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Effect of heating on Maillard reactions in milk

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Heated milk is subject to the Maillard reaction; lactose and lysine residues in milk proteins (mainly casein) are the reactants. An overview is given of the early, advanced and final stages of the Maillard reaction as it occurs in milk. The early Maillard reaction is confined to the formation of the protein-bound Amadori product lactulosyllysine. Breakdown of the Amadori product leads to formation of all kinds of advanced Maillard reaction products such as lysylpyrraline, pentosidine, hydroxymethylfurfural, (iso)maltol, furfurals and formic acid. The content of these compounds in heated milk is, however, very low (with the exception of formic acid), and does not correspond to the breakdown of Amadori product in quantitative terms. The final stage, in which melanoidins (brown pigments) are formed and protein polymerization occurs, is largely unknown from a chemical point of view, let alone quantitatively. The conclusion can only be that not all important compounds are yet identified. Some experimental data for heated milk are given to illustrate the various stages of the Maillard reaction in heated milk. A kinetic analysis of the Maillard reaction is difficult because it is such a complicated reaction with many parallel and consecutive steps; in addition, one of the reactants, lactose, is also subject to another reaction, namely isomerization followed by degradation. The kinetics can be tackled by kinetic, multiresponse modelling, and this approach is illustrated. It appears that the temperature dependence of the (early) Maillard reaction is lower than for the simultaneously occurring isomerization reactions of lactose. The use of several components formed in the Maillard reaction to evaluate the heat intensity given to milk is discussed.

INTRODUCTION

The Maillard reaction, a chemical reaction between amino groups and reducing sugars, is very significant for foods because it strongly affects the quality. In the case of milk, amino groups are mainly lysine residues in milk proteins (the content of free amino acids in milk is quite low; Walstra and Jenness, 1984). Lysine residues in caseins seem to be more reactive than in serum proteins, while κ-casein seems to be the most reactive casein (Turner *et al.*, 1978). The reducing sugar in milk is lactose, a disaccharide of glucose and galactose (the monosaccharides glucose and galactose are found in raw milk only in very low concentrations).

The Maillard reaction is sometimes subdivided into three stages: the early Maillard reaction, the advanced Maillard reaction and the final Maillard reaction (Mauron, 1981). This classification merely serves as a way of systematizing a complicated reaction such as the Maillard reaction. Recently, another type of classification has been proposed (Yaylayan, 1997). For the time being, the first mentioned classification is used here. The early Maillard reaction consists of condensation of the reducing sugar with the amino group and leads, via formation of a Schiff's base and the Amadori rearrangement, to the so-called Amadori product. In milk, this Amadori product is lactulosyllysine (bound to protein). The advanced Maillard reaction consists of the breakdown of the Amadori product (or other products related to the Schiff's base) into numerous fission products of the sugar–amino compound. The final Maillard reaction consists of the condensation of amino compounds and sugar fragments into polymerized protein and brown pigments, called the melanoidins, the chemical nature of which is still rather obscure. (It is quite remarkable that the Maillard reaction is frequently referred to as the non-enzymatic browning reaction while little is known about the actual browning part.)

The consequences of the Maillard reaction for milk and milk products are considerable:

(a) loss of nutritive value due to blockage of lysine residues which are no longer available for digestion (early, advanced and final Maillard reaction, Finot, 1990), reduced digestibility and inhibition of enzymes (Friedman, 1996*a*,*b*)

(b) flavour compounds are formed: these are mainly low molecular fission compounds from degradation of the Amadori compund (advanced Maillard reaction, e.g. Danehy, 1986)
(c) antioxidative compounds are formed in the advanced Maillard reaction (Namiki, 1988; Bressa *et al.*, 1996)
(d) mutagenic (Shibamoto, 1982), as well as antimutagenic (Kato *et al.*, 1987; Yen *et al.*, 1992) and anticarcinogenic compunds (Aeschbacher, 1990), are formed. However, it should be noted that Berg *et al.* (1990) could not detect any mutagenicity in normally heat-treated milk, only in milk burnt to the pan. Formation of heterocyclic amines during heating (Jägerstad *et al.*, 1991) is not relevant for milk, on the one hand because heating temperatures applied in dairy technology are too low for formation of heterocyclic amines, and on the other hand because an important precursor for heterocyclic amines, creatin(in)e, is not present in significant quantities in milk.
(e) antibacterial compounds may be formed (Einarsson *et al.*, 1983)
(f) antigenicity of heated cow's milk may be less for people allergic to cow's milk (Friedman, 1996*a*)
(g) milk proteins polymerize because of the Maillard reaction (e.g. Zin El-Din *et al.*, 1991; Ames, 1992; Zin El-Din and Aoki, 1993; Henle *et al.*, 1996)
(h) a brown colour develops due to melanoidins (advanced and final Maillard reaction, e.g. Patton, 1955; Namiki, 1988; Ames, 1992).

This paper attempts to summarize the present knowledge about the Maillard reaction as it occurs in heated milk and milk products.

DESCRIPTION AND ANALYSIS OF THE STAGES OF THE MAILLARD REACTION

Early stage

In the early Maillard reaction, lactose (as an aldehyde in the open chain form) forms a Schiff's base with the amino group of protein-bound lysine and is subsequently transformed via the Amadori rearrangement into the protein-bound Amadori product (1-deoxy-1-amino-) lactulosyllysine (Fig. 1) (in the Amadori rearrangement, an aldose sugar is converted to a ketose). According to Mauron (1981), the Amadori product in milk is rather stable as long as the heating conditions are not too drastic or the reaction time not very prolonged. The largest consequence in this stage is loss of lysine availability; there is hardly any colour or flavour formation.

The Amadori product can actually be measured, e.g. by amino acid analysis, after enzymatic breakdown of the protein (Henle *et al.*, 1991). This is, however, a rather time-consuming analysis and therefore another analysis method is frequently chosen, namely the furosine method. Furosine is an artificial amino acid that arises from acid hydrolysis (e.g. 6 N HCl at 110°C during 24 h) of the Amadori product (Finot *et al.*, 1981). Furosine can easily be determined by reversed-phase HPLC (Resmini *et al.*, 1990) and ion-exchange chromatography (Hartkopf and Erbersdobler, 1993), while capillary electrophoresis is also possible (Tirelli and Pellegrino, 1995). A problem with the furosine method is that the conversion factor for calculating the content of the Amadori product from the furosine content is somewhat uncertain. About 30–40% of the Amadori product is converted into furosine (Finot *et al.*, 1981; Erbersdobler, 1986; Molnár-Perl *et al.*, 1986; Furth, 1988), 50% in lysine and a minor reaction product is pyridosine, another artificial amino acid (Fig. 2). A different method for estimating the amount of Amadori product is via its oxidation by periodic acid into carboxymethyllysine (CML, Fig. 2) and subsequent acid hydrolysis of the protein (Badoud *et al.*, 1990, 1991, 1996). CML can be determined by HPLC (Badoud *et al.*, 1990, 1996; Hewedy *et al.*, 1994) or GLC (Büser and Erbersdobler, 1986; Badoud *et al.*, 1991). An indirect way to estimate the Amadori product in heated milk is to determine the content of available lysine by some chemical method, as the amount of unavailable lysine and formation of Amadori compound are similar in the early stage (Berg and Van Boekel, 1994); however, at prolonged heating lysine loss does not correspond any longer to formation of Amadori compound, on the one hand because lysine becomes involved in advanced and final Maillard reactions, and on the other hand, because the Amadori product is broken down again (Berg and Van Boekel, 1994). The extent of glycosylation can also be measured via immunochemical methods (e.g. Matsuda *et al.*, 1992; Fogliano *et al.*, 1997).

Yet another indirect way is to induce formation of hydroxymethylfurfural (HMF) from the Amadori product by boiling in oxalic acid, as a result of which part of the Amadori compound is transformed into HMF (Fig. 2). However, the yield of HMF is only about 10% of the content of the Amadori compound (Furth, 1988), while it was also noted that there was not so much difference between the levels of HMF directly formed during the actual heat treatment of milk ('free HMF') and that formed by boiling the heated milk after heat-treatment with acid ('total HMF') (Berg, 1993). Morales *et al.* (1997) have clearly shown that, in milk, the major part of 'total' HMF is induced from lactose due to the boiling in oxalic acid, and not from protein-bound Amadori product. Nevertheless, HMF is frequently used as a heat-damage indicator (Burton, 1984; Pellegrino *et al.*, 1995*a*) since Keeny and Basette (1959) proposed a colorimetric method for measurement of HMF. This colorimetric method is, however, not very specific. A more specific HPLC method is now available (Van

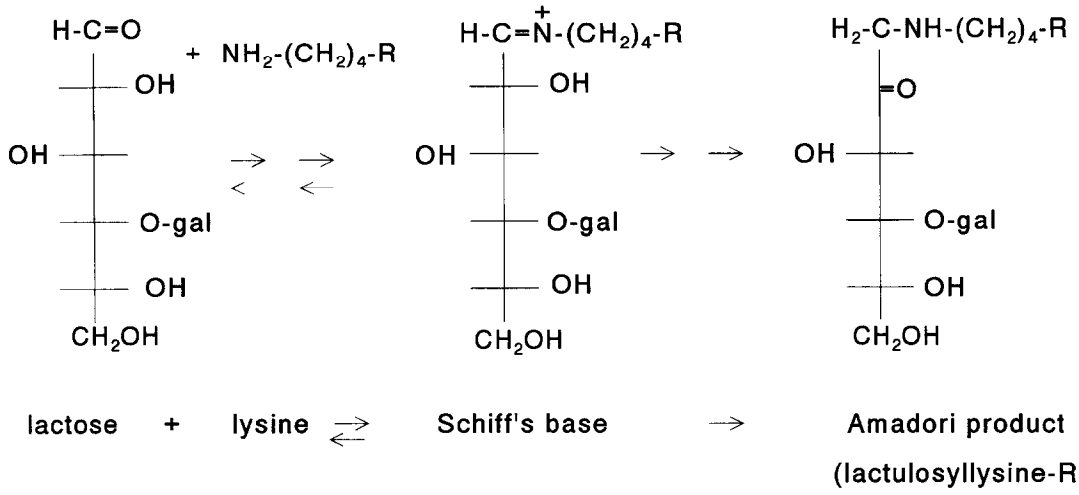

Fig. 1. Schematic overview of the early Maillard reaction in milk, leading to the Amadori product. (gal = galactose, R = protein chain).

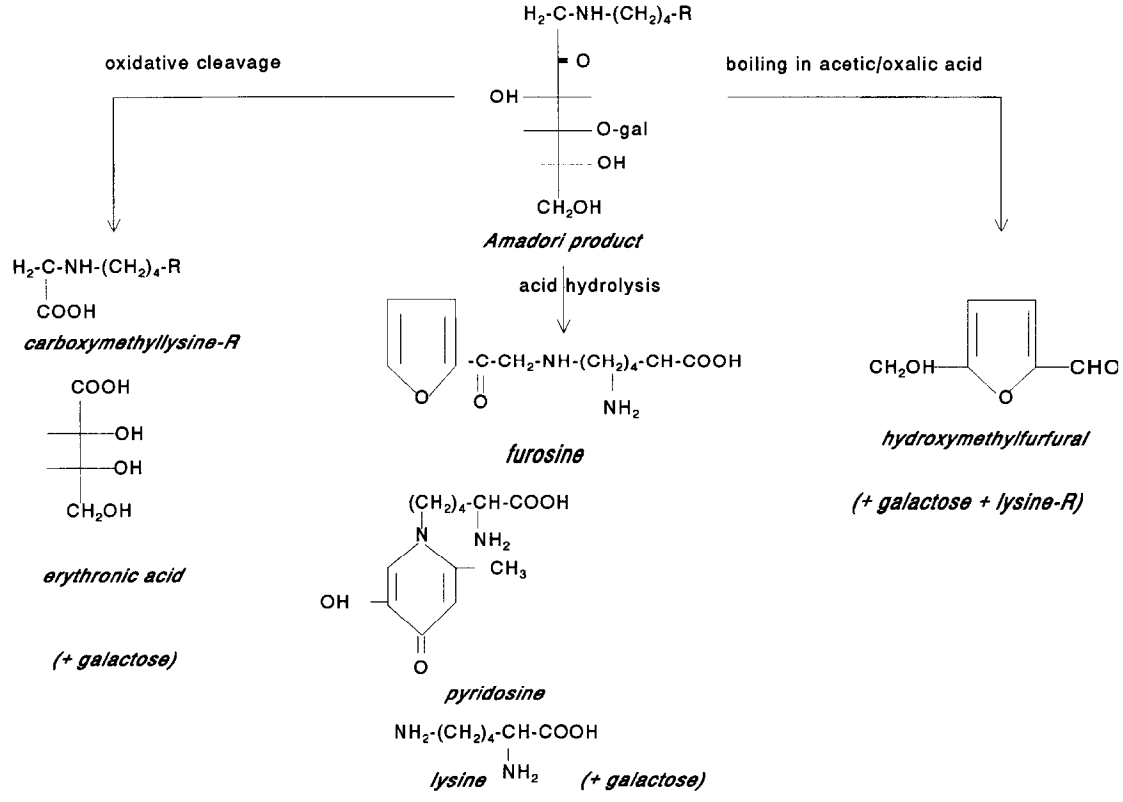

Fig. 2. Degradation of the Amadori product via oxidative cleavage, acid hydrolysis, or boiling in acetic/oxalic acid. (gal = galactose, R = protein chain).

Boekel and Zia-ur-Rehman, 1987; Morales *et al.*, 1992, 1995, 1997).

Advanced stage

In the advanced stage, the Amadori product is subject to breakdown, probably via the enol forms of the Amadori compound (Mauron, 1981) (Fig. 3). There are two general breakdown routes, namely the 3-deoxyosone-pathway via the 1,2 enolization route, chiefly at pH < 7, and the 1-deoxyosone-pathway via the 2,3 enolization route, chiefly at neutral and alkaline pH (Mauron, 1981; Ledl and Schleicher, 1990; Nursten, 1990; O'Brien, 1995), while a third route, the 4-deoxyosone-pathway is significant for disaccharides at slightly alkaline conditions (Pischetsrieder and Severin, 1996). Each route leads to deoxyosones, very reactive intermediates (Fig. 3), the formation and content of which are difficult to determine because of their reactive nature. Enolization, cyclization, and elimination of water

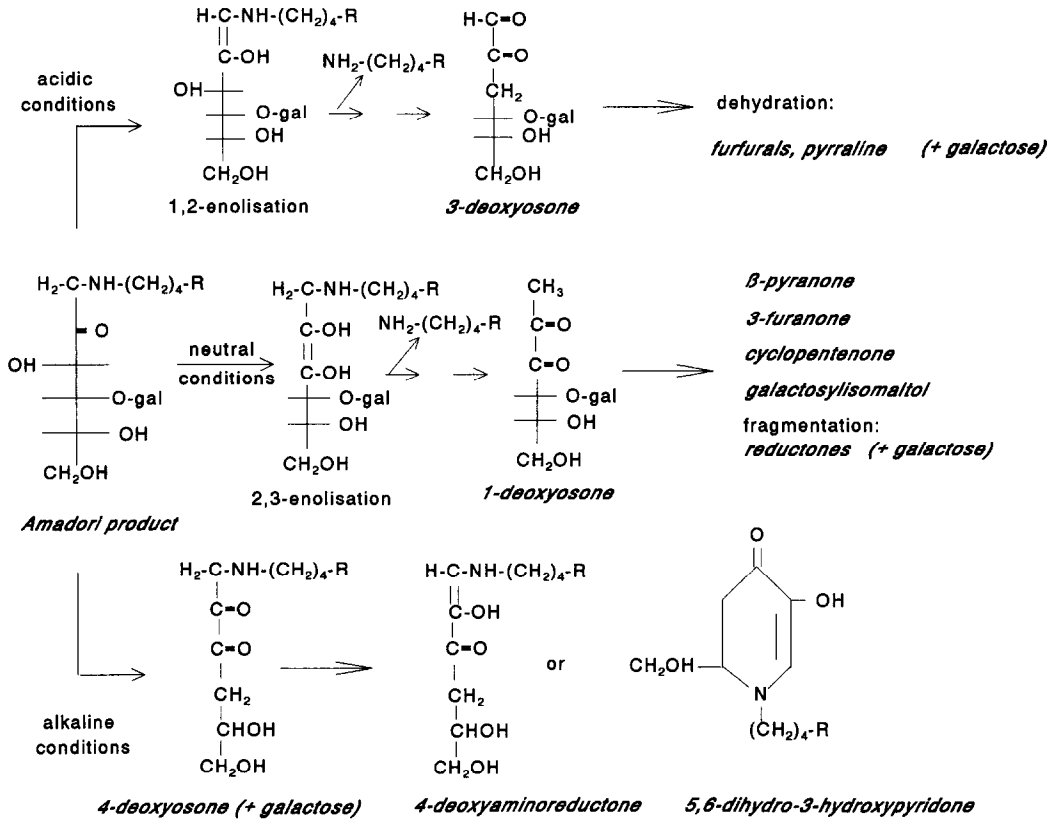

Fig. 3. Breakdown of the Amadori product in the advanced Maillard reaction under acidic, neutral and basic conditions (gal = galactose, R = protein chain).

represent the main reaction pathways for transformation of deoxyosones (Lederer *et al.*, 1993). The three routes give different types of reaction products. The 3-deoxyosone-pathway leads to products such as hydroxymethylfurfural (HMF) and pyrraline; this route is not specific for disaccharides and occurs at acidic conditions, and is therefore not so important for milk. Products such as HMF, furfural and furfurylalcohol, and lysylpyrraline are indeed formed in heated milk but only in very small amounts (Berg, 1993; Morales and Van Boekel, 1996). The 1-deoxyosone-pathway is the most important pathway at neutral pH; in view of the pH of milk, pH 6.6, degradation of the Amadori compound in milk would thus be mainly via the 2,3 enolization route. Disaccharides (like lactose) differ in this pathway from monosaccharides (like glucose) due to the 1,4 glycosidic linkage in lactose. Cyclization and enolization of the 1-deoxyosone leads to 5 or 6-membered rings, (β-pyranone and 3-furanone, respectively), while fragmentation results in reductones and α-dicarbonyls. β-pyranone and 3-furanone are typical disaccharide products (Fig. 4) but they are not very stable (Pischetsrieder and Severin, 1996). β-pyranone isomerizes to cyclopentenone and both products are converted into galactosylisomaltol (Fig. 4). In the presence of aminogroups, β-pyranone, cyclopentenone and galactosylisomaltol easily condense to nitrogen containing products such as acetylpyrrole, pyridinium betaine and furanone-amine, some of which may further react with proteins, possibly to form cross-links (Pischetsrieder and Severin, 1996). Acetylpyrrole (Fig. 4) is a fairly stable end product of lactose-Maillard reactions (Pischetsrieder and Severin, 1996). The third degradation route for the Amadori product, the 4-deoxyosone pathway, is significant for disaccharides under alkaline conditions, and therefore probably not so important for milk. The two products that are formed, 4-deoxyaminoreductone and 5,6-dihydro-3-hydroxypyridone (Fig. 3) are degraded again upon prolonged heating (Pischetsrieder and Severin, 1996).

Berg and Van Boekel (1994) determined some breakdown products of isolated protein-bound lactulosyllysine and they found galactose and formic acid as main reaction products (among, probably numerous, unidentified minor reaction products). A small amount of lactose was also formed but no lactulose; hence, lactulose is not formed by hydrolysis of the Amadori compound as has been suggested by Adachi and Patton (1961). Perhaps, the lactose formed originated from a small amount of Schiff's base left in the Amadori preparation.

In research related to the Maillard reaction *in vivo*, compounds are isolated that are commonly referred to as AGEs (advanced glycosylation end products). Some of these compounds have also been identified in milk. One of them is carboxymethyllysine (CML, already mentioned above) which is formed in heated milk from

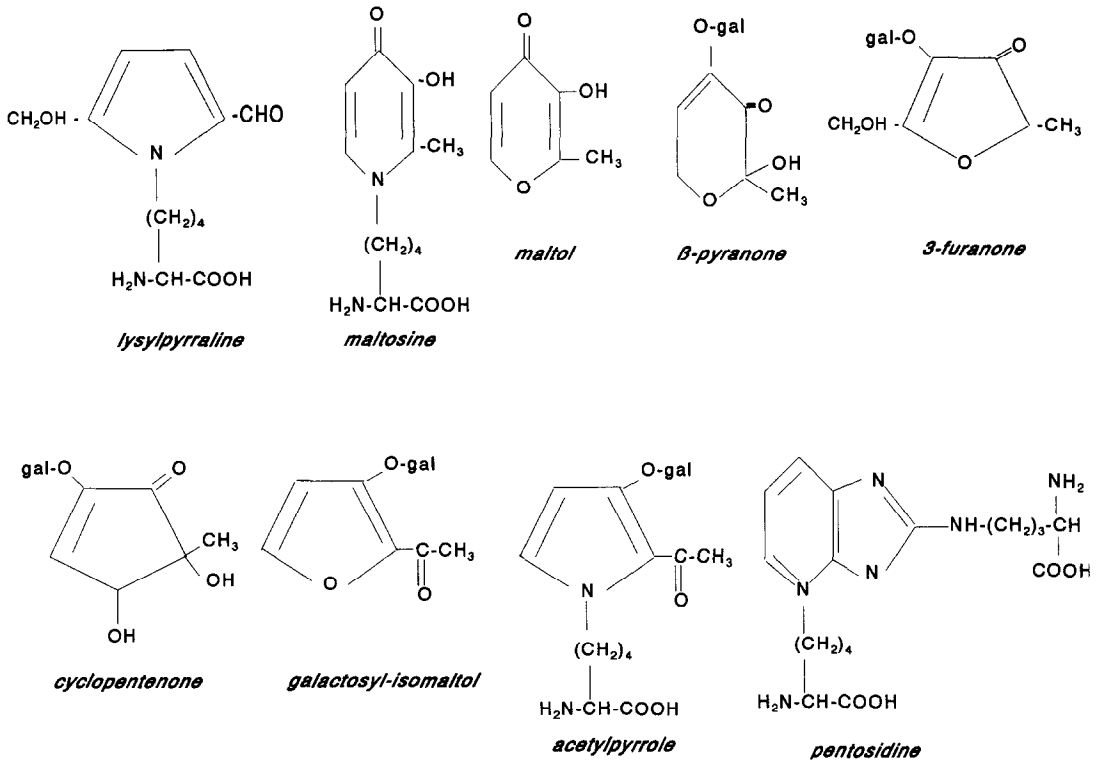

Fig. 4. AGE (advanced glycosylation end) products formed in the advanced Maillard reaction, as found in heated milk.

the Amadori product but only in very low amounts (a few μmol/l, Hewedy *et al.*, 1994). It is only formed in the presence of oxygen. Concomitantly with the formation of CML, erythronic acid and galactose are formed (Fig. 2). (Incidentally, it was recently found that CML is not only formed from oxidation of the Amadori component but also via a pre-Amadori pathway (Glomb and Monnier, 1995). At any rate, the CML content in milk is low.) Analysis of protein-bound CML (hence without prior hydrolysis) is possible using antibodies (Gempel *et al.*, 1994, Ikeda *et al.*, 1996). Another breakdown product of the Amadori product is hydroxymethylfurfural (HMF, already mentioned above). Again, HMF is only a minor breakdown product (μmol/l) while the content of the Amadori product amounts to mmol/l (Berg and Van Boekel, 1994, please note that this paper contains a printing error in Figures 1, 2, 4–7: the values should be in mmol/l rather than μmol/l). Moreover, HMF is not only formed from the Amadori product but also from sugars (Berg, 1993, Morales *et al.*, 1997). (As noted above, CML and HMF can also be induced from the Amadori compound by periodate oxidation and boiling in acid, respectively, to be used as a marker for the Amadori compound.)

Another AGE product is lysylpyrraline (Fig. 4). It was inferred from kinetic studies (Morales and Van Boekel, 1996) that the key compound in the formation of lysylpyrraline is probably 3-deoxyosone (Fig. 3). According to Morales and Van Boekel (1996), the main source of 3-deoxyosone is the sugar and not the Amadori compound in milk and milk-like systems. The content of lysylpyrraline in heated milk was found to be quite low, in the order of μmol/l (Henle *et al.*, 1994, Morales and Van Boekel, 1996), probably because this pathway occurs at acidic conditions (Fig. 3). Nevertheless, it was concluded by Morales and Van Boekel (1996) that lysylpyrraline could be used as a marker for the advanced stage of the Maillard reaction in heated milk.

Pentosidine is another AGE compound, a five-carbon sugar covalently linking lysine to arginine (Fig. 4). Its presence is reported in systems containing pentoses such as ribose (Sell and Monnier, 1989), but it is also formed in low yield from glucose, fructose, ascorbate, Amadori compounds and 3-deoxyglucosone (Dyer *et al.*, 1991). Its content in milk is very low, in the order of a few nmol/l (Henle *et al.*, 1996, 1997). A related AGE compound was recently described as maltosine, chiefly formed at pH > 7 (Fig. 4, Henle *et al.*, 1994). Its content in milk was therefore very low if present at all.

Other AGE compounds occur in milk, as is already known for a long time (Patton, 1955). Among them are compounds such as maltol and furfurals. Maltol is specific for disaccharides and is formed from sugars through base-catalysed 2,3 enolization, but can probably also be formed directly from the Amadori product (Yaylayan and Mandeville, 1994). Related compounds (Fig. 4) are β-pyranone found in milk (Ledl *et al.*, 1986), galactosylisomaltol and a cyclopentenone (Kramhöller *et al.*, 1992); galactosylisomaltol is formed from the pyranone (Ledl *et al.*, 1986); these compounds are formed via the 1-deoxyosone-pathway, as discussed

above. They are probably formed only in low amounts, which is not to say that they are not important.

The contents of known AGEs in heated milk are thus generally very low and do not seem to be important from a quantitative point of view. However, the amounts of cross-linked protein found in heated milk are such that the contents of cross-link compounds must be higher than discovered until now; it probably means that important compounds are not yet identified (Henle *et al.*, 1996, 1997). New cross-linking compounds are discovered from time to time, especially for the Maillard reaction *in vivo*, see for instance, Nagaraj *et al.* (1996). It remains to be established whether or not such cross-links are also relevant for heated foods.

Final stage

This is the stage in which brown pigments (melanoidins) are formed (from reactive compounds formed in the advanced stage, some of which have been identified, Lederer *et al.*, 1993) and in which protein cross-linking occurs. This final stage is not very well characterized from a chemical point of view (Mauron, 1981; Ames, 1992; Rizzi, 1997). Melanoidins are of a high molecular weight (up to about 100 000), and they contain nitrogen by definition. However, brown pigments are also formed without nitrogen, from sugar degradation. In the case of milk it has been noted already for a long time that brown components are mainly bound to protein (Patton, 1955), but the nature of the bonds is not clear. It is to be expected that lysine residues are involved in melanoidins, because lysine loss increases also during the final stage.

A recent overview of methods for analysing Maillard products in foods in the early, advanced and final stage can be found in Olano and Martínez-Castro (1996). Coloured Maillard reaction products, including their analysis, have recently been reviewed by Rizzi (1997).

SOME EXPERIMENTAL DATA FOR HEATED MILK

Figure 5 shows the loss of lactose and lysine (plotted as unavailable lysine) and the simultaneous formation of the Amadori compound (as calculated from the furosine content) at 120 and 130°C. At 120°C unavailable lysine paralleled formation of the Amadori product, while at 130°C unavailable lysine was higher than formation of Amadori product. This suggests that at 130°C degradation of the Amadori product was faster than its formation, and/or that lysine residues were involved in the advanced stages of the Maillard reaction. Note that both at 120 and 130°C loss of lactose was higher than formation of Amadori product. This is due to the fact that besides the Maillard reaction lactose is also subject to isomerization and degradation, and this contribution is quantitatively more important (Berg, 1993; Berg and

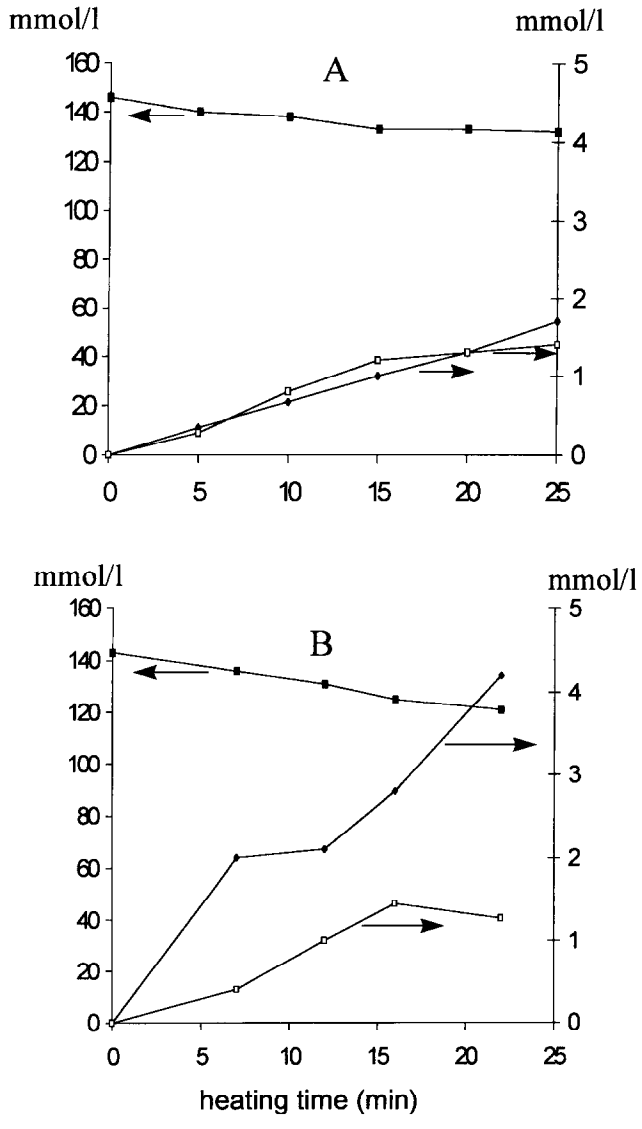

Fig. 5. Lactose (■), unavailable lysine (◆) and lactulosyllysine (□) in milk heated at 120 (A) and 130°C (B).

Van Boekel, 1994). The amounts of Amadori product depicted in Fig. 5 (in the order of 1–2 mmol/l) correspond to what has been reported for in-bottle sterilized milk, while the contents of Amadori product in direct-heated UHT milk are in the order of 0.1–0.2 mmol/l and in indirect-heated UHT milk 0.6–0.9 mmol/l (as calculated from furosine contents (e.g. Nangpal and Reuter, 1990; Nangpal *et al.*, 1990; Corzo *et al.*, 1994; Pellegrino *et al.*, 1995*a*,*b*; Van Renterghem and De Block, 1996).

Figure 6 gives an impression of the formation of HMF and lysylpyrraline at 130°C. Comparison with Fig. 5 shows the quantitative differences between the Amadori compound and advanced Maillard products such as HMF and lysylpyrraline.

The Maillard reaction gives rise to browning. This can be measured as absorption at 420 nm (Olano and Martínez-Castro, 1996) or by using various instrumental methods. Examples for milk can be found in, for example, Kessler and Fink (1986), Andrews and

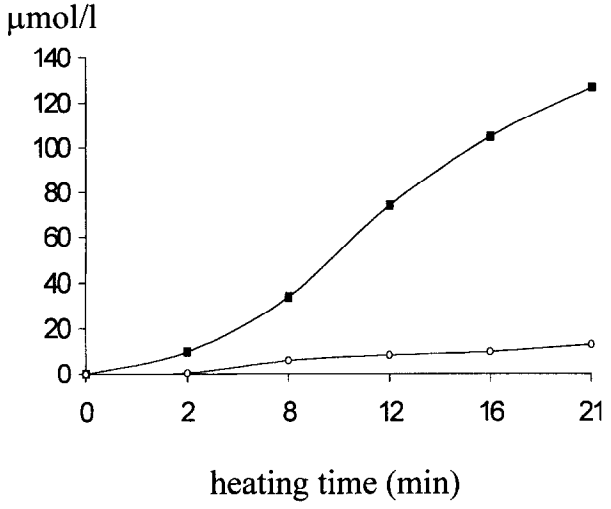

Fig. 6. Formation of 'total' HMF (■) and lysylpyrraline (○) in milk-resembling (lactose-casein) solutions heated at 130°C.

Morant (1987), Rhim *et al.* (1988), Rampilli and Andreini (1992). As already noted, not much is yet known about the chemistry of the browning components and certainly not in milk. It has been stated that fluorescent compounds are formed prior to brown compounds (Baisier and Labuza, 1992). Morales *et al.* (1996) studied 'free' fluorescence (i.e. not protein bound) in milk and milk-like systems. Figure 7 gives an impression of the extent of formation of brown and fluorescent pigments in heated lactose–casein systems (which behave similarly to milk as far as the Maillard reaction is concerned), released after enzymatic digestion of protein (thus including brown and fluorescent compounds not bound to protein). In agreement with

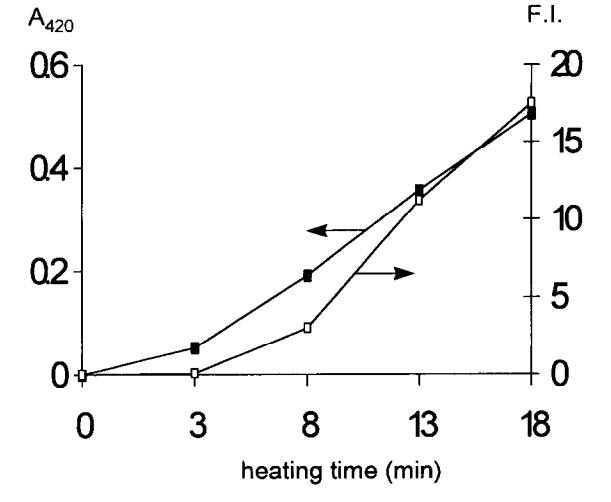

Fig. 7. Formation of brown colour (■, absorption at 420 nm, A_{420}) and fluorescence (□, excitation wavelength 347 nm, emission wavelength 415 nm, F.I. = % fluorescence intensity relative to a quinine sulphate solution, Morales *et al.*, 1996) in milk-resembling (lactose-casein) solutions heated at 130°C. Protein was enzymatically hydrolysed after heating to release protein-bound fluorescent and brown components.

earlier findings (Patton, 1955), browning and fluorescence compounds were to a large extent bound to protein. The 'free' fluorescence and brown colour was less than 1% than that of protein-bound (measured in the filtrate remaining after precipitation of protein). The pigments are likely to be linked covalently via lysine-residues in protein, but non-covalent interaction cannot be excluded.

KINETICS

It is of the utmost importance for the food technologist to be able to control the extent of the Maillard reaction. For that reason, kinetic data are necessary. This is not easy for a complicated reaction such as the Maillard reaction is and the use of simple kinetics, description in terms of zero-, first- or second-order reaction is not adequate (Van Boekel, 1996*a*). One of the complications is that lactose is not only a reactant in the Maillard reaction but is also subject to isomerization/degradation reactions (Berg and Van Boekel, 1994, Van Boekel, 1996*b*). Basically, this is the base-catalysed Lobry de Bruin-Alberda van Ekenstein degradation (Berg, 1993). In the case of milk, protein acts as the catalyst. The resulting reaction products of the isomerization are lactulose (a disaccharide of galactose and fructose) and epilactose (disaccharide of galactose and mannose); epilactose is present only in small amounts (Olano and Calvo, 1989). Degradation products arise from enolization of lactulose according to Berg and Van Boekel (1994) and reaction products are galactose, tagatose (tagatose only in very small amounts, Troyano *et al.*, 1992*a*), formic acid, and five carbon fragments such as deoxypentulose (Troyano *et al.*, 1992*b*) and deoxyribose (Berg and Van Boekel, 1994), while it is very likely that smaller fragments (C_2–C_4) are also formed. Formic acid is formed in relatively large amounts and is largely responsible for the heat-induced pH decrease in heated milk, along with changes in salt-equilibria (Van Boekel *et al.*, 1989; Berg, 1993). The pH decrease makes things even more complicated as the pH affects the Maillard reaction as well as isomerization, although in a different way: isomerization increases more strongly with pH than does the (early) Maillard reaction (Nangpal and Reuter, 1990; Pellegrino *et al.*, 1995*b*). Another disturbing effect is that degradation products of the sugar reactions may interfere in the Maillard reaction; one example was already given in discussing lysylpyrraline formation, where it was indicated that 3-deoxyosones from sugars react with lysine residues (Morales and Van Boekel, 1996).

In an attempt to tackle the complicated kinetics, lactose reactions were divided into two main reactions: (1) isomerization/degradation, and (2) the Maillard reaction (Berg, 1993; Van Boekel, 1996*b*). Kinetic parameters were obtained by kinetic modelling. Basically this procedure consists of the following steps:

(a) determination of the main reactants and products;
(b) proposition of reaction mechanisms;
(c) building of a kinetic model based on the proposed reaction mechanism;
(d) fitting of the kinetic model to the data.

For lactose reactions in milk, steps 1 and 2 have been studied and summarized by Berg and Van Boekel (1994): see Fig. 8. A kinetic model (step 3) is built by setting up coupled ordinary differential equations which are subsequently solved by numerical integration. The fitting in step 4 is done by applying the appropriate statistical techniques as indicated by Van Boekel (1996*a*,*b*). This procedure appears to be quite powerful because it becomes immediately obvious whether or not a kinetic model is valid. If it is not, the model can be adjusted easily and fitted again. In this way, kinetics is also useful for obtaining information on reaction mechanisms. It should be realized also that the model of Fig. 8, complicated as it is, is still a simplification (as is every model), but it may give more insight than the use of simple kinetics. Figure 9 gives an example how the model of Fig. 8 fits the experimental data, and it can be seen that the fit appears to be reasonable. From temperature studies in the range of 100–150°C it was found that the activation energy for isomerization/degradation (about 130 kJ/mol) was higher than that for the (early) Maillard reaction (about 100 kJ/mol). This implies that the Maillard reaction is more noticeable at lower temperatures (say < 100°C) than isomerization and vice versa. It was also clear from the kinetic studies that, at least at temperatures above 100°C, isomerization reactions are far more important from a quantitative point of view than the Maillard reaction. This is not to say that Maillard reactions are not important above 100°C, because the consequences are still considerable even though the quantitative significance is less.

Heat-damage indicators

Several heat-induced markers have been proposed in the past to control and check the heat treatments given to milk and milk products, and quite a few of them are

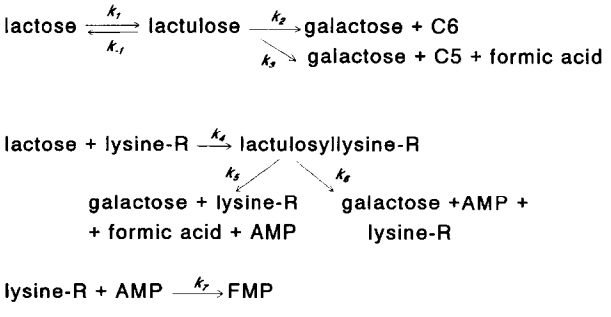

Fig. 8. Kinetic model of the reaction network of lactose in heated milk. (AMP = advanced Maillard products, FMP = final Maillard products).

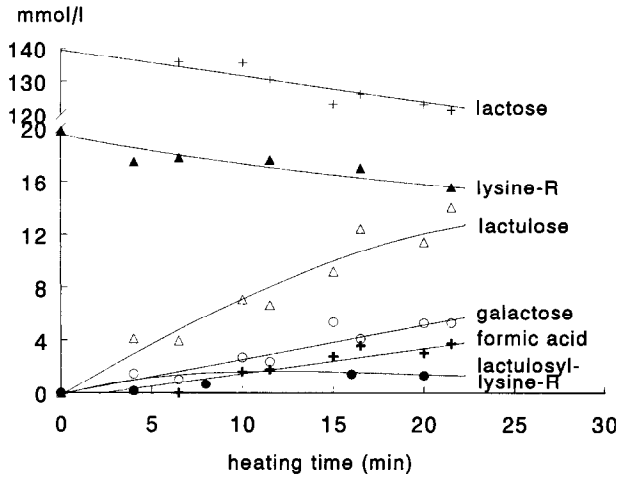

Fig. 9. Fit of the kinetic model presented in Fig. 8 to experimental data for milk heated at 130 °C. Drawn lines are the fits predicted by the model.

related to the Maillard reaction (Burton, 1984; Pellegrino *et al.*, 1995*a*). It is perhaps of interest to review them in view of the above information. Table 1 gives an overview. (Strictly speaking, lactulose is not a Maillard reaction product, but it is mentioned in Table 1 because it is sometimes used in combination with Maillard reaction products.) Furosine and carboxymethyllysine can be used as a marker for the early Maillard reaction but the Amadori product from which they are derived is not a stable end product, as noted above, and consequently their concentration changes during storage, reflecting the ongoing MR. Lactulose is also not an end product, but it changes less during storage at room temperature (or lower) than does furosine (because the temperature dependence for isomerization is higher). Several workers have proposed using the ratio of furosine to lactulose as heat-induced marker (Corzo *et al.*, 1994; Pellegrino *et al.*, 1995*b*; Van Renterghem and De Block, 1996). HMF has been used frequently in the past because it was easy to measure, but it can be formed both in isomerization/degradation reactions and in the Maillard reaction, and there is always the difficulty between free HMF (marker for the advanced Maillard reaction) and total HMF (marker for the Amadori product, hence the early Maillard reaction). It seems that the use of lactulose and furosine gives better perspectives for evaluating a heat treatment (Pellegrino

Table 1. Overview of heat-damage indicators related to the Maillard reaction (MR)

Indicator	Measure for
Furosine	Early MR
Carboxymethyllysine	Early MR
Lactulose	Isomerization of lactose
HMF, galactose, tagatose	Advanced MR, isomerization
Lysylpyrraline	Advanced MR
Brown colour, fluorescence	Final MR

et al., 1995*a,b*). Markers for the advanced Maillard reaction such as lysylpyrraline have been proposed but are not yet in use. Measurement of colour and fluorescence could also be used.

It may be of interest to note that the fat content of milk and milk products has an effect on several heat-damage indicators, as shown by Pellegrino (1994). Fat globules apparently have some suppressing effect on the heat load given to a product, possibly because of a turbulence depressing effect, making heat transfer less efficient.

In comparing direct UHT heating (steam injection or infusion) with indirect UHT heating, furosine and lactulose values are generally lower for direct UHT milk, even if given the same equivalent heat load. This is due to dilution of the milk by steam, resulting in reduction of the concentration of reactants. If this dilution effect is taken into account, kinetics for both direct and indirect UHT are about the same according to Nangpal and Reuter (1990).

The Maillard reaction in dried milk products is much faster than in milk, and this has of course an effect on heat-induced markers such as lactulose and furosine (Pellegrino *et al.*, 1995*a,b*; Van Renterghem and De Block, 1996). The increased Maillard reaction in dried milk products is frequently ascribed to lowered water activity, but it should be realized that the increase is not so much due to a water activity effect as to an increase in the concentration of reactants as a result of drying (at very low water content the rate of Maillard reaction decreases again because the diffusion of reactants then becomes rate limiting).

In general, the pH optimum for the Maillard reaction is between pH 8 and 10 (Labuza and Baisier, 1992). This pH range is not relevant for milk products, but it indicates that changes in pH can have a large effect on reaction rates. As noted above, pH has a stronger effect on lactose isomerization (lactulose formation) than on the early Maillard reaction (as measured by furosine) (Nangpal and Reuter, 1990; Pellegrino *et al.*, 1995*b*). Consequently, the ratio lactulose/furosine is strongly pH dependent.

It is perhaps worthwhile mentioning that microwave heating has the same effect as conventional heating as measured by some markers discussed above (Meißner and Erbersdobler, 1996; Lopez-Fandiño *et al.*, 1996).

CONCLUSIONS

The Maillard reaction has considerable consequences for the quality of heated milk and milk products in terms of colour, flavour and nutritional value. The early stage of the Maillard reaction does not yet give rise to colour and flavour changes and results in only very limited lysine loss. This is the case for minimally heat-treated milk (e.g. a few seconds at 145°C) such as direct heat-treated UHT milk (steam injection or infusion). Then again, advanced and final stages may occur during subsequent storage of direct-heated UHT milk, depending on storage time and temperature. In milk heated more intensively, advanced and final stages of the Maillard reaction will occur during heating, as well as during storage, giving rise to formation of brown colour and flavour components, as found in in-bottle sterilized milks and intensely heated indirect UHT milk.

The kinetics of lactose reactions, including the Maillard reaction, revealed that (i) lactose isomerization is quantitatively more important than the Maillard reaction at sterilization temperature (for instance 80% isomerization versus 20% Maillard at 120°C), (ii) lactose isomerization is more strongly temperature dependent than the Maillard reaction. It can also be concluded that, as yet, not much is known about the advanced and final stage of the Maillard reaction in heated milk. The advanced and final stages are particularly important in view of protein polymerization due to the Maillard reaction and research is on the way to characterize this in more detail.

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