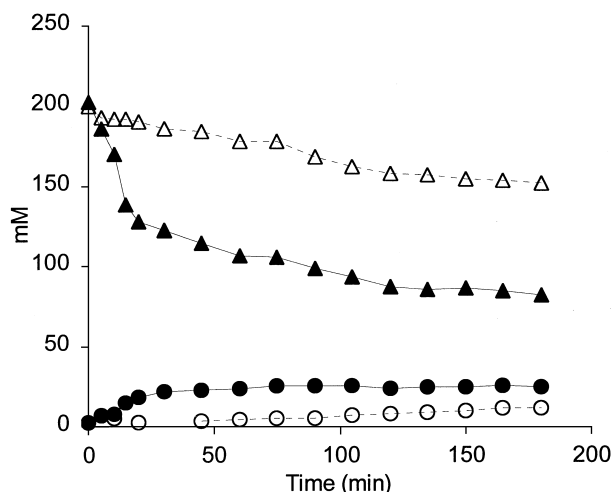


Figure 3. Isomerisation of glucose into fructose in glucose/glycine model system (200 mM, in phosphate buffer 0.1M, pH 6.8) when heated at 90°C (broken line) and 120°C (continuous line) (Δ glucose, 90°C; \blacktriangle glucose, 120°C; \circ fructose, 90°C; \bullet fructose, 120°C).

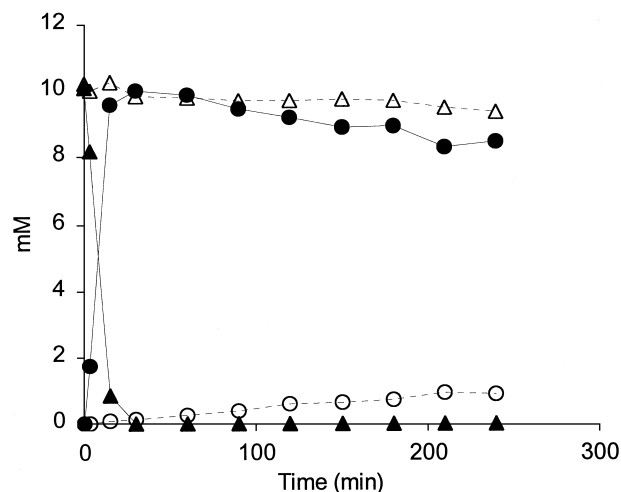


Figure 4. Glycine regeneration from Amadori decomposition when heated at 90°C (broken line) and 120°C (continuous line) (Δ Amadori, 90°C; \blacktriangle Amadori, 120°C; \circ glycine, 90°C; \bullet glycine, 120°C).

- Degradation of sugars into organic acids, namely formic acid, and C_5 fragments, designed as intermediate Maillard products, not easily experimentally accessible. Possible C_5 fragments are 2-deoxyribose [18] and 3-deoxypentulose [57]. Formic acid is supposedly formed via the 1,2-enediol by C_1 – C_2 cleavage [55].
- The Maillard reaction between sugars and amino groups leading to the so-called Amadori product, followed by its degradation into breakdown products, from which currently only acetic acid was identified. However, 1-deoxy and 3-deoxyglucosones are likely key intermediates of this pathway [13,37].
- The complete regeneration of glycine from the Amadori product. From studies with Amadori product (fructosylamine) heated at pH 7, on its own, at different temperatures, when the degradation of Amadori product starts, the regeneration of glycine is very prominent (Fig. 4).
- The interaction of breakdown products with amino groups into advanced Maillard products, such as colour compounds, known as melanoidines. The colour (measured as absorption A at 420 nm) is related to the melanoidins concentration, by the molar extinction coefficient E and can be expressed in terms of the number of glucose molecules incorporated, as described by Wedzicha and co-workers [58,59]. In this way, colour can be linked quantitatively to sugar losses.

As secondary reactions were identified:

- formation of hydroxymethylfurfural (not significant here because of the neutral pH range);

- isomerisation of glucose via 2,3-enolization (acetic acid is believed to be a degradation product of 2,3-enediol by C_2 – C_3 cleavage [55]);
- the Maillard reaction of fructose formed via isomerisation (not significant because of the low amount formed compared to glucose).

The big advantage of this approach is that the initial, intermediate and final stages become linked in this way, as well as the common intermediates stemming from sugar reactions, and this allows the interpretation of the quantitative importance of the different reaction paths.

Influence of critical process parameters

Various product and processing variables influence the Maillard reaction [60,61]. Temperature and pH are believed to play a crucial role. By manipulating these variables, the balance of the various chemical pathways making up the Maillard reaction changes.

Influence of temperature

Temperature and duration of heating were studied by Maillard [2] himself, who reported that the rate of the reaction increases with temperature. Many workers have confirmed this observation [7,9]. An increase in temperature leads to an increase of the reactivity between the sugar and the amino group. The temperature dependence of a reaction rate constant k is often described by the well-known Arrhenius equation:

$$k = A \cdot \exp\left(-\frac{E_a}{RT}\right) \quad (5)$$

where k is the rate constant; A the so-called frequency factor; E_a the activation energy; R the gas constant ($8.3 \text{ Jmol}^{-1} \text{ K}^{-1}$) and T is the absolute temperature (K).

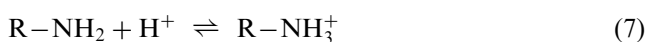
However, it can also be described by the transition-state theory developed by Eyring:

$$k = \frac{k_B T}{h} \exp\left(-\frac{\Delta G^\ddagger}{RT}\right) = \frac{k_B T}{h} \exp\left(-\frac{\Delta H^\ddagger}{RT}\right) \exp\left(\frac{\Delta S^\ddagger}{R}\right) \quad (6)$$

where k_B is Boltzmann's constant ($1.4 \times 10^{-23} \text{ JK}^{-1}$); h is Planck's constant ($6.6 \times 10^{-34} \text{ Js}$); R is the gas constant ($8.3 \text{ J mol}^{-1} \text{ K}^{-1}$); T is the absolute temperature (K); ΔH^\ddagger is the activation enthalpy (J mol^{-1}); ΔS^\ddagger is the activation entropy ($\text{J mol}^{-1} \text{ K}^{-1}$); ΔG^\ddagger is the activation Gibbs energy (J mol^{-1}). The relationship between rate constant and temperature is frequently taken by the Arrhenius equation. Still, it is an over simplification [53]. In the fundamental Eyring relation, the reaction rates are determined by changes in activation entropy and enthalpy. Based on experimental data these parameters can be determined and the influence of temperature on the reactants reactivity modelled. It is therefore preferable to use the Eyring equation when studying more fundamental reaction kinetics, since not only an interpretation of the reaction rate data in terms of thermodynamic properties is considered, but also an insight on the reaction mechanism is given.

Influence of pH

Just as for temperature, the reactivity of the sugar and amino group is also highly influenced by the pH. The open chain form of the sugar and the unprotonated form of the amino group, considered to be the reactive forms, are favoured at higher pH. Studying the two reactants separately, the following equilibrium can be written for the amino group:



The lower the pH, the more protonated amino group is present in the equilibrium and therefore, less reactive with the sugar. This equilibrium is dependent on the pH and the pKa of the amino group. The pKa of an amino group is defined as the pH value where 50% of the amino group is protonated. The pKa for the amine group in glycine is 9.6. Fig. 5 shows the effect of pH on the amount of glycine available in the unprotonated form. The percentage of unprotonated amino group at room temperature and pH 7 is less than 1% and it decreases with lower pH values. Its dependence on the temperature is not yet known, however its pH dependence can be estimated by the plotted trend. In many articles the amino group effective concentration is considered to be equal to the total concentration. This assumption could be valid for high pH values, but not for neutral/acid conditions. A thorough study is still needed.

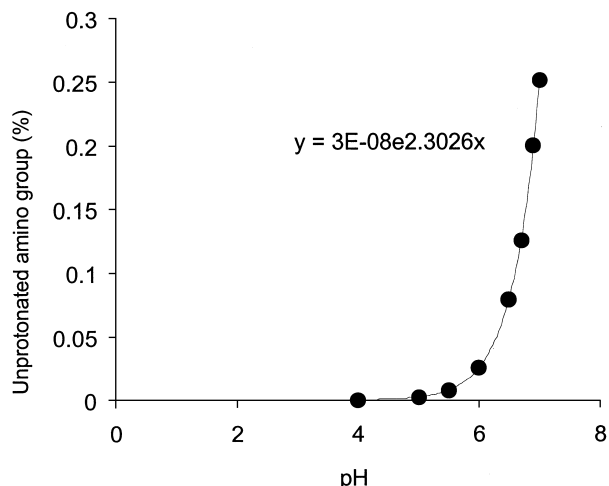


Figure 5. Effect of pH on the effective concentration of the amino group of glycine.

As for the other reactant, a similar approach can be used. The reducing sugar (ring form) in solution is in equilibrium with its open chain form, ionised form, enediol anion and isomers [55,62]. When we look to the isomerisation step, the equilibrium is established through the enediol anion, from the ionisation of the sugar. The pH dependence for isomerisation can be illustrated using the general model of Fig. 6. As the ionisation of the sugar in alkaline medium is faster with respect to subsequent enediol anion formation it can be deduced [55] that :

$$\frac{-d[S_t]}{dt} = A \times \frac{[\text{OH}^-]}{\frac{k_w}{k_{as}} + [\text{OH}^-]} [S_t] \quad (8)$$

where the constant A comprises the elementary rate constants relating enolisation and isomerisation equilibrium, k_w is the water constant and k_{as} the sugar alkaline degradation constant. It follows from De Bruin [55] that the concentration of the enediol is proportional to the sugar concentration, and therefore the enediol anion can be eliminated. The then pseudo first order rate constant includes the OH^- concentration and is dependent on the enolisation rate constant. A quantitative relation is thus given in eqn (8), expressing the dependence of sugar disappearance on pH, as far as isomerisation is concerned. For the reaction with the

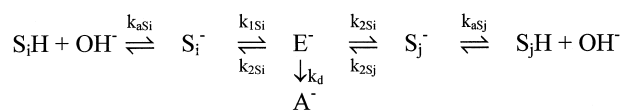
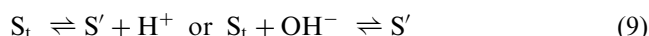


Figure 6. General model for alkaline isomerisation and degradation of monosaccharides [55]. S_iH and S_jH : monosaccharides, S_i^- and S_j^- : monosaccharides anions, E^- : enediol anion, A^- : acidic degradation products.

amino group, the open chain form (S'), which is in equilibrium with the ring form, is required:



The proportion between the sugar concentration and the open ring remains unknown. Taking the same line of thinking as for the derivation of eqn. (8), different assumptions should be taken into account. If the equilibrium in eqns (7) and (9) is very fast (high pH), the effective concentration is directly proportional to the total concentration. However, this is still a controversial issue. According to Namiki [33], the maximum condensation rate, involving aldoses and amines, occurs when the product of the concentrations [$>C=O$][RNH_2] is maximum. It is believed that the condensation reaction is initiated by an attack of a nucleophilic amino nitrogen, with an unshared electron pair, on the carbonyl carbon. Protonation of the carbonyl group should enhance its reactivity to the nucleophilic reagent. The rate of condensation would therefore, reach a maximum at weakly acidic pH.

This dependence of reactivity of both principal reactants on pH explains qualitatively the dependence of the Maillard reaction on pH. A thorough study is still needed and we are currently working on it.

Numerical and statistical procedure

After proposing a reaction mechanism, where the influence of critical process parameters (like pH and temperature) is identified, a model can be built by setting up differential equations for every step in the reaction network. To clarify this, Fig. 7 shows the kinetic model employed by us, adapted from Martins *et al.* [63]. The coupled ordinary differential equations (ODEs) describe the degradation/formation of the intermediates over time. For the glucose degradation, for example, we can write:

$$\frac{dS'}{dt} = -k_1[S'] + k_2[SI'] - k_3[S'] - k_5[S'][A] \quad (10)$$

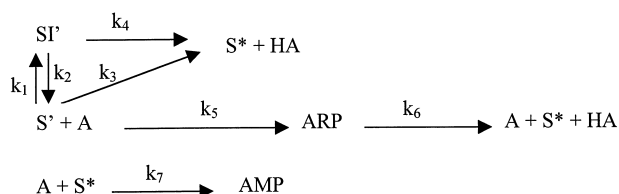


Figure 7. Kinetic model used to describe the parallel interaction of sugar isomerisation/degradation and Maillard reaction. S' : open chain form of the sugar, SI' : open chain form of sugar isomers, S^* : reactive sugar fragments, HA organic acids formed (acetic + formic acid), ARP: Amadori product, AMP: advanced Maillard products (melanoidins among others) adapted from Martins *et al.* [63].

This can be done for each component and the ODEs can then be solved by numerical integration. Several algorithms are available and they should be able to handle stiff differential equations [64].

Once the model (i.e. the integrated rate equations) is proposed, it should be fitted to the experimental data. In literature, the fit criterion is not always clearly indicated. The usual procedure seems to be the method of least squares minimization. In the above example, there are several responses at the same time (the concentrations of components S' , SI' , S^* , A, HA, ARP, AMP at each time interval studied). As mentioned by Van Boeckel [54] there are several, rather strict, requirements for application of least squares, in particular in the case of multiresponse modelling. It turns out that for cases of multiresponse modelling the fit criterion to be used depends on the experimental error structure of the data. The variance-covariance matrix of the experimental errors is of importance. The least-squares minimisation can be attempted but the variance of each response must be known, which is not always possible. In this case, the best-fit criterion is the minimisation of the determinant of the matrix of cross-products of the various responses, so-called dispersion matrix. As a result, maximum likelihood estimate of the parameters would be obtained, even if the variance-covariance matrix is unknown. Moreover, if a fit seems adequate, this means that there is consistence between the data and the model. On the other hand if it appears to be bad, the model is obviously wrong and should be adjusted. This approach of numerical integration followed by fitting to the data is flexible and powerful because changing relevant differential equations can easily test different models. The quality of experimental data is therefore very important, in particular for the validity of the studied parameters.

Often, various mechanistic models can be generated. Alternative models may be formed from a candidate model by adding or deleting parameters. Criteria to identify a preferred model and assess its adequacy are desired. Questions like “which model is most probable according to the data?” or, “do any of the models represent the data adequately?” can arise. Stewart *et al.* [65] addressed this problem for models of a multi-response analysis. If more models seem to fit adequately we can search for model discrimination by Bayesian analysis (probability share). Judgement of the performance of the model: scrutiny of the residuals is recommended along with one of the multivariate tests of goodness of fit. This test is installed in a software package named Athena Visual Workbench (www.athena.com). This goodness-of-fit test gives a sampling probability by which the adequacy of a model can be judged and then the probability function used as provided in the software package to see if the chosen model is good enough. A small value of this probability (0.01

or less) cast doubts on the hypotheses, whereas a value nearer to 1.0 supports it. The software package also offers several statistical indicators that allow judgement on the identifiability of parameters. For instance, a large confidence interval and high correlation coefficients may indicate that parameters are redundant (or that the data do not contain enough information to allow estimation).

Conclusion

The Maillard reaction is a cascade of consecutive and parallel reaction steps, whose complexity has been illustrated. It is of utmost importance for the food technologist to be able to control the extent of the Maillard reaction. For that reason, kinetic data are needed. The kinetic approach tends to present a much simpler view of the mechanism, because it is based only on the rate-determining steps of the reaction. It is powerful because rate-determining steps provide control points. The important requirement is to be able to identify these steps correctly and, when such knowledge is based on formation or loss of characterised intermediates, the multiresponse kinetics approach becomes more fundamental than the traditional global zero-, first or second-order approach. It is both helpful for deriving relevant kinetic parameters as well as for obtaining insight into reaction mechanisms. If a model is not consistent with the data, a new model can be proposed and easily tested by computer simulation. The only requirement is software that is capable of numerical integration of differential equations and the application of the appropriate statistical methods for multiresponse modelling. Such software is now available (www.athenavizual.com) and we are currently applying this to the Maillard reaction of glucose and glycine.

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References

- Hodge, J.E. (1953) 'Chemistry of Browning Reactions in Model Systems' in *J. Agric. Food Chem.* 1, 928–943
- Maillard, L.-C. (1912) 'Action Des Acides Aminés Sur Les Sucres. Formation Des Melanoidins Par Voie Methodique' in *Compt. Rend.* 154, 66–68
- Ericksson, C., ed (1981) 'Maillard Reaction in Food: Chemical, Physiological and Technological Aspects' in *Progress in Food and Nutrition Science* 5, Pergamon Press
- Waller, G.R. and Feather, M.S. (eds) (1983) *The Maillard Reaction in Foods and Nutrition*, ACS Symp. Ser. 215, Washington, DC
- Fujimaki, M., Namiki, M. and Kato, H. (1986) 'Amino-carbonyl Reactions in Foods and Biological Systems, Developments in Food Science 13', Elsevier, Amsterdam
- Finot, P.A., Aeschbacher, H.U., Hurrell, R.F. and Liardon, R. (1990) 'The Maillard Reaction in Food Processing, Human Nutrition and Physiology', Birkäuser Verlag, Basel, Switzerland
- Labuza, T.P., Reineccius, G.A., Monnier, V.M., O'Brien, J. and Baynes, J.W. (1994) 'Maillard Reactions in Chemistry, Food and Health', Royal Society of Chemistry, Cambridge, UK
- Ikan, R. (1990) 'The Maillard Reaction: Consequences for the Chemical and Life Sciences', John Wiley and sons Ltd, Chichester, UK
- O'Brien, J., Nursten, H.E., Crabbe, M.J.C. and Ames, J.M. (1998) 'The Maillard Reaction in Foods and Medicine', Royal Society of Chemistry, Cambridge, UK
- McWeeny, D.J., Knowels, M.E. and Hearne, J.F. (1974) 'The Chemistry of Non-enzymic Browning and Its Control by Sulphites' in *J. Sci. Food Agric.* 25, 735–746
- Ghiron, A.F., Quack, B., Mahinney, T.P. and Feather, M.S. (1988) 'Studies on the Role of 3-deoxy-D-erythro-glucosulose (3-glucosone) in Nonenzymatic Browning. Evidence for Involvement in a Strecker Degradation' in *J. Agric. Food Chem.* 36, 677
- Huber, B. and Ledl, F. (1990) 'Formation of 1-amino-1,4-dideoxy-2,3-hexodiulose and 2-aminoacetylfurans in the Maillard Reaction' in *Carbohydrate Res.* 204, 215–220
- Tressl, R., Nittka, C. and Kersten, E. (1995) 'Formation of Isoleucine-specific Maillard Products from [1-¹³C]-D-glucose and [1-¹³C]-D-fructose' in *J. Agric. Food Chem.* 43, 1163–1169
- Yaylayan, V.A. and Huyghues-Despointes, A. (1996) 'Retrospective Studies with Various Labeled D-[¹³C] Glucose' in *J. Agric. Food Chem.* 44, 672–681
- Berg, H.E. and Van Boekel, M.A.J.S. (1994) 'Degradation of Lactose During Heating of Milk. Reactions Pathways' in *Netherlands Milk and Dairy Journal* 48, 157–175
- Van Boekel, M.A.J.S. and Brands, C. (1998) 'Heating of Sugar-casein Solutions: Isomerization and Maillard Reactions' in *The Maillard Reaction in Foods and Medicine* (O'Brien, J., Nursten, H.E., Crabbe, M.J.C., Ames, J.M., eds), pp. 154–158, Royal Society of Chemistry, Cambridge, UK
- Yaylayan, V.A. (1997) 'Classification of the Maillard Reaction: A Conceptual Approach' in *Trends Food Sci. Technol.* 8, 13–18
- Berg, H.E. (1993) *Reactions of Lactose During Heat Treatment of Milk: A Quantitative Study*, PhD thesis, Wageningen University, NL
- Molero-Vilchez, M.D. and Wedzicha, B.L. (1997) 'A New Approach to Study the Significance of Amadori Compounds in the Maillard Reaction' in *Food Chem.* 58, 249–254
- Fu, M.X., Wells-Knecht, K.J., Blackledge, J.A., Lyons, T.J., Thorpe, S.R. and Baynes, J.W. (1994) 'Glycation, Glycooxidation, and Cross-linking of Collagen by Glucose: Kinetics, Mechanisms, and Inhibition of Late Stages of the Maillard Reaction' in *Diabetes* 43, 676–683
- Buckholz, L.L. (1988) 'The Role of Maillard Technology in Flavoring Food Products' in *Cereal Foods World* 33, 547–551
- Ho, C.T. (1996) 'Thermal Generation of Maillard Aromas' in *The Maillard Reaction: Consequences for the Chemical and Life Sciences* (Ikan, R., ed), pp. 27–53, John Wiley and sons Ltd, Chichester, England
- Taylor, A.J. and Mottram, D.S. (1996) *Flavour in Science. Recent Developments*, Royal Society of Chemistry, Cambridge, UK
- Chuyen, N.V. (1998) 'Maillard Reaction and food processing. Application aspects' in *Processed-Induced Chemical Changes in Food* (Shahidi, F., Ho, C.-T., Chuyen, N. eds), pp. 213–235

- 25 Lane, M.J. and Nursten, H.E. (1983) 'Variety of odors produced in Maillard model systems and how they are influenced by reaction conditions' in *The Maillard Reaction in Foods and Nutrition*. ACS symp. series, 215, 141–158
- 26 Fors, S. (1983) 'Sensory properties of volatile Maillard reaction products and related compounds. A literature review.' in *The Maillard Reaction in Foods and Nutrition*. ACS symp. series, 215, 185–286
- 27 Teranishi, R., Wick, E.L. and Hornstein, I. (1999) *Flavour Chemistry. Thirty Years of Progress*, Kluwer Academic/Plenum Publishers, New York
- 28 Baisier, W.M. and Labuza, T.P. (1992) 'Maillard Browning Kinetics in a Liquid Model System' in *J. Agric. Food Chem.* 40, 707–713
- 29 Ledl, F. and Schleider, E. (1990) 'New Aspects of the Maillard Reaction in Foods and in the Human Body' in *Angew. Chem., Int. Ed. Engl.* 29, 565–594
- 30 Ames, J.M., Apriyantono, A. and Arnoldi, A. (1993) 'Low Molecular Weight Colored Compounds Formed from Xylose-lysine Model Systems' in *Food Chem.* 46, 121–127
- 31 Tressl, R., Wondrack, G.T., Garbe, L-A., Krüger, R-P. and Rewicki, D. (1998) 'Pentoses and Hexoses as Sources of New Melanoidin Like Maillard Polymers' in *J. Agric. Food Chem.* 46, 1765–1776
- 32 Feather, M.S. (1985) 'Some Aspects of the Chemistry of Non-enzymatic Browning (Maillard reaction)' in *Chemical Changes in Food during Processing*, pp. 289–303, AVI publ. Co
- 33 Namiki, H.E. (1988) 'Chemistry of Maillard Reactions: Recent Studies on the Browning Reaction Mechanism and the Developments of Antioxidants and Mutagens' in *Adv. Food Research* 32, 115–184
- 34 Friedman, M. (1996) 'Food Browning and Its Prevention: An Overview' in *J. Agric. Food Chem.* 44, 631–653
- 35 Rizzi, G.P. (1997) 'Chemical Structure of Coloured Maillard Reactions Products' in *Food Reviews International* 13, 1–28
- 36 Hashiba, H. (1982) 'The Browning Reaction of Amadori Compounds Derived from Various Sugars' in *Agricultural and Biological Chemistry* 46, 547–548
- 37 Hoffman, T. (1999) 'Quantitative Studies on the Role of Browning Precursors in the Maillard Reaction of Pentoses and Hexoses with L-alanine' in *Eur. J. Food Technol.* 209, 113–121
- 38 Chuyen, N.V., Utsunomiya, N. and Kato, H. (1991) 'Nutritional and Physiological Effects of Casein Modified by Glucose Under Various Conditions on Growing and Adults Rats' in *Agric. Biol. Chem.* 55, 657–664
- 39 Pellegrino, L., Van Boekel, M.A.J.S., Gruppen, H., Resmini, P. and Pagani, M.A. (1999) 'Heat-induced Aggregation and Covalent Linkages in β -casein Model System' in *Int. Dairy J.* 9, 255–260
- 40 Nagao, M., Takahashi, Y., Yamanaka, H. and Sugimura, T. (1979) 'Mutagens in Coffee and Tea' in *Mutat. Res.* 68, 101–106
- 41 Arvidsson, P., Van Boekel, M.A.J.S., Skog, K. and Jagerstad, M. (1998) 'Formation of Mutagenic Maillard Reaction Products' in *The Maillard Reaction in Foods and Medicine* (O'Brien, J., Nursten, H.E., Crabbe, M.J.C., Ames, J.M., eds), pp. 219–224, Royal Society of Chemistry, Cambridge, UK
- 42 Lee, H., Jiaan, C.Y. and Tsai, S.J. (1992) 'Flavone Inhibits Mutagen Formation During Heating in a Glycine/creatinine/glucose Model System' in *Food Chem.* 45, 235–248
- 43 Yen, G.C., Tsai, L.C. and Lill, J.D. (1992) 'Antimutagenic Effect of Maillard Browning Products Obtained from Amino Acids and Sugars' in *Food and Chemical Toxicology* 30, 127–132
- 44 Lee, I.E., Chuyen, N.V., Hayase, F. and Kato, H. (1994) 'Desmutagenicity of Melanoidins Against Various Kinds of Mutagens and Activated Mutagens' in *Biosci, Biotechnol. Biochem.* 58, 21–23
- 45 Brands, M.J., Alink, G.M., Van Boekel, M.A.J.S. and Jongen, W.M.F. (2000) 'Mutagenicity of Heated Sugar-casein Systems: Effect of the Maillard Reaction' in *J. Agric. Food Sci.* 48, 2271–2275
- 46 Griffith, T. and Johnson, J.A. (1957) 'Relation of the Browning Reaction to Storage of Sugar Cookies' in *Cereal Chem.* 34, 159–169
- 47 Chuyen, N.V., Ijichi, K., Umetsu, H. and Moteki, K. (1998) 'Antioxidative Properties of Pproducts from Amino Acids Or Peptides in the Reaction with Glucose' in *Process-induced Chemical Changes in Food* (Shahidi, F., Ho, C-T., Van Chuyen, N., eds), pp. 201–235, Advances in Experimental Medicine and Biology 434, Plenum Press, New York
- 48 Ames, J.M. (1998) 'Applications of the Maillard Reaction in the Food Industry' in *Food Chem.* 62, 431–439
- 49 Shahidi, F., Ho, C-T. and Van Chuyen, N. (eds) (1998) *Process-Induced Chemical Changes in Food* in Advances in Experimental Medicine and Biology 434, Plenum Press, New York
- 50 Sloof, M., Tijskens, L.M.M. and Wilkinson, E.C. (1996) 'Concepts for Modelling the Quality of Perishable Products' in *Trends Food Sci. Technol.* 7, 165–171
- 51 Nicolai, B.M. and De Baerdemaeker, J. (eds) (1998) *Food Quality Modelling*, European Commission COST Action 915 'Consumer-oriented Quality Improvement of Fruit and Vegetable Products', Brussels, Belgium
- 52 Buchanan, R.L. (1993) 'Predictive Food Microbiology' in *Trends Food Sci. Technol.* 4, 6–11
- 53 Van Boekel, M.A.J.S. and Walstra, P. (1995) 'Use of Kinetics in Studying Heat-induced Changes in Foods' in *Heat-induced Changes in Milk*, 2nd ed (Fox, P.F., ed), pp. 22–50, IDF special issue 9501, International Dairy Federation, Brussels
- 54 Van Boekel, M.A.J.S. (1996) 'Statistical Aspects of Kinetic Modelling for Food Science Problems' in *J. Food Sci.* 61, 477–489
- 55 De Bruijn, J.M. (1986) *Monosaccharides in Alkaline Medium: Isomerization, Degradation, Oligomerization*, PhD Thesis, University of Technology, Delft
- 56 Van Boekel, M.A.J.S. (1998) 'Effect of Heating on Maillard Reactions in Milk' in *Food Chem.* 62, 403–414
- 57 Hollnagel, A. (2000) *Contribution to the Chemistry of Non-Enzymatic Browning of Oligomer Carbohydrates*, PhD Thesis, University of Berlin (in German).
- 58 Davies, C.G.A., Wedzicha, B.L. and Gillard, C. (1997) 'Kinetic Model of Glucose-glycine Reaction' in *Food Chem.* 60, 323–329
- 59 Leong, L.P. (1999) *Modelling the Maillard Reaction Involving More than One Amino Acid*, PhD Thesis, University of Leeds
- 60 Ames, J.M. (1990) 'Control of the Maillard Reaction in Food Systems' in *Trends Food Sci. Technol.* 1, 150–154
- 61 Labuza, T.P. and Baisier, W.M. (1992) 'The Kinetics of Non-enzymatic Browning' in *Physical Chemistry of Foods* (Schwartzberg, H., ed), pp. 595–640, Dekker, New-York
- 62 Angyal, J.S. (1992) 'The Composition of Reducing Sugars in Solution: Current Aspects' in *Adv. Carbohydrate Chem. Biochem.* 42, 19–35
- 63 Martins, S.I.F.S., Van Boekel, M.A.J.S. and Jongen, W.M.F. (2000) 'Kinetic Modelling: A Tool to Understand Maillard Reaction Mechanisms' in *Czech. J. Food Sci.* 18, 281–282
- 64 Stewart, W.E., Caracotsios, M. and Sørensen, J.P. (1992) 'Parameter Estimation from Multiresponse Data' in *AIChE J.* 38, 641–650
- 65 Stewart, W.E., Shon, Y. and Box, G.E.P. (1998) 'Discrimination and Goodness of Fit of Multiresponse Mechanistic Models' in *AIChE J.* 44, 1404