Reactions of lactose during heat treatment of milk:
a quantitative study

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H.E. Berg

Reactions of lactose during heat treatment of milk:
a quantitative study

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
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1. Het kwantitatieve effekt van de Maillard-reaktie tijdens het verhitten van melk is aanzienlijk kleiner dan tot nu toe verondersteld.
   Dit proefschrift.

2. Het modelleren van chemische reakties is een krachtig hulpmiddel bij het ophelderen van gecompliceerde reaktienetwerken.
   Dit proefschrift.

3. De mate van vorming van hydroxymethylfurfural is niet geschikt als indikator voor de intensiteit van de hittebehandeling van melk.
   Dit proefschrift.

4. De conclusie van McGookin and Augustin (1991) dat de pH-verandering als gevolg van verhitten van een caseïne-suiker mengsel voornamelijk kan worden toegeschreven aan de Maillard-reaktie is niet juist.
   B.J. McGookin and M.A. Augustin. 1991. J. Dairy Research 58: 313.

5. De koe heeft meer voor de mensheid betekend dan de mensheid voor de koe.

6. De emancipatie van de vrouw kan leiden tot produktie-inefficiëntie bij de bakkers.
   F.A.J.M. van Welie en R.A.G. Menting. 1993. Bakkerswereld 21: 14.

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8. Zolang de antimutagene werking van caseïne niet verklaard is, kan de vorming van eventueel mutagene verbindingen in verhitte melk niet aangetoond worden.
   H.E. Berg et al. 1990. J. Food Science 55: 1000.

9. Door de afname van de verkoop van krentenbrood mist de bakker de krenten in de pap.
   Bakkerswereld.

10. Bij de berekening van de concentratie aan caseïnomacropeptiden zoals die vrijkomen bij de enzymatische stremming van melk dient rekening te worden gehouden met de volume-uitsluiting en sterische uitsluiting van wei-eiwitten ten opzichte van para-caseïne; hetzelfde geldt voor de overgang van eiwit uit de kaasmelk in de kaas.
    G. van den Berg et al., 1992. Neth. Milk Dairy J. 46: 145.

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Stellingen behorende bij het proefschrift "Reactions of lactose during heat treatment of milk: a quantitative study" door H.E. Berg. Wageningen, 5 april 1993.

ABSTRACT

Berg, H.E. (1993). Reactions of lactose during heat treatment of milk: a quantitative study. Ph. D. thesis, Agricultural University, Wageningen. (pp, English and Dutch summaries).

Keywords: lactose, lactulose, galactose, formic acid, isomerization, degradation, Maillard reaction, modelling, milk.

The kinetics of the chemical reactions of lactose during heat treatment of milk were studied. Skim milk and model solutions resembling milk were heated. Reaction products were determined and the influence of varying lactose, casein and fat concentration on the formation of these products was studied. It was observed that lactose isomerized into lactulose, and subsequently degraded into galactose, formic acid, deoxyribose, hydroxymethylfurfural, furfural and furfuryl alcohol; lactose also reacts with lysine-residues to form lactulosyllysine-residues (early stage of the Maillard reaction). From these results, a model describing the steps in the reaction network of the degradation reactions of lactose during heating of milk was proposed.

It was tried to model the degradation of lactose by computer simulation in order to predict the quantities of the various degradation products in the course of time. The model appeared to fit the experimentally obtained results reasonably well. Altogether, the hypothesized mechanism for degradation of lactose appeared adequate to explain the observations for milk and model solutions resembling milk. Mathematical modelling thus allowed rigorous checking of a proposed complicated reaction network in foods. In the case of milk it has been found that, from a quantitative point of view, the isomerization reaction is much more important than the Maillard reaction in the degradation of lactose during heat treatment of milk.

VOORWOORD

Nu dit proefschrift af is, wil ik iedereen bedanken die op enigerlei wijze heeft bijgedragen aan het totstandkomen van dit boekje of bij het uitvoeren van het hierin beschreven onderzoek. Allereerst natuurlijk mijn promotor, Pieter Walstra, en mijn co-promotor, Tiny van Boekel. Zij hebben het initiatief tot dit onderzoek genomen en het met enthousiasme begeleid. Vooral de inbreng van Tiny is erg groot geweest. Met name het programmeerwerk boeide hem zodanig dat hij daarin dan ook vele (vrije) uren heeft gestopt; Tiny bedankt! Ook voor alle opbouwende kritiek tijdens de schrijffase. Verder wil ik Henk Jansen en Henk van der Stege, de toeleveranciers van de voor dit onderzoek zo noodzakelijke ondermelk, bedanken. Zij hadden altijd wanneer ik dat wenste weer een litertje ondermelk voor me. Tevens wil ik hen bedanken voor de begeleiding bij het verhitten van melk in het UHT-apparaat. In het kader van hun doctoraalvak hebben Jan Spoelstra, Roelof Timmer, Marjan Vrins, Henk Wymenga, Theo Verwaaijen en Yves de Groote een bijdrage aan dit onderzoek geleverd. In het kader van een afstudeeropdracht van de HALO heeft Eddy van den Brand meegewerkt aan dit onderzoek.
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1 INTRODUCTION

1.1 General introduction

Often, milk is heat-treated to obtain a safe and healthy product with a much longer shelf-life than raw milk. This is due to heat inactivation of micro-organisms and enzymes. However, heating will also induce changes which are not desired and will result in loss of quality. Especially chemical changes are responsible for off-flavour and colour development. To produce food of high quality, it is necessary to know the changes that occur during heat treatment. In this study, we are interested in the role of lactose in the deterioration of milk quality.

The milk can be pasteurized, (conventionally) sterilized or UHT-heated (Ultra-High-Temperature). Low-pasteurization is carried out at rather low temperatures (e.g., 15 s at 74°C); most microorganisms but not spores are then killed and some enzymes are inactivated but almost no chemical reactions take place. High-pasteurization (e.g., 15 s at 90°C) is more intensive. During (conventional) sterilization, the milk is heated for a longer time at high temperature (e.g., 20 min. at 120°C); all microorganisms and spores are then killed and more chemical reactions occur. UHT treatment means a shorter time (a few seconds) at a higher temperature (e.g., 140°C); microorganisms (including spores) are killed but chemical reactions are minimized due to a very different temperature sensitivity of most chemical reactions as compared to microbial inactivation.

In milk several chemical reactions take place during heat treatment. Here, we focus on reactions of lactose. The first reaction to mention is the Maillard reaction, a reducing sugar (in milk: lactose) and an amino group (in milk: lysine residues) condensate to a glycosylamine which is a very reactive compound. This condensation is followed by a series of other chemical reactions; the reaction products cause changes in flavour and taste, formation of a brown colour and loss of nutritive value. The formation of toxic or mutagenic compounds is reported as a result of the Maillard reaction. It has been suggested that early intermediate Maillard reaction products are mutagenic (Shinohara et al., 1980; Shibamoto, 1982; Mihara and Shibamoto, 1980). Berg et al. (1990) studied mutagenicity in heated milk, but found no mutagenic response in heated milk or model systems. They concluded that there were either no mutagens formed in the heated milk or any mutagens formed were adsorbed or complexed with casein.

A second reaction path is the degradation of lactose during isomerization reactions. Lactose is isomerized to lactulose and/or degraded to galactose and other compounds. During these reactions, acids are also formed, which may influence the stability of the milk.

Much research is done on the formation of chemical compounds in milk, especially on the use of such compounds as an indicator of the intensity of the heat treatment. However, little research is done on the kinetics of the complex degradation reactions of lactose which take place during heat treatment. In order to describe these kinetics a detailed inventory of the reactions taking place has to be performed. First, a review of literature on these subjects is given.

1.2 Review of literature on sugar reactions

Sugar degradation

Lactose is a disaccharide, consisting of two monosaccharide units condensed with the loss of one molecule of water. Lactose, properly called 4-O-β-D-galactopyranosyl-D-glucopyranose, is heterogeneous because it consists of two different monosaccharides; galactose and glucose joined in a β-1,4-glycosidic linkage. Lactose is a reducing sugar, as it still has a free hemiacetal linkage between carbons 1 and 5 of the glucose moiety. Chemical reactions involve this linkage, as well as the β-1,4-glycosidic linkage between both sugar rings, the hydroxyl groups and the -C-C- bonds within the rings. Lactose can undergo reactions typical of aldehydes. First the degradation of monosaccharides in alkaline solutions and the Maillard reaction in general will be discussed, after that, some special degradation products of lactose studied in this research. As the degradation products found in heated lactose solutions appeared to be mostly the same as those mentioned in the degradation route of monosaccharides in alkaline medium, which was thoroughly investigated by de Wit (1979) and de Bruijn (1986), we will pay attention to the alkaline degradation routes.

De Bruijn (1986) wrote a thesis on the behaviour of monosaccharides in alkaline medium; isomerization, degradation, oligomerization. Monosaccharides in aqueous alkaline medium and at moderate temperature (3-80°C) undergo both reversible and irreversible transformations (Figure 1.1).

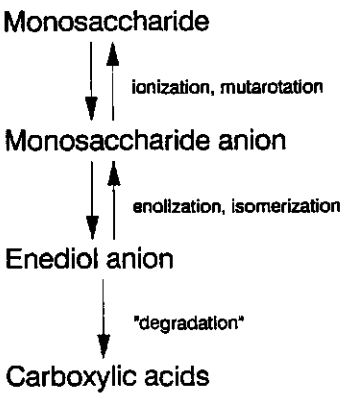


Figure 1.1 Overall reaction scheme of monosaccharides in alkaline medium (de Bruijn, 1986)

Reversible reactions include ionization, mutarotation and enolization, the latter resulting in interconvertible monosaccharides. The isomerization via the enolization reaction is accompanied by irreversible transformation of the monosaccharides into carboxylic acids; this is generally known as the alkaline degradation reaction. Enediol anions are considered common intermediates in both isomerization and degradation reactions. Much research has been done to obtain a better understanding of the fundamental aspects of the reactions of monosaccharides in alkaline solution. Initial transformations such as ionization, mutarotation, enolization and isomerization have been extensively studied in recent years and are now reasonably well understood. However, only part of the degradation reactions have been elucidated until now, because of the complexity of these reactions. The enolization followed by isomerization is known as the "Lobry de Bruyn-Alberda van Ekenstein transformation" henceforth referred to as the LA-transformation, this is a base-catalysed enolization of an aldose or ketose to the enediol, followed by isomerization. Glucose, mannose and fructose are in equilibrium with the 1,2-enediol (Figure 1.2).

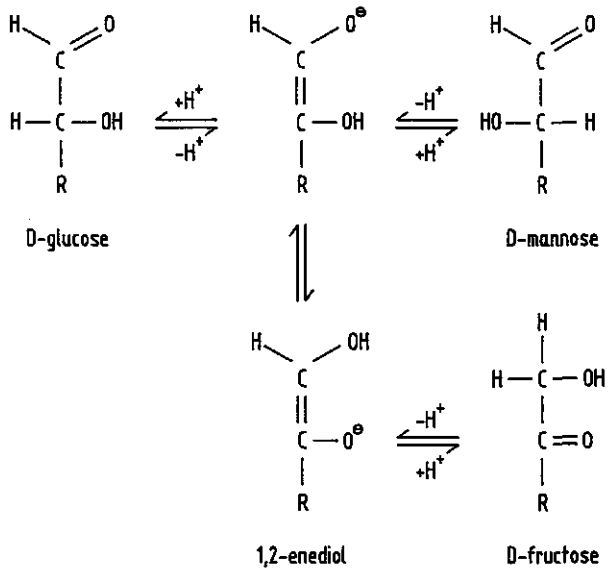
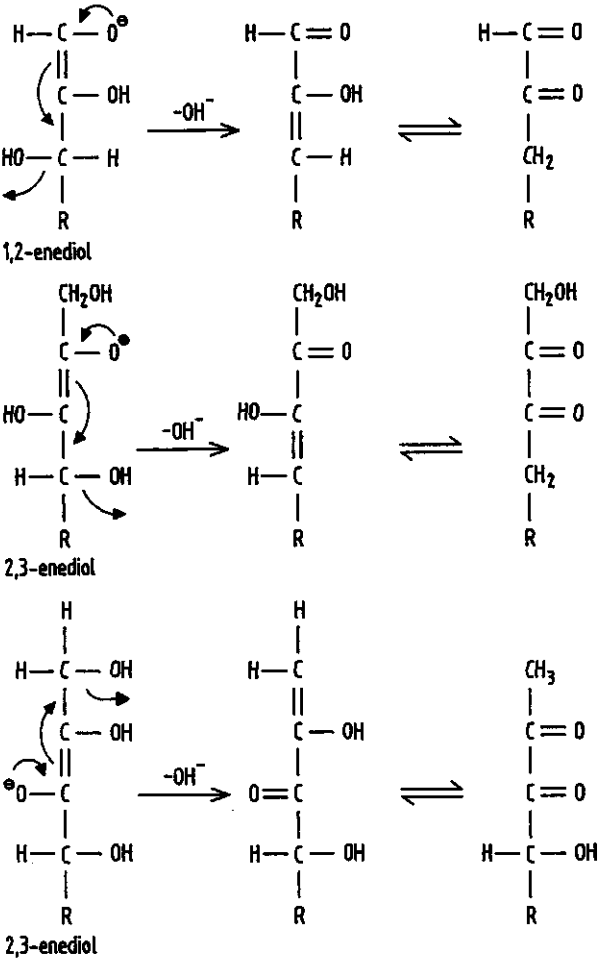


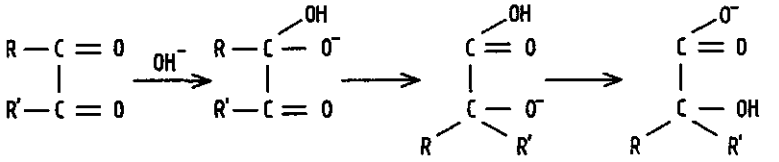
Figure 1.2 Formation of the 1,2-enediol anion as intermediate in the Lobry de Bruyn-Alberda van Ekenstein transformation

The influence of temperature on the LA-transformation is not given by de Wit (1979) and de Bruijn (1986), and neither by Speck (1958) who wrote a review on the LA-transformation. De Bruijn (1986) mentioned a number of reviews published since 1950 on the alkaline degradation of carbohydrates. It is suggested that 1,2- and also 2,3-enedioles are the key intermediates in both isomerization and degradation reactions of monosaccharides (Speck, 1958). Those enediol anions must be considered the starting intermediates in the alkaline degradation reactions; they lead to carboxylic acids as the final stable degradation products via several pathways (Figure 1.3).

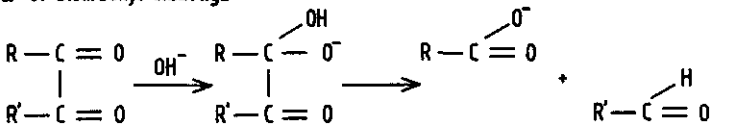
I β-elimination



II Benzilic acid rearrangement



II α-Dicarbonyl cleavage



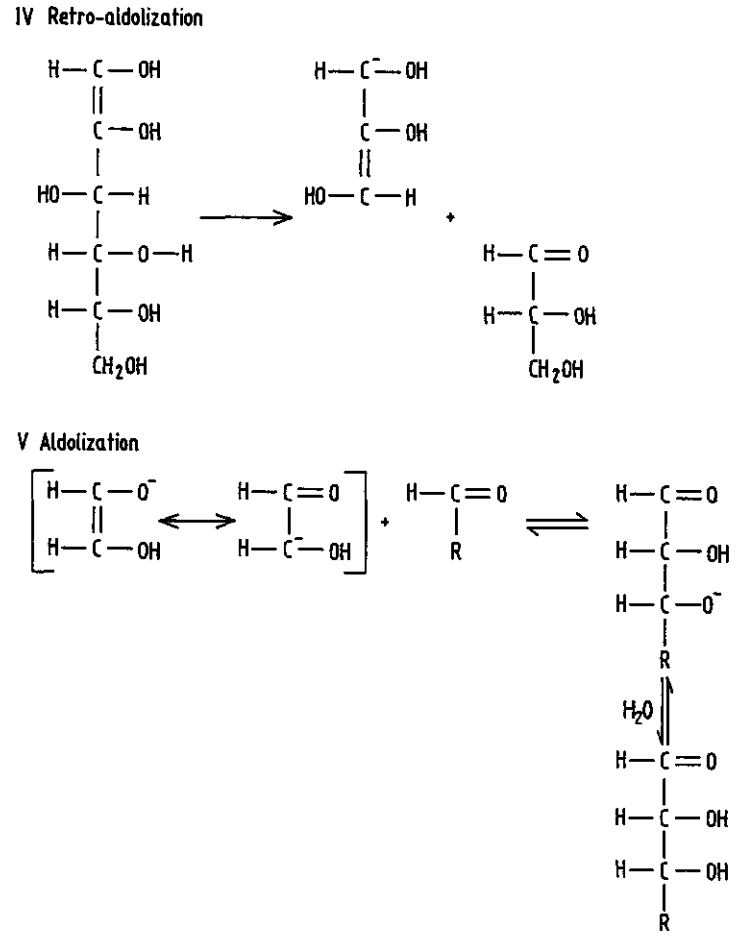


Figure 1.3 Rearrangements in aqueous alkaline medium (de Bruijn, 1986)

The enediols may undergo β-elimination resulting in dicarbonyl compounds. The α-dicarbonyl compounds are unstable under basic conditions and undergo either a benzylic acid rearrangement resulting in a meta-, iso- or saccharinic acid, or a cleavage reaction towards a carboxylic acid and an aldehyde. The 1,2-enediol can also undergo a retro-aldol reaction, giving two trioses. Finally, aldolization reactions of carbonyl compounds, formed from the starting hexose, are also important in the degradation reactions (de Wit, 1979). De Wit also gave a simplified scheme of the degradation of hexoses which demonstrates the alkaline degradation to be a dynamic interconversion of C-2 to C-6 monosaccharides, resulting in the irreversible formation of carboxylic acids.

The Maillard reaction

The Maillard reaction (non-enzymatic browning reaction) is the name for a complex of reactions starting with reactions of reducing sugars with compounds having free amino groups. Reactive intermediates are formed by a variety of pathways and these can yield flavour components and brown melanoidins of higher molecular weight. Sometimes formation of these compounds is desirable (e.g. in baking of bread) but it can also lead to a reduction of quality (Baltes, 1982). The reaction is named after the French chemist Louis Maillard, who first described the formation of brown pigments or melanoidins when heating a solution of glucose and lysine. The difference between the Maillard reaction and caramelisation is that the latter occurs when pure sugars are heated. Most reactions involved in thermal degradation of sugars are also observed in the Maillard reaction. Many chemical reactions that occur in pure sugars only at very high temperatures take place at much lower temperatures after the sugars have reacted with amino acids. The transformation of an aldose into a ketose via the formation of the N-glycoside during the Maillard reaction is analogous to the LA-transformation observed when sugars are in alkaline solution (Mauron, 1981). Hodge (1953) wrote an extensive review on the Maillard reaction, and his scheme of the Maillard reaction pathways is still widely used. The Maillard reaction can be divided into three stages: the early, advanced and final Maillard reactions.

Early Maillard reaction

The first step involves the condensation reaction between the carbonyl group of the sugar and the amino group of the protein (Figure 1.4).

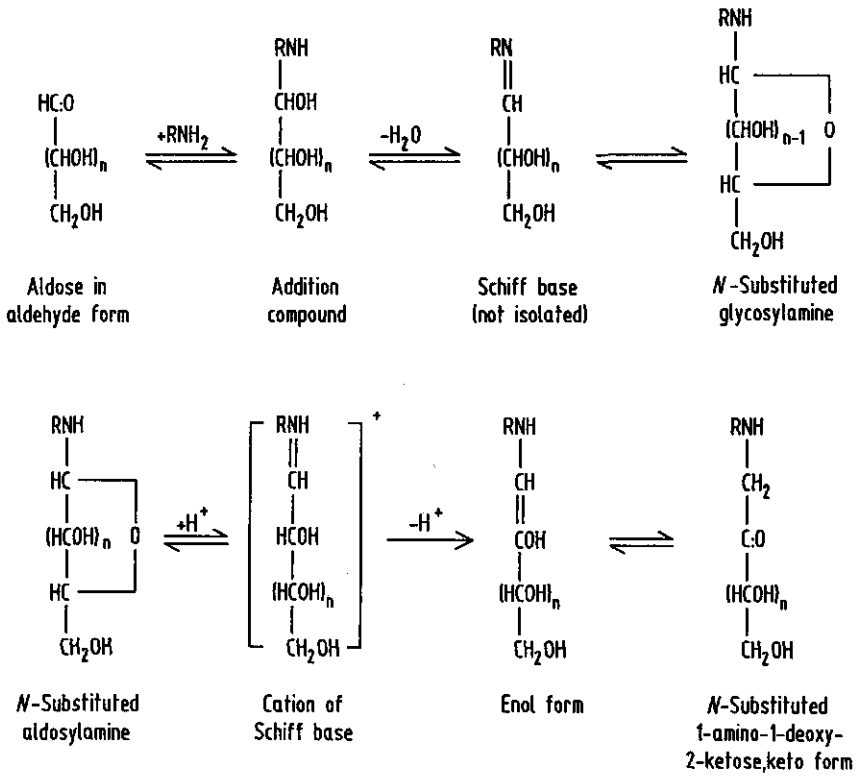


Figure 1.4 Early Maillard reaction (Hodge, 1953)

The amines are acting as nucleophiles and depending on the pH, as bases or acids (Ledl, 1990). The condensation starts with an attack of the unshared electron pair of a nucleophilic amino nitrogen on the carbonyl carbon (Figure 1.5). Protonation of the carbonyl group enhances its reactivity to the nucleophilic reagent whereas protonation of the nitrogen inhibits the attack on the carbonyl group. The optimum pH for this reaction is when the product of the concentrations (in fact activities) $[>C{=}O]$ $[RNH_2]$ is at maximum. As these concentrations (activities) vary in opposite direction with pH, the rate of the condensation reaches its maximum at a weakly acidic pH (Namiki, 1988).

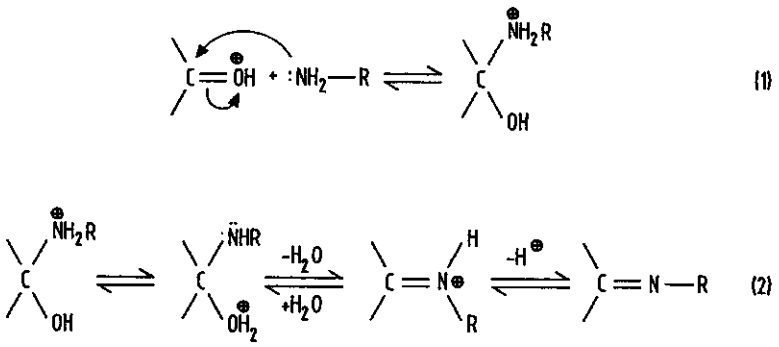


Figure 1.5 Condensation of carbonyl compounds with amino compounds (Namiki, 1988)

After addition of the amine to the carbonyl group one molecule of water is eliminated to form a Schiff base, which undergoes cyclization to the N-substituted glycosylamine. This reaction is reversible as the glycosylamine can be hydrolysed in aqueous solution. The glycosylamines derived from amines show a certain stability, those from amino acids are almost immediately converted into the 1-amino-1-deoxy-2-ketose by the Amadori rearrangement. This reaction is catalysed by weak acids and the carboxyl group of the amino acid provides the necessary protons (Mauron, 1981). Heyns et al. (1970) found the Amadori rearrangement to be accelerated by electron attracting groups in the ω-position of the sugar moiety. They also found that the formation of the glycosylamine and the Amadori rearrangement go faster in the case of an aldose in the furanose form than in the pyranose form of the Amadori product. The Amadori rearrangement is the key step in the early Maillard reaction; it involves the transition from an aldose to a ketose-sugar derivative. A reaction between ketoses and amines usually involves the formation of ketosylamines followed by the Heyns rearrangement to form 2-amino-2-deoxyaldoses (Mauron, 1981, Matsuda et al., 1991). Baltes (1982) reported the ring opening of the glycosylamine to be rate limiting for the course of the Amadori rearrangement. He also reported the degradation of the Amadori compound itself to take place especially fast when it exists in the furanose form or in the open chain form. However, not all authors have the same opinion on the effect of ring opening. Bunn and Higgins (1981) and Watkins et al. (1987) also concluded that the rate of reaction of the various monosaccharides correlated strongly with the

extent to which the sugar exists in the open chain in the monosaccharide/protein solutions they used, but Westphal and Kroh (1985) found the cyclic β-conformation to be most reactive in carbohydrate/phenylalanine model systems. If the mechanism depicted in Figure 1.5 is the correct one, we feel that reaction via the open chain is more likely, because the C1 carbon atom may be more electrophilic in the open chain form than in the ring structure.

So, as nucleophiles the amines rearrange with aldoses to the amino ketoses and with ketoses to the amino aldoses (Ledl, 1990). These products are rather stable; under mild heating conditions the Maillard reaction stops at this stage. These early Maillard reactions do not cause browning nor give flavour to the food (Mauron, 1981).

Advanced Maillard reactions

Starting with the relatively stable Amadori compounds there are three main pathways for the advanced Maillard reactions. In the first pathway the 1-amino-1-deoxy-2-ketose enolizes in position 2-3 irreversibly and eliminates the amine from C1 to form a methyl α-dicarbonyl intermediate (Hodge, 1967), which further reacts to fission products like C-methyl-aldehydes, keto-aldehydes, dicarbonyls and reductones (Figure 1.6).

The second pathway starts with the formation of a 1,2-enediol of the Amadori compound by the elimination of the hydroxy group at C3, followed by deamination at C1 and addition of one molecule water yielding the 3-deoxyhexosulose. After dehydration flavour compounds like 2-furaldehydes (e.g. 5-hydroxymethyl-2-furaldehyde, HMF) are formed (Hodge, 1967). Which of these two pathways occurs is mainly determined by pH, a low pH favouring 1,2-enolization and a higher pH 2,3-enolization. The 3-deoxyhexosones contain a α-dicarbonyl group on C1 and C2 and the methyl α-dicarbonyl intermediate contains it at C2 and C3. Both types of α-dicarbonyl intermediates may provide flavour compounds. These compounds are also known as caramelization products of sugars. In the absence of amines 1,2-enolization of the sugar results in the same transformation products; but the condensation with amino compounds allows the enolization and elimination to take place near neutral pH and at much lower temperatures (Hodge, 1967).

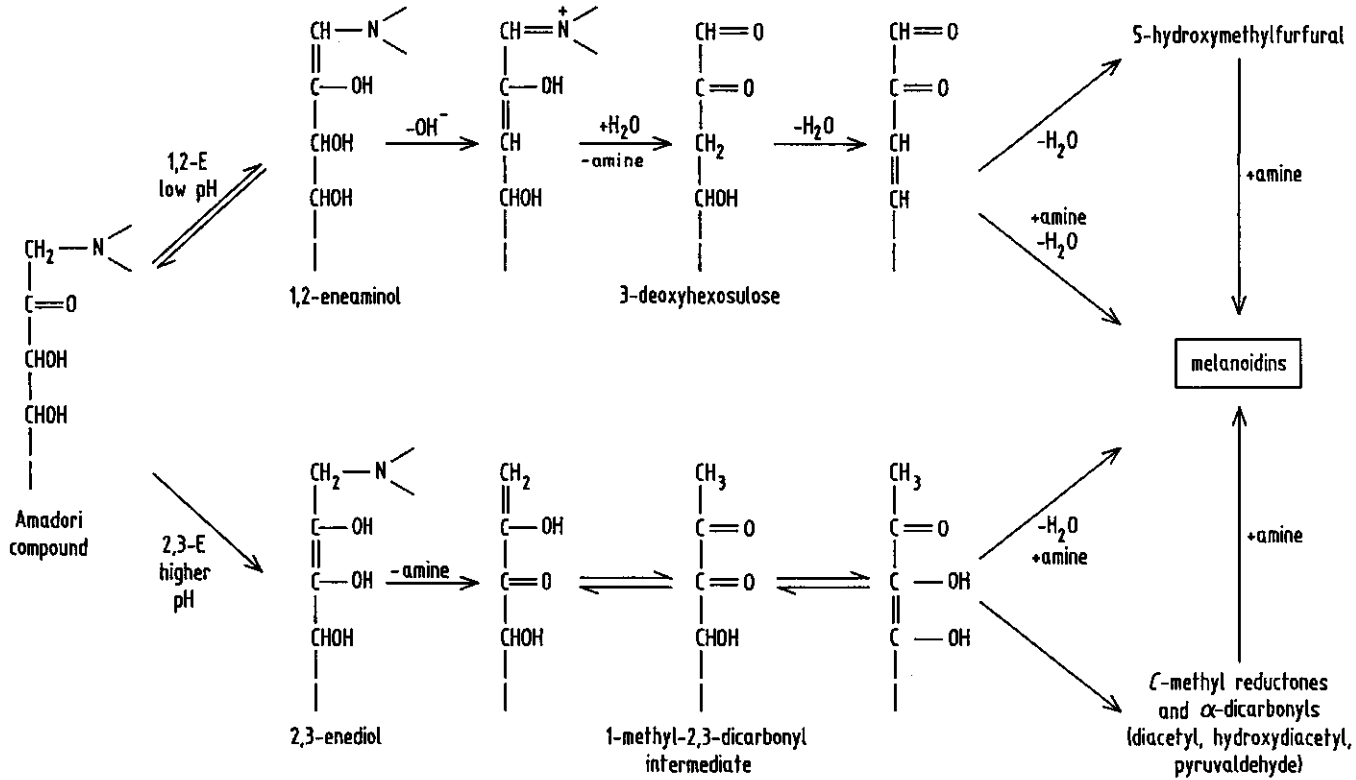


Figure 1.6 Advanced Maillard reactions: Two major pathways from Amadori compounds to melanoidins (Hodge, 1967)

The third reaction pathway is the Strecker degradation. This reaction involves the oxidative degradation of amino acids by the α-dicarbonyls and other conjugated dicarbonyl compounds formed during the above mentioned pathways 1 or 2. Amino acids react with α-dicarbonyl compounds to form Schiff bases which enolize into amino acid derivatives that are easily decarboxylated. The new Schiff base with one carbon less is then easily split hydrolytically into the amine and aldehyde, which correspond to the original amino acid with one carbon atom less. Most of the carbon dioxide evolved in the Maillard reaction originates from the carboxyl groups of the amino acids. The amino ketones and aldehydes formed in the Strecker degradation are very reactive: pyrazines are formed from the amino ketones and they are well known as aroma compounds in roasted products. The aldehydes and amino ketones also condense with themselves, furfurals or other dehydration products to form melanoidins and pyrazines, well known as aroma carriers of roasted products. During the later stage of the advanced Maillard reaction a great number of heterocyclic compounds are formed, they are largely responsible for the roasted, bready and nutty flavours in heated foods (Mauron, 1981). Because the formation of these aroma compounds at lower temperatures seems to be less probable, these compounds ought to appear less frequently during food storage and mild heat treatments (Baltes, 1982). However, Patton (1955) emphasized that there are substantial indications of the importance of the Strecker degradation to the flavour of dairy products.

Kato et al. (1986, 1988, 1989) described also a pathway for the degradation of the Amadori product. They investigated the amino-carbonyl reaction between proteins and reducing and non-reducing sugars. Ovalbumin was used as a protein and they investigated the reaction with glucose, mannose, galactose, talose, lactose, 4-O-methyl-D-glucose, maltose, cellobiose, isomaltose and melibiose. They found no large differences in the amino group decrease among these mixtures but they did find remarkable differences in browning and protein polymerization. Galactose and talose induced browning and cross-linking more strongly than glucose and mannose did and lactose did induce them much less than glucose or galactose did. The differences in the advanced Maillard reaction were explained by the stability of the Amadori compound. Because 4-O-methyl-D-glucose reacted in much the same way as lactose, it was suggested that the C-4 hydroxy group (which is not available in lactose) plays an important role in the advanced stages of the Maillard reaction. Cleavage between the C3-C4 bond is proposed as the

mechanism. Melibiose and isomaltose also induced browning and polymerization very strongly whereas maltose, cellobiose and lactose did not. This indicated that the terminal pyranoside groups bonded at the C-4 hydroxy group of glucose retard further degradation to brown compounds and polymers.

Final Maillard reactions

During the final stage of the Maillard reaction the brown melanoidins are formed. These are polymerization products that are not dialysable and are supposed to have a molecular weight above 1000. The structures of these melanoidins are very complex and not much is known about the chemistry of the formation of these polymers (Mauron, 1981).

Characterization of the Maillard reaction

The final Maillard reaction is easily recognized in foods, because of the formation of melanoidins and aroma compounds. But when these reactions have taken place, the Maillard reaction is already in a final stage and the food may be spoiled. Also the presence of HMF merely shows that the Maillard reaction has already occurred or that sugar containing, acidic solutions have been heated. Therefore, it appeared necessary to detect the early stages of the Maillard reaction. Proof of the presence of the initial, colourless Amadori compounds provides a method to detect the early stages of the Maillard reaction and they may also provide an indicator of the heat treatment. The furosine method is a method to determine early Maillard products; this method is described later in this review.

1.2.1 Isomerization of lactose during heating of milk

Lactulose is absent in raw milk, and is formed during heat treatment. Much research has been done on the formation of lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose) during heat treatment. Lactulose was isolated by Montgomery and Hudson (1930) from a solution of lactose in saturated limewater. They suggested that it was formed by the LA-transformation of lactose (Figure 1.7).

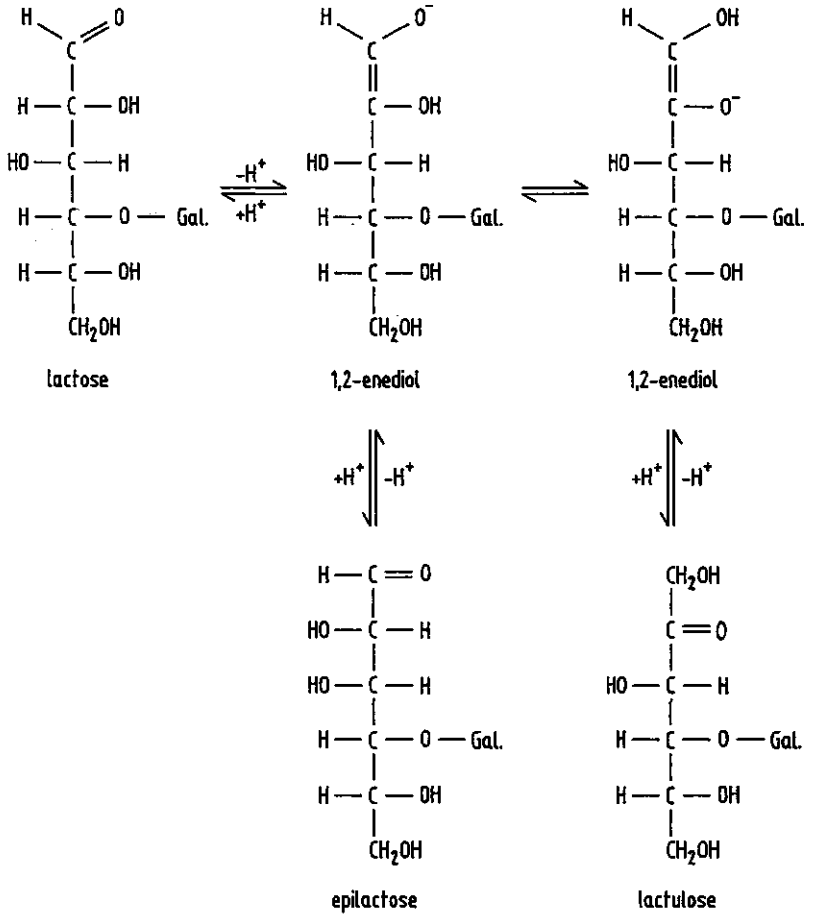


Figure 1.7 Lobry de Bruyn-Alberda van Ekenstein transformation of lactose into lactulose and epilactose. Gal. = galactose moiety

Epilactose is also formed by the LA-transformation, but in much smaller amounts than lactulose and is discussed in 1.2.3.

Lactulose was also suggested to be formed during the Maillard reaction (Adachi and Patton, 1961).

Adachi (1956) isolated two glycosylamines after tryptic hydrolysis of evaporated milk and dried skim milk. He found one of them containing glucose and galactose residues, the other galactose and a ketose sugar. He concluded that lactose had condensed with the ϵ-NH_2 group of lysine residues to form a glycosylamine. Part of the glycosylamine was transformed into lactulosyl-lysine via the Amadori rearrangement.

Richards and Chandrasekhara (1960) identified compounds formed in skim milk powder during storage; these compounds are also known as products of the alkaline degradation of lactose. As the decomposition of an amino-sugar complex could cause the browning in the stored skim milk powder, but -according to these authors- could not lead to the presence of compounds normally associated with the alkaline degradation of lactose, they suggested that these degradation products of lactose are not formed from an amino-sugar complex but by degradation of lactose, catalysed by the free basic amino groups of casein. This can also be concluded from results of Richards (1956), who found that no sugar epimeres formed in a "dry" glucose-glycine mixture under neutral conditions. Such a degradation would follow a sequence of reactions similar to that postulated by Corbett and Kenner (1953) for the degradation of lactose with strong alkali (Figure 1.8).

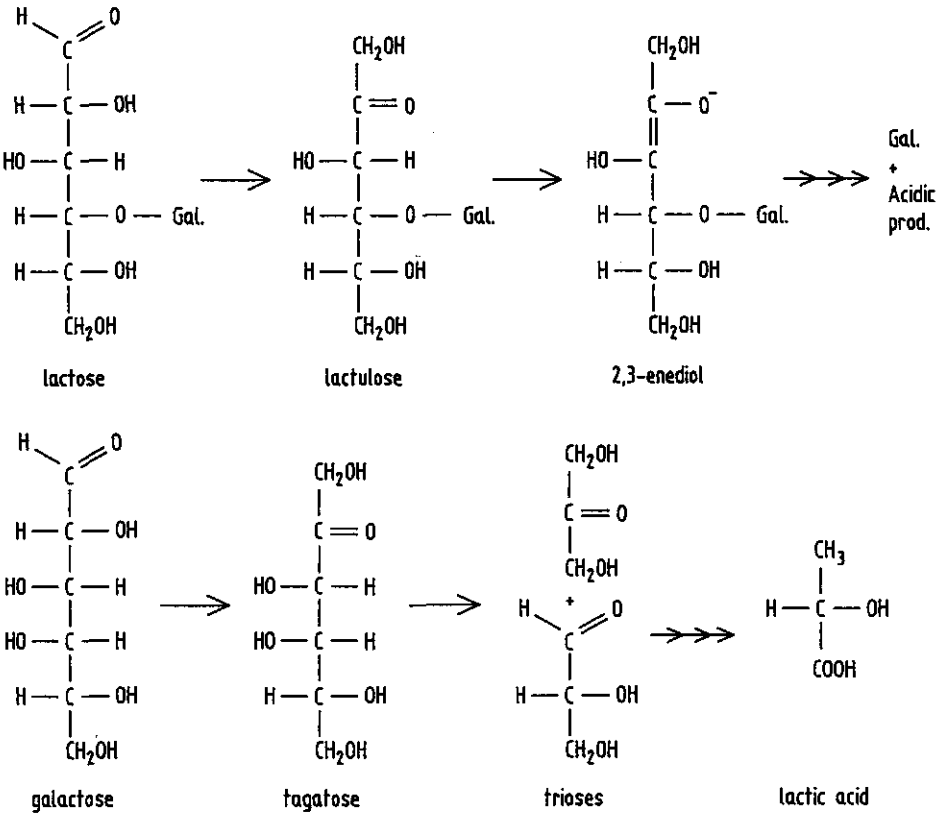


Figure 1.8 Degradation of lactose with strong alkali (Richards and Chandrasekhara, 1960)

Lactose is epimerized to lactulose, and the fructose moiety of lactulose is further degraded resulting in acidic products, while galactose is split off. The galactose then partly epimerizes to tagatose, which can be degraded into trioses. Those trioses can be oxidized to acids, for example lactic acid. The products formed are very reactive and can react in the Maillard reaction to form brown components. Richards (1963) found a close relation between the formation of 1-amino-1-deoxy-2-ketoses and the degradation of free NH_2 groups in dried skim milk powder and "dry" lactose-casein mixture. After 60 days at 45°C, the concentration of 1-amino-1-deoxy-2-ketoses decreased, but without an increase of free amino groups. Therefore, he concluded that the degradation did not take place by hydrolysis of the amino-carbonyl compound. This is in agreement with Hodge (1955) who concluded the sugar-amino linkage of the amino-carbonyl compound in general to be stable to hydrolysis but the bond could be broken by dehydration resulting in a free sugar group.

Effect of protein

Martinez-Castro et al. (1986) and Andrews and Prasad (1987) proposed that the formation of free lactulose in heated milk proceeds exclusively by the LA transformation, catalysed by the milk salt system. To study the effect of free ϵ-amino groups on the formation of lactulose, Olano et al. (1989) heated lactose in buffer solutions containing variable amounts of N-α-acetyl-L-lysine. The lactulose concentration decreased by adding increasing amounts N-α-acetyl-L-lysine; this is the same result as found by Andrews and Prasad (1987) in concentrated milks. Greig and Payne (1985) showed that lactulose was formed mor e rapidly in milk ultrafiltrate than in milk. They concluded that casein, or a component of casein, inhibits the formation of lactulose. Andrews and Prasad (1987) compared the lactulose formation during heat treatment in ultrafiltrate with that in milk diluted with ultrafiltrate. They found the lactulose content of the ultrafiltrate to be much lower than that of milk diluted with ultrafiltrate, suggesting that the milk protein may have had a catalytic effect on lactulose formation. This is contrary to the results of Greig and Payne (1985). Calvo and Olano (1989) also heated ultrafiltrate, milk and concentrate and found the lactulose concentration the highest with the lowest protein concentration. The lactulose concentration increased with increasing heat treatment and increasing pH. Andrews and Prasad (1987) showed that an increasing amount of protein resulted in a decrease in lactulose

concentration. So, a small amount of protein increased the lactulose level and increasing amounts of protein reduced the lactulose level. As the apparent activation energies for formation of lactulose in milk (127.8 ± 6.4 kJ/mol) and in ultrafiltrate (131.0 ± 2.5 kJ/mol) were almost the same, they concluded that protein did not catalyse lactulose formation. They proposed that the decrease of lactulose level found with the increase of protein concentration is due to increased formation of the lactosyl-amino compounds which would reduce the substrate concentration for lactulose formation. It can also be due to the condensation of lactulose with an amino group, which would remove free lactulose from solution. Or it may be a combination of both factors. From the results of Andrews and Prasad (1987) two conclusions could be drawn: First, the fact that a considerable amount of lactulose was found in heated ultrafiltrate indicates that the free amino groups of milk protein are not a necessary catalyst for lactulose formation, as Richards and Chandrasekhara (1960) suggested. However, it remains possible that milk protein acts as a catalyst. Second, they concluded from their results that lactulose is not formed as a result of hydrolysis of lactulosyl-lysine because this would then be formed in greater amounts in concentrated milks. We feel that this is not necessarily true, because a greater amount of protein possibly increases the degradation of the formed lactulose. However, Andrews and Prasad (1987) did not determine the formation of degradation products of lactulose; they did not even mention the possibility of lactulose degradation.

Effect of pH and minerals

Adachi and Patton reported in 1961 that lactulose formation increased with rise of the pH of the milk from 6.6 to 7.0. This is quite conceivable because the isomerization of reducing sugars is favoured at higher pH values. This was also suggested by Overend et al. (1961) who found that for simple sugars the transformation from cyclic to reducible form (open chain) was enhanced with increasing pH. Martinez-Castro and Olano (1980) found the degree of isomerization of lactose decreasing markedly at lower pH values (6.0 to 6.5). Geier and Klostermeyer (1983) also studied the influence of pH on the lactulose formation. They adjusted raw skim milk with initial pH 6.70 to pH values between 6.59 and 6.72 prior to sterilization at 120°C for 10 min. They found a 28% decrease at pH 6.59 and a 9% increase at pH 6.72 compared to the lactulose formation in the original milk. However, UHT milks with pH values in a similar range due to different

pre-processing storage conditions of the same milk showed only slight differences in the lactulose contents. Martinez-Castro and Olano (1980) found that addition of Na_2HPO_4 caused a slight increase of lactulose formation, but this was probably due to the rise of pH caused by Na_2HPO_4 addition.

Martinez-Castro et al. (1986) heated several buffer solutions containing 5% lactose. Buffer solution A (Na_2HPO_4/NaH_2PO_4), B (Na_2HPO_4/sodium citrate), C (Simulated Milk Ultrafiltrate, SMUF) and D (SMUF without $CaCl_2$ and $MgCl_2$) were made. After heat treatment of these solutions, lactulose, galactose and epilactose had formed. Concentration and type of buffer seemed to have no influence, only pH was a source of variation. With increasing pH, the formation of lactulose, galactose and epilactose increased. When solution C was heated above 100°C a precipitate containing calcium phosphate was formed:

$$Ca^{2+} + H_2PO^-_4 \rightarrow CaHPO_4 + H^+$$

which partly redissolved on cooling (Nieuwenhuijse et al., 1988). So, during heat treatment of solution C the pH dropped, resulting in a lower content of lactulose as compared to solutions A and B. In solution D no precipitation was observed and more lactose was transformed than in solution C, but slightly less than in solutions A and B. The concentration of lactulose formed in solution C was comparable with that in milk, the concentration in solution D was considerably higher. Phosphates and citrates are responsible for the buffer capacity of the solution and thus affect the lactulose formation in an indirect way. Andrews and Prasad (1987) also reported a catalytic effect of citrate and phosphate buffers on the formation of lactulose, presumably by acting as bases. As the amount of lactulose formed in solution C was in the range of the lactulose concentration in heated milk, Martinez-Castro et al. (1986) suggested that most of the lactulose in heated milk is formed through the LA-transformation of lactose due to the buffering of milk, as the absence of protein in these systems did not seem to have any influence on the lactulose formation.

Olano et al. (1987) also studied the effect of pH and calcium on the isomerization of lactose during heat treatment of simulated milk ultrafiltrates. They used two kinds of SMUF; one a normal Jenness and Koops buffer (SMUF-A) and

one without $CaCl_2$ and $MgCl_2$ (SMUF-B). After heat treatment, they found lactulose, galactose and epilactose. The formation of lactulose increased with pH. At pH > 7 a large effect was found, but pH values within the range found in normal milk samples had only a small influence, if any. The lactulose formation in SMUF-A was considerably less than in SMUF-B. This inhibiting effect of calcium was also observed by Martinez-Castro et al. (1986). It was suggested that this effect was caused by a drop of pH induced by precipitation of calcium phosphate (Walstra and Jenness, 1984).

Effect of fat content

Andrews (1984) compared the lactulose formation in whole and separated milk processed in the same UHT-plant. No large difference was apparent. Therefore, he concluded that the fat content of the milk did not have any influence on the lactulose formation during heat treatment. Geier and Klostermeyer (1983) reported the same results. This is in contrast with results by De Koning et al. (1990), who studied the effect of the fat content on the properties of UHT-milk. They heated milk with 1.5 and 3% fat (direct heat treatment for 2.5 and 15 sec at 145°C and indirect heat treatment for 15 and 30 sec at 142°C). The lactulose content of the heated milk with 3% fat appeared to be 40-50% higher than the lactulose content of UHT-milk with 1.5% fat. No explanation for this large unexpected effect of fat was given. More recent (not yet published) results indicate that the presence of fat reduces the heat load during UHT heating, possibly due to a turbulence depressing effect of fat (van Boekel, 1992, private communication).

Effect of O_2

The oxygen level also can play a role in the degradation of lactose during heating of milk.

Isbell (1976) described a mechanism for the degradation of reducing sugars by oxygen. The oxidation of D-fructose by oxygen in alkaline solution can be described by four reaction paths. The 1,2-enediol of D-fructose yielded 77% of D-arabinonic acid and formic acid and 10% of D-glyceric acid and formic acid; the 2,3-enediol yielded 5% of D-erythronate and glycolate and 5% glycolate and formic acid. The remaining 3% of D-fructose was converted into saccharinic acids and other products. However, Patton (1955) concluded from several studies on the role of oxygen, that the browning of milk is independent of the oxygen level.

The solubility of O_2 in water decreases with increasing temperature; at 100°C it is only 0.76 mmol/l (BINAS, 1977). So, during heating at high temperatures, the oxygen level probably does not play a very important role. Fink (1984) studied the correlation between concentration of thiamin, lysin, ascorbic acid, free SH-groups and oxygen level during storage of the milk. He found no influence of oxygen on thiamin and lysin concentration, but found that in the presence of sufficient oxygen ascorbic acid and free SH-groups were totally oxidized.

Reactivity of lactose and lactulose

Olano and Martinez-Castro (1981) compared the reactivities of lactose and lactulose. They dissolved both disaccharides in aqueous salt solutions. Considerable isomerization into other disaccharides was found in lactose solutions, whereas formation of monosaccharides and loss of carbohydrates was more significant in lactulose solutions. These results suggest that lactulose is mainly degraded by β-elimination and subsequent degradation (Figure 1.3) and that lactose degradation seems to occur through previous transformation into lactulose by the LA-transformation (Figures 1.2 and 1.7).

1.2.2 Galactose formation

Richards (1963) stored dried skim milk and a "dry" lactose-casein mixture at 45°C and 75% R.H. He found a maximum galactose concentration after 50 days storage (0.33 mmol galactose/g total N for skim milk powder and 0.50 mmol galactose/g total N in the "dry" lactose-casein mixture). The galactose formation was much higher than the lactulose and tagatose formation for both systems. In dried skim milk, 0.035 mmol lactulose/g total N and 0.011 mmol tagatose/g total N were formed and in "dry" lactose-casein mixture 0.015 mmol lactulose/g total N and 0.006 mmol tagatose/g total N. This is contrary to the results of Corbett and Kenner (1953), who found the formation of lactulose to be higher than that of galactose, but this was under very different circumstances, because they estimated the sugars after degradation of lactose in lime water. Richards (1963) dialysed dried skim milk (stored for 22 days) and a "dry" lactose-casein mixture (stored for 10 days) until no sugars were detected any more. Then he stored the dialysed and subsequently freeze-dried materials again. After 10 days storage no sugars were detected. After 31 days galactose was detected but no lactulose. The colour

increased during storage (most for dried skim milk) and free HMF was formed (0.46 μmol/g in dried skim milk and 0.18 μmol/g in "dry" lactose-casein mixture).

Two mechanisms were suggested to explain the formation of lactulose, galactose and tagatose from lactose in milk:

1. Richards and Chandrasekhara (1960) postulated that these compounds are formed by degradation of lactose catalysed by the free amino groups of casein.
2. Adachi and Patton (1961) postulated that lactulose is formed by the hydrolytic degradation of the Amadori product of lactose-casein.

As Richards did not find any lactulose in the dialysed materials, he concluded that lactulose is probably formed only by the base catalysed degradation of lactose and that galactose is formed both by the base catalysed degradation of lactulose and by the breakdown of the protein-sugar complex. This may also explain why he found more galactose than lactulose. Because he found a relationship between the HMF and galactose concentrations (though not equimolar), he postulated that galactose is formed mainly by the breakdown of the 1-amino-1-deoxy-2-ketoses.

Olano and Calvo (1989) found that the galactose formation during heat treatment of milk increased with increasing temperature and time. Calvo and Olano (1989) studied the effect of the initial galactose concentration (35.2 to 45.6 μmol/100 ml). They found that in this concentration range the galactose concentration did not affect the amount of galactose present in heated milks. They found that galactose formation also increased with increasing pH. When milk, ultrafiltrate and concentrate were heated, galactose concentration increased with protein concentration and with heat treatment, whereas the lactulose concentration was highest at the lowest protein concentration. They presumed that part of the galactose originated from the reaction of lactose with the free amino groups of lysine followed by the further degradation of lactulosyl-lysine formed. But when they added free α-acetyl-lactulosyl-lysine to milk, the amount of galactose formed after heat treatment did not increase. However, lactulosyl-lysine in milk is bound to casein and α-acetyl-lactulosyl-lysine is a low-molecular weight compound. When they heated galactose solutions in the presence of casein and whey proteins, the degradation of galactose was reduced as compared to the degradation of galactose in SMUF. In a model solution containing proteins heated in the absence of lactose, no galactose was formed. Olano et al. (1989) estimated the galactose, epilactose and lactulose contents after heat treatment of a lactose buffer solution with variable amounts of N-α-acetyl-L-lysine. They found an increase in galactose and

epilactose concentration when 1.28 mmol/100 ml was added to the buffer, but the formation of these sugars was reduced on further addition of N-α-acetyl-lysine. This is similar to the effect of protein concentration on the lactulose formation as found by Andrews and Prasad (1987). Olano et al. (1989) reported an increase of the galactose concentration with severity of heating (e.g., 69.4 μmol/100 ml at 4 s 140°C and 117.7 μmol/100 ml at 20 min 120°C). As the glucose concentration remained almost unaltered (about 0.12 mmol/l), they concluded that most of the galactose must have been formed through degradation of the reducing group of lactose, resulting in saccharinic acids and galactose according to Corbett and Kenner (1953). However, they did not determine saccharinic acids experimentally.

1.2.3 Formation of epilactose

Epilactose is a disaccharide formed from lactose by isomerization via 1,2-enolization (Figure 1.7). Martinez-Castro and Olano (1980) isolated an unknown disaccharide from the reaction mixture obtained after epimerization of lactose. Acid hydrolysis of the unknown disaccharide resulted in a hexose mixture with equal quantities of galactose and mannose. The disaccharide was assigned to be epilactose (4-O-β-D-galactopyranosil-D-mannopyranose). In milk the isomerization of lactose into lactulose and epilactose increased with pH, but the lactulose/epilactose ratio also increased with pH (from 6.0 at pH 6.6 to 11.2 at pH 7.5 at 120°C). Olano et al. (1989) found epilactose in all samples of in-container sterilized milks, and no epilactose was detected in dried, pasteurized or UHT milk. Hence they concluded that epilactose determination could be a suitable procedure to distinguish UHT from sterilized milks. Olano and Calvo (1989) studied the kinetics of epilactose formation. They found concentrations varying from 0.03-1.0 mmol/l after heating times varying from about 1700 s at 100°C to 600 s at 150°C. They supposed that formation of epilactose was a first order reaction.

1.2.4 Formation of formic acid

During heat treatment of milk formic acid is formed. There is very little recent literature on this subject. Nef (Nef, 1907) postulated that in alkaline solutions of D-glucose and D-galactose a series of enediols is formed, the 1,2-, 2,3- and 3,4-form. From the 3,4-enediol pyruvic aldehyde is formed. At lower temperatures and

lower alkalinity pyruvic aldehyde is degraded into formic and acetic acid, at higher temperatures and alkalinity into lactic acid. If the 1,2-enediol is split at the double bond, formaldehyde and the appropriate pentose are formed. Formic acid is formed from the formaldehyde, so both the 1,2- and the 3,4-enediol are sources of formic acid (Evans et al., 1926).

Whittier and Benton (1927) studied the formation of acid in milk by heating. They continued heating after coagulation had taken place and found that the hydrogen ion concentration continued to follow the same curve after precipitation of the casein. They concluded that the source of the acid is a constituent of the serum. Investigators of the mechanism of sugar oxidation have found that lactose is very easily oxidized (Whittier and Benton, 1927). Under weakly oxidizing conditions formic acid is always one of the acids produced. Levulinic acid is characteristic of acid oxidations of lactose, saccharinic acids of alkaline oxidations. When 5% lactose was added to skim milk practically twice as much acid was produced as in heated normal skim milk. If solutions of 5% lactose containing the same concentrations of total phosphate, total citrate and of hydrogen ions as are present in normal milk were heated, the changes in hydrogen ion activity and acidity were very similar to those in milk under the same time and temperature conditions of heating (Whittier and Benton, 1927). They concluded that lactose was the principal source of acid formed in heated milk. When lactose was replaced by sucrose no acid was produced. The loss of lactose was sufficient to account for more than four times the amount of acid formed.

Gould (1945a) found the acidity produced in whey about one ninth as compared to milk under similar heating conditions. From these results he concluded that removal of the casein and the minerals associated with the casein (they probably meant micellar calcium phosphate) greatly reduced the heat production of acid. The lactic acid created was less than 5% of total acid produced.

Kometiani (1931) found the formation of formic acid in heated milk to represent about 20-25 percent of the total lactose loss. After 3 hours at 100°C, 4.8 mmol/l was found and after 30 min at 120°C, 9.1 mmol/l. He also found the amount of lactic acid formed to be four to five times the amount of formic acid and together they account for the total loss of lactose.

Gould (1945b) measured the amount of formic acid obtained from the distillate. He found 2.8 to 3.4 mmol/l for skim milk heated at 116°C for two hours. The formic acid represented 80-85% of the total volatile acidity.

Gould and Frantz (1946) found 2.2 mmol/l for milk heated 1 hour at 116°C and 4.7 mmol/l for milk heated 2 hours at 116°C. They concluded that the distillation procedure is not a highly quantitative method as they found a recovery of 66.5%. They also found the proportion of formic acidity to titrable acidity larger when the heating time progressed. This may be due to a relatively larger production of formic acid than other acids or to lesser so-called "non-acid" changes; these are changes which may affect the titrable acidity, not necessarily involving organic acid production. Salt changes, for example, may occur early during the heating period and these changes may affect the acidity.

Patton (1950b) described the possible mechanism of the degradation of lactose into formic acid and furfuryl alcohol (Figure 1.9) at pH 6-8; at pH-values below 6, HMF is supposed to be formed without formic acid.

Patton and Flipse (1957) added lactose-1-C^{14} to condensed skim milk to investigate the degradation products of lactose during heat treatment (4 hours at 121°C). They found radioactivity in maltol and formic acid but not in furfuryl alcohol; this means that furfuryl alcohol is derived from carbon atoms 2 to 6 of the glucose moiety of lactose and the formic acid from carbon 1.

Morr et al. (1957) described a chromatographic method to analyse acids. The recovery of formic acid was still poor; 59.2%. After six hours heating of skim milk at 100°C about 75% of the acid formed was identified as formic acid. The formation of acetic and formic acids was closely related to browning. They measured the formation of formic acid in normal skim milk and in phosphate-treated (0.5% disodium phosphate was added just prior to heating) skim milk. The phosphate-treated samples exhibited acid concentrations that were two to three times that of the normal skim milk samples (especially in the case of formic, acetic and lactic acid).

Marsili et al. (1981) developed a high performance liquid chromatography (HPLC) method to determine organic acids. The recovery of formic acid was about 98%. The method is well suited to the analyses of organic acids in dairy products. We used this method in the present study.

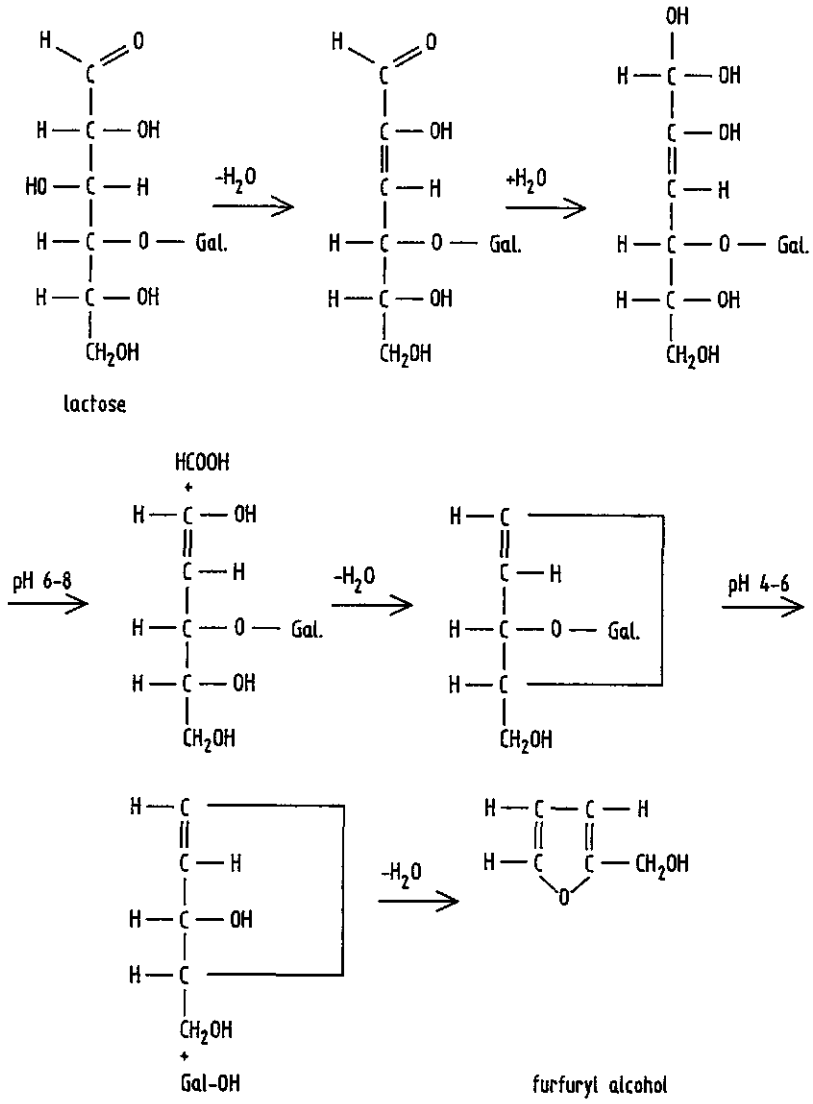


Figure 1.9 Formation of formic acid and furfuryl alcohol from lactose according to Patton (1950b)

1.2.5 Formation of HMF

One of the compounds involved in browning is 5-hydroxymethyl-2-furfural (HMF). Formation of HMF in the Maillard reaction is described by Hodge (1967); HMF is formed from the 1,2-enediol (Figure 1.6). Patton (1950a) studied the formation of HMF in heated skim milk, in model systems containing lactose and

glycine and in model systems containing lactose, glucose or galactose and casein. They found HMF formation during heating of all these systems. They also heated a control sample containing lactose without protein, and this solution showed only little discoloration and no HMF could be recovered. So, they concluded that the conversion of lactose into HMF is facilitated by the presence of glycine, casein or heat degradation products of casein, and that HMF formation is associated with browning in these systems. Further research of Patton (1950b) showed the importance of pH and buffer capacity of the heated systems. Condensed skim milk and weakly alkaline lactose solutions produced both HMF and furfuryl alcohol, acidified condensed skim milk and neutral or acidic lactose systems yielded HMF but no furfuryl alcohol. Their findings also showed that both HMF and furfuryl alcohol are produced in pure lactose solutions having the required pH and buffer capacity. In milk various protein groups and salts create such conditions.

Lee and Nagy (1990) studied the relative reactivities of sugars in the formation of HMF in sugar-catalyst model systems. As fructose is less stable than glucose at pH 3.5 and enolizes faster than glucose, it is five times more reactive. The rate of HMF formation from sucrose was less than from fructose but more than from glucose, which is probably due to the fructose portion of sucrose. The sucrose, a nonreducing sugar, is first hydrolysed to the reducing sugars. They also studied the effect of acids, minerals and amino acids on the formation of HMF from fructose, glucose and sucrose. Three amino acids were used; alanine, aspartic acid and γ-aminobutyric acid. The rate of HMF formation from glucose or sucrose was slightly enhanced by the presence of amino acids. The formation of HMF from fructose did not change with addition of amino acids. It was suggested that the catalytic effects of amino acids were less important to fructose because it contained a high percentage of the acyclic form.

A lot of research is done on the question whether or not HMF is a suitable indicator of the heat treatment applied. Konietzko and Reuter (1986) studied the total HMF formation in UHT milk and concluded that it can be used as a parameter for control of the heat treatment applied to the milk. Investigations of Fink and Kessler (1986) showed that HMF could be measured simply and rapidly and that the HMF value was well suited for distinguishing between UHT milk and sterilized milk. Fink and Kessler (1988) compared four parameters that may be suitable as indicators for control of the heat treatment; lactulose, HMF, colour and serum protein denaturation. They found HMF value and lactulose concentration to be the

most suitable, but, as lactulose determination (enzymatically) was rather slow and HMF value determination was cheap, easy and quick, this appeared to be the most useful method to estimate the severity of heat treatment of milk and to distinguish between UHT milk and sterilized milk. Dehn-Müller et al. (1988) found the HMF method to be a useful alternative for the expensive and difficult furosine method, which is discussed later in this review.

Kind and Reuter (1990) reported the suitability of HMF values for detecting the heat treatment of UHT milks to be limited, because they also found a HMF content in the raw milk, which is not constant and because they found the temperature-time-conditions of commercial UHT plants are partly in a range in which formation of HMF is non-linear with time.

It remains a problem, however, how HMF contents can be quantitatively related to the Maillard reaction. Dehn-Müller et al. (1988) found a fairly good correlation between furosine and HMF concentrations in UHT milk. From the furosine concentration the losses in available lysine can be calculated (Erbersdobler, 1986).

1.2.6 Maillard reaction in milk

Patton and Flipse (1953) showed that heating of casein and lactose-1-C^{14} in milk resulted in an amino-carbonyl complex in which C^{14} activity was found. This complex was recovered by exhaustive dialysis and degraded by further heat treatment. Lactose was not found after degradation, but a slightly brown colour was formed, suggesting that the complex was destroyed without regeneration of lactose.

Nielsen et al. (1963) also added lactose-1-C^{14} to fresh skim milk before heating. After heating and exhaustive dialysis they also found radioactivity in the protein fraction. This indicated that a relatively large molecule had been formed in which lactose had participated.

Turner et al. (1978) studied the interaction of lactose with proteins in model systems and skim milk during UHT processing. They found that casein incorporated five to six times the amount of C^{14} as compared to α-lactalbumin or β-lactoglobulin. This cannot be explained by the primary structure of the proteins. Probably the accessibility of the lysyl residues is different for the various proteins. Studying the incorporation of C^{14}-lactose into skim milk proteins showed the radioactivity associated with κ-casein to be much more (65%) than the radioactivity associated

with β-casein and α_{s1}-casein. It was suggested that the greater incorporation of lactose in κ-casein also results from differences in accessibility of the reactive lysyl group (Turner et al., 1978).

Furosine

The evaluation of the extent of the Maillard reaction in milk has always been a problem. Proof of the presence of initial Amadori products would be a method which recognises the early stages of the Maillard reaction. For milk, the general method until about 1980 was measurement of HMF or was based on the measurement of available or reactive lysine (Hurrell et al., 1979). Finot et al. (1981) described a method based on the measurement of the Amadori compound lactulosyllysine, which is a biologically unavailable molecule formed from lactose and lysine. Lactulosyllysine is analysed by means of the so-called furosine method. During acid hydrolysis of the milk sample, furosine is formed from lactulosyllysine, and it can be determined by amino acid analysers and by HPLC (Erbersdobler, 1986). Finot et al. (1981) found the furosine method an excellent tool to measure the blocked lysine and thus the Maillard reaction in milk. However, a problem in the determination of furosine is the fact that there is until now no commercially available, stable and pure standard. Erbersdobler (1986) stated that furosine is formed out of protein-bound fructoselysine at a constant level of 40% (Figure 1.10). The furosine concentration was calculated from the peak area using the response factor for arginine as comparison, as arginine is the closest amino acid to furosine in the amino acid chromatogram, and an additional factor of 0.9 was introduced. So a conversion factor of 0.36 for the formation of furosine was assumed. This factor has also been estimated by others and varied from 0.29 to 0.36. The method using a amino acid analyzer is rather complicated and not really reliable as the furosine concentration in the milk is not determined on the basis of a pure furosine standard.

Chiang (1983) described a simple HPLC procedure for determination of furosine, but again quantification is a problem.

Resmini et al. (1990) described a direct HPLC method to determine furosine in milk and dairy products. They used 2-acetylfuran as external standard for routine quantification purposes. The response factor of 2-acetylfuran was found to be comparable to that of furosine. If their results are correct, the data reported till now, obtained by ion exchange chromatography and gas liquid chromatography

using traditional amino acids as standard, are underestimated at least by a factor of 2.

However, we feel that the furosine method is not yet adequate to determine lactulosyllysine, for two reasons. One reason is that the response factor of furosine in the chromatogram can not be determined accurately enough; the second reason is that the conversion factor of furosine content to lactulosyllysine concentration was found to vary substantially (Erbersdobler, 1986).

Henle et al. (1991a) also described a method to determine furosine, pyridosine, lysinoalanine and common amino acids by amino acid analysis. The furosine and pyridosine contents were calculated by taking standards of lysine and arginine, respectively, as references. They found a ratio of furosine to pyridosine different from previously published values. Henle et al. (1991b) also described a new method for the determination of modified and unmodified lysine in heat-treated milk products. This method is based on the direct measurement of enzymatically released lactulosyllysine as well as lysine, after complete enzymatic hydrolysis, via ion exchange chromatography. The lactulosyllysine contents were calculated by

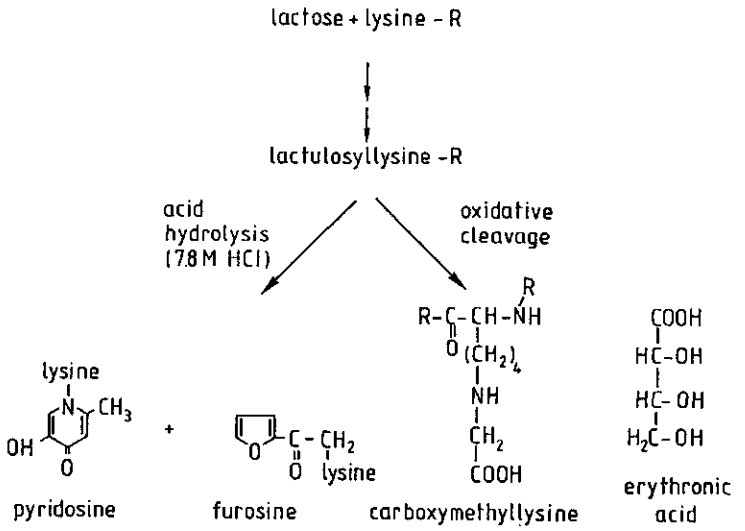


Figure 1.10 Initial steps of the Maillard reaction with the formation of furosine (after hydrolysis with 7.8 M HCl) as well as of N-ε-carboxymethyllysine (CML) and erythronic acid (Erbersdobler and Dehn-Müller, 1989)

taking a standard of lysine as reference. They suggested that the method might

serve as an alternative procedure to the furosine method, as the latter may lead to a significant under-estimation of lysine damage.

Carboxymethyllysine

Büser and Erbersdobler (1986) found a new peak in the gas liquid chromatogram of hydrolysed milk and identified it as N-ϵ-carboxymethyllysine (CML). They found a good linear correlation with an average ratio of 1:3 between CML and furosine in several milk products. However, this ratio is not always constant and conditions for the formation of CML seem to depend on oxidative processes and on the presence of glycosylated lysine (Büser and Erbersdobler, 1986). CML is formed by oxidative cleavage of fructoselysine into erythronic acid and CML (Figure 1.10), and it seems to be an interesting indicator of heat damage, as the formation of CML increased with increasing heat treatment (Erbersdobler and Dehn-Müller, 1989). Badoud et al. (1990) described the use of CML to measure the blockage of free ϵ-amino groups of lysine residues, which is important from a nutritional point of view. The protein-bound Amadori compounds were degraded with periodic acid to release CML upon acid hydrolysis. CML was quantified by reversed-phase HPLC after precolumn derivatization. Lüdemann and Erbersdobler (1990) measured the formation of CML and fructoselysine (fructoselysine by means of the furosine method) in various model systems. They concluded that CML may be helpful in characterising heat damage, namely by detecting oxidative influences. CML is more heat resistant than fructoselysine and, thus, can also be used as an indicator of severe heat damage.

Ames mutagenicity assay

Ekasari et al. (1986) found that heat treatment of orange juice induced mutagenicity under special conditions. The mutagenicity was dose related and related to the time of heating. They ascribed the mutagenicity to the early Maillard products and suggested the use of the Ames test as a measurement for the heat damage of orange juice. Following Ekasari et al. (1986), we studied whether the bacterial mutagenicity assay of Ames could also be used as a method to measure the extent of the early Maillard reaction in milk (Berg et al., 1990). However, no mutagenic response was found in heat treated milk or model solutions of lactose and casein and lactose and lysine. One of the reasons is that casein appears to be a very effective antimutagenic agent. However, since also no response was found

with lactose and lysine, it appears that the use of the Ames test for measuring the extent of the Maillard reaction is not well suited, and may only be useful in the specific case of orange juice.

1.3 Effect of temperature

This study is concerned with the degradation of lactose during heating. Clearly, heating promotes this degradation. A very obvious explanation for this is that, in general, reaction rates increase with temperature. The temperature dependence of a reaction rate constant k can generally be described by the transition-state theory developed by Eyring:

$$k = \frac{k_B T}{h}\exp(-\frac{\Delta G^{\ddagger}}{RT}) = \frac{k_B T}{h}\exp(-\frac{\Delta H^{\ddagger}}{RT})\exp(\frac{\Delta S^{\ddagger}}{R}) \qquad (1.1)$$

k_B	= Boltzmann's constant = $1.4 \cdot 10^{-23}$	$(J.K^{-1})$
h	= Planck's constant = $6.6 \cdot 10^{-34}$	(J.s)
R	= gas constant = 8.3	$(J.mol^{-1}.K^{-1})$
T	= absolute temperature	(K)
$\Delta H^{\ddagger}$	= activation enthalpy	$(J.mol^{-1})$
$\Delta S^{\ddagger}$	= activation entropy	$(J.mol^{-1}.K^{-1})$
$\Delta G^{\ddagger}$	= activation Gibbs energy	$(J.mol^{-1})$

In this way, kinetic data are interpreted in terms of thermodynamic properties: reaction rates are determined by changes in activation entropy and enthalpy.

However, the relationship between the rate constant and temperature is frequently taken to follow the well known Arrhenius equation:

$$k = A'\exp(-\frac{\Delta E_a}{RT}) \qquad (1.2)$$

ΔE_a	= activation energy	$(J.mol^{-1})$
A'	= frequency factor	

The Arrhenius equation is an empirical equation and appears to fit many reactions; it is, however, an over-simplification. In principle the more fundamental Eyring relation is to be preferred (van Boekel and Walstra, 1989).

In general, the activation enthalpy (energy) is not very high for bimolecular reactions, in which case the breaking of old bonds and the forming of new ones are highly concerted and synchronous, so that the overall energy requirements are modest. This does not mean that bimolecular reactions will always be very fast, because the molecules have to meet each other (mostly, however, the actual chemical transformation is rate limiting); generally, however, their rates are less temperature dependent than those of unimolecular reactions. The activation entropy for bimolecular reactions is usually quite negative (unfavourable) because translational and rotational entropy of the two reactants is lost; if the activation enthalpy would not be low, these reactions would be immeasurably slow. For unimolecular reactions, the activation entropy depends on intramolecular changes, but usually these reactions do not have a serious entropy constraint. The reactions described in this thesis are either monomolecular (e.g. isomerization) or bimolecular (initial Maillard reaction).

Effect of temperature on isomerization and degradation

Apart from the general effect that temperature has on reaction rates, it is conceivable that activities of reactants increase with temperature. In this respect, it must be realized that a reducing sugar in solution exists in a number of states that are in equilibrium, including two pyranoses (α, β), two furanoses (α, β), an open-chain carbonyl and the hydrated form of the open-chain carbonyl. For most aldoses, the open-chain carbonyl and its hydrated form represent less than 1% of the equilibrium mixture at room temperature; for ketoses, the proportion is somewhat higher. Assuming that reactions proceed via the open-chain carbonyl (which thus determines the activity of the sugar), changes in the above mentioned equilibria cause changes in activity. Unfortunately, there is very little literature on the effect of temperature on the equilibria.

De Wit (1979) found no effect of temperature in the range of 5-80°C on the alkaline degradation of glucose.

Overend et al. (1961) studied the factors which are likely to alter the stability of the ring-form to that of the open-chain. They found an increase in the transformation from cyclic to reducible form at higher temperatures (up to 60°C)

for D-ribose and 2-deoxy-D-ribose. Hayward and Angyal (1977) also found an increase of open chain and furanose form with higher temperatures (up to 60°C). Wertz et al. (1981) found the α-β pyranose-pyranose equilibrium to be hardly temperature dependent, but the pyranose-furanose equilibrium was.

Effect of temperature on the Maillard reaction

Temperature and duration of heating are obviously the most important reaction conditions influencing the course of the Maillard reaction and were studied by Maillard himself, who reported that the rate of reaction increases with temperature. Many workers have confirmed this observation (Mauron, 1981). However, they did not give an explanation. Maillard browning has a relatively high temperature coefficient, the Q_{10} is usually in the range 3-6 (Nursten, 1986). An increase in temperature will probably lead to an increase in activities of lactose and the amino groups.

1.4 Conclusion from literature

According to literature several pathways for lactose degradation are possible:

1 - Lactose → Lactulose → Galactose + Saccharinic Acids + Formic Acid
Lactulose ↑↓ Epilactose

2 - Lactose + Lysine → Lactulosyllysine → Galactose + HMF + Lysine
→ Galactose + Furfural + Formic acid + Lysine

3 - 1 and 2 occur simultaneously

It can be concluded from literature that a possible relation between isomerization and the Maillard reaction has hardly been studied, and that kinetics have only been studied for isolated reactions, not for a series of mutually dependent, simultaneous reactions. It also follows from the literature survey that the pH has a large effect on both the Maillard reaction and the isomerization reactions. A complication thus arises because the pH changes as the very result of lactose degradation.

Another conclusion from the literature is that the open chain form of the sugar is the reactive compound. As there is only a small percentage of a sugar in the

open chain form, there is only a small percentage of a reactive form. However, the equilibrium between ring form and open chain form is established rather fast, so when the open chain has reacted, the equilibrium will soon be recovered and, thus, there will always be a percentage open chain form present in the solution.

1.5 Outline of this thesis

The objective was to determine the kinetics of the chemical reactions associated with lactose degradation that take place during heat treatment of milk. The available literature at the moment this study was started clearly pointed to the Maillard reaction playing a very important role in the lactose degradation. Initially, therefore, we focused on the Maillard reaction, including the possible toxicological effects. The results are published elsewhere (Berg et al., 1990); no toxicological effects could be found. Furthermore, from the results of our experiments it appeared that isomerization followed by degradation may well be much more important in a quantitative sense than the Maillard reaction. Therefore, during the study the emphasis was shifted from the Maillard reaction towards the isomerization reactions.

Experiments were performed in which milk was heated in a glycerol-bath and in a pilot-plant UHT-apparatus. Model solutions resembling milk were also heated in a glycerol-bath. Reaction products and changes in pH, were analysed and an attempt was made to study the kinetics of these reactions in connection with each other.

The methods used for heating milk and model solutions and the analytical methods are described in chapter 2. In chapter 3 the results on milk and model solutions heated in a glycerol-bath are described. The results of the UHT treatments are given in chapter 4 and the reaction kinetics are discussed in chapter 5.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Milk

Fresh cow's milk was obtained from the farm of Wageningen Agricultural University. Raw skim milk was used in most experiments.

2.1.2 Sodium caseinate (Cas)

Sodium caseinate was obtained from DMV Campina, Veghel, Holland. This is a spray-dried milk protein powder containing 94.5% protein (N x 6.38) in the dry matter, 5.2% water, 4.1% "ash" and 0.8% fat.

2.1.3 Chemicals

All chemicals used were of analytical grade and were obtained from Merck, except the following ones:

d-Galactose	Difco 0163-15
Crocein Orange G (Acid Orange 12)	Janssen Chimica 18.936.21

2.1.4 Preparation of Jenness and Koops (JK) buffer

To prepare a salt solution which simulates that of milk serum the method of Jenness and Koops (1962) was followed with some minor adjustments. In 10 l buffer the following salts were dissolved:

KH_2PO_4	15.80 g	Merck 4873
K_3citrate.H_2O	5.08 g	Merck 4956
2 Na_3citrate.11H_2O	21.20 g	Merck 6431
K_2SO_4	1.80 g	Merck 5153
$CaCl_2.2H_2O$	13.20 g	Merck 2382
Mg_3citrate.H_2O	5.02 g	BDH 29098
K_2CO_3	3.00 g	Merck 4928
KCl	10.78 g	Merck 4936

The salts were dissolved one by one in the above order, the next salt was added when the first was completely dissolved. Mg_3citrate was first dissolved in 2 l water under slight heating, and then added. K_2CO_3 was also first dissolved in a little water and was added to the solution made up to 9 l; very carefully with a pipette drop by drop to prevent precipitation. The solution was kept cool and the pH was adjusted to 6.6 just before use. The pH was adjusted with 1 to 1.5 N KOH.

2.1.5 Preparation of model solutions

Model solutions representing simplified milk systems were used. Mostly, JK-buffer was used as salt solution and sugars were dissolved in the JK-buffer. In the case of model solutions containing protein and sugar, sugar and sodium caseinate were dissolved in JK-buffer by stirring overnight in the cold (4°C). The next day the pH was adjusted to 6.6.

2.1.6 Water

The water used was demineralized water, only for HPLC eluent demineralized water filtered over a 0.2 μm filter was used.

2.1.7 HPLC equipment

Two HPLC systems were used, the first consisting of an SP8100 pump-oven-injector (fixed loop, 20 μl), an SP8110 autosampler (both from Spectra Physics), an ERC-7510 RI-detector (Erma optical works) or a Spectroflow 757 UV-visible variable wavelength detector (Kratos Analytical), an SP4200 computing integrator (Spectra Physics) and an Epson personal computer with chromatography software (WINNER, Spectra Physics). The second system consisted of a 4110 pump (Kipp & Zonen), a Marathon autosampler (Spark Holland, fixed loop, 20 μl), an SP8440 UV-visible variable wavelength detector (Spectra Physics) and an SP4290 computing integrator (Spectra Physics) connected to the same personal computer as the first system.

2.1.8 GLC equipment

GLC was performed using a GLC 5890 gas chromatograph (Hewlett Packard), H_2 gas was used as carrier gas and a katharometer was used as detector.

2.1.9 Spectrophotometer

For measuring the extinction of the dye solution a Carl Zeiss M4 QIII spectrophotometer was used.

2.2 Methods

2.2.1 Analytical methods

2.2.1.1 Milko scan

The Milko scan 104 A/B (Foss Electric, Denmark) was used for determination of fat, protein and lactose contents of the raw skim milk. The apparatus measures infrared absorption at different wavelengths and is calibrated against milks having a known composition.

2.2.1.2 pH determination

The pH of the raw skim milk and model solutions was always measured before heating at 20 °C. About thirty minutes after heat treatment the pH was measured again at 20 °C. The equipment used consisted of a Radiometer PHM62 Standard pH Meter.

2.2.1.3 Determination of lysine

The method described by Hurrell et al. (1979) was followed for the lysine determination, with some minor adjustments. This is a dye-binding procedure; the dye Crocein Orange (sodium-6-hydroxy-5-phenylazo-2-naphthalenesulfonate, $[C_{16}H_{11}N_2O_4SNa]$) binds to the basic amino acid units in the protein. To prepare the dye solution 1.3627 g (3.89 mmol/l) Crocein Orange, 20.0 g oxalic acid dihydrate, 3.4 g potassium dihydrogen phosphate $[KH_2PO_4]$ and 60 ml glacial acetic acid were made up with water to 1000 ml. The method requires two absorption

measurements: One on the unmodified sample (the "A" reading) and one on the sample after treatment with propionic anhydride which neutralizes the basicity of the ϵ-NH_2 groups of lysine units in the protein by propionylation (the "B" reading). The first measurement gives histidine + arginine + lysine and the second histidine + arginine, so the difference between the two measurements gives the lysine concentration. For the "B" measurement propionylation was carried out by shaking 1 ml sample, 1 ml sodium acetate solution (16.4%, w/w) and 100 μl propionic anhydride for 15 min in a 25 ml glass flask. Then 10 ml of dye solution were added and this mixture was again shaken for 1 hr. For the "A" measurement the sample was treated in the same way, but instead of propionic anhydride 100 μl water were added, and the solution was shaken for just 1 hr. The mixture was centrifuged for 10 minutes at 3000 rpm, the supernatant was diluted 50 fold with water and its absorbance was measured at 475 nm. The dye concentration was determined from a calibration curve and the amount of dye bound was calculated from the difference between the "A" and "B" reading. From this, the lysine concentration in the sample was calculated.

Furosine

In the study described in this thesis, an attempt was made to synthesize furosine in order to determine the furosine concentration by HPLC. It appeared, however, impossible to isolate pure furosine. A close relationship between the height of the supposed furosine peak and the intensity of heat treatment was found, but the furosine concentration could not be determined.

2.2.1.4 Determination of sugars

For the determination of sugars the provisional International Standard 147, 1991 of the IDF was used. This specifies a HPLC method for the determination of the lactulose content of heated milk. Simultaneously, lactose, lactulose, galactose (tagatose and desoxyribose) can be determined on this column. The reagent in the sugar determination is used to remove fat and proteins. To prepare this reagent 91.0 g of zinc acetate dihydrate [$Zn(CH_3COOH)_2.H_2O$, Merck 8802], 54.6 g of phosphotungstic acid x-hydrate [$H_3(P(W_3O_{14})_4).xH_2O$, Merck 883] and 58.1 ml of glacial acetic acid [CH_3COOH] were dissolved in water and made up to 1000 ml. As standard samples three sugar solutions containing each lactose, lactulose and galactose in water were used in the concentration range expected in the heated

milk samples. Least squares linear regression analysis of peak height versus concentration was performed by the integrator. To prepare the test sample, 15 g was weighed in a 50 ml flask. No volume correction was made, as this was negligible.

HPLC conditions:

For sugar determination an ion-exchange column was used (Aminex HPX-87P, 300 mm x 7.8 mm i.d., Bio-Rad) with a guard column (filled with 65% AG 3-X4A, OH^- and 35% AG 50W-X4, H^+, Bio-Rad) (Brons and Olieman, 1983). The guard column was kept at ambient temperature and the analytical column was kept in a water bath of 75°C. The eluent used was water. The flow rate was 0.4 ml/min. The sugars were detected by monitoring the refractive index.

2.2.1.5 Determination of organic acids

To determine organic acids 15 g of sample was weighed in a volumetric flask, it was made up to 50 ml with 0.5 M perchloric acid ($HClO_4$) and mixed. After that it was filtered in a glass funnel through filter paper S&S 589[5], the first 5 ml of filtrate being discarded. The filtrate was ready to be injected onto the HPLC column.

HPLC conditions:

For organic acid determination an ion-exchange column was used (Aminex HPX-87H, 300 mm x 7.8 mm i.d., Bio-Rad), with guard column (filled with AG 50W-X4, H^+, Bio-Rad). The eluent used consisted of 0.01 N sulphuric acid. The flow rate was 0.6 ml/min. The acids were detected by their UV absorbance at 220 nm.

GLC conditions:

Formic acid was also determined by gas-liquid chromatography (GLC). A DB-wax megabor column (30 m x 0.545 mm ID, df = 1.0 μm)
was used with temperature limits 20-230°C. The oven temperature was 100°C, the injector temperature 150°C and the detector temperature 200°C.

Titration

To determine total acid formation in heat-treated milk titrations were

performed. A sample of 25 ml of raw and heated milk was titrated with 0.1 N NaOH to pH 8.3. From the difference in used NaOH between raw and heated milk the total amount of acid formed was calculated.

2.2.1.6 Determination of HMF and furfural

For the HMF determination the method of van Boekel and Zia-Ur-Rehman (1987) was followed with some minor adjustments. 5 ml sample was mixed with 1 ml 15 N acetic acid. The tube was covered to prevent evaporation and was heated in a boiling water bath for 15 min; after cooling in ice water 1.5 ml 67% (w/v) trichloroacetic acid (TCA) was added and the contents of the tube were mixed well. The mixture was filtered through filter paper S&S 589[5]. The filtrate was then ready to be injected onto the HPLC column. The filtrate had to be kept cool as much as possible. In this way so called "total HMF" and "total furfural" was determined, to determine "free HMF" and "free furfural" the heating procedure was omitted.

A second method used to determine the free HMF and furfural concentration was the same sample pre-treatment as performed for the formic acid determination. The filtrate was injected on both the organic acids and the HMF HPLC column.

Sometimes, HMF was found in unheated samples, probably due to the sample treatment which includes a heating step. In that case the results of the heated samples were corrected by subtracting the HMF value of the unheated sample from the HMF value of the heated samples.

HPLC conditions:

For HMF determination a reversed phase column (Lichrosorb RP-8, 250 mm x 4 mm i.d., Merck, with guard column, filled with pellicular reverse phase material, Chrompack) was used. The eluent used consisted of 7.5% methanol in water. The flow rate was 0.8 ml/min. HMF and furfural were detected by their UV absorbance at 280 nm.

2.2.1.7 Determination of furfuryl alcohol

The heated milk was precipitated with 0.5 M perchloric acid ($HClO_4$) and mixed. The mixture was filtered through filter paper S&S 589[5]. The filtrate was then ready to be injected onto the HPLC column.

HPLC conditions:

For furfuryl alcohol determination the same reversed phase RP8 column was used as described in 2.2.1.6. The eluent used was 4% methanol in water. Furfuryl alcohol was detected by its UV absorbance at 220 nm.

2.2.2 Dialysis

The milk or model solution was dialysed in the cold (4°C) against Jenness and Koops buffer; the buffer was changed twice a day. 200 ppm sodium azide was added to prevent microbial growth. After 5 days no lactose could be detected in the dialysate and dialysis was stopped.

2.2.3 Diafiltration

As dialysis took a long time it was tried to perform diafiltration. Ultrafiltration was carried out with an Amicon apparatus (CH2A) and an Amicon H1P10-20 membrane. The retentate was made up to its original volume with JK-buffer; this was repeated until no lactose could be detected in the retentate. However, the problem with this method was that the membrane fouled and the filtration proceeded very slowly.

2.2.4 Heating methods

2.2.4.1 Sterilization

The samples were heated for various times (0-60 min) at 110-150°C in a glycerol bath, in tightly stoppered stainless steel tubes (7 x 120 mm or 10 x 170 mm, about 4.5 and 13 ml, respectively); the reported heating times include the heating-up period of about 1.5 minutes in the case of the small tubes and about 2-3 minutes in the larger tubes. The initial temperature increase during heating was calculated for both the small and the large tubes. If it is postulated that the temperature is uniform throughout the tubes at any instant the following equation can be used (Hiddink, 1975):

$$V\rho c_p \frac{dT}{dt} = UA(T_w - T) \Rightarrow \frac{T_w - T}{T_w - T_0} = \exp\left(\frac{-UAt}{V\rho c_p}\right) \qquad (2.1)$$

V	= volume liquid	(m^3)
ρ	= density	($kg.m^{-3}$)
c_p	= heat capacity of liquid	($J.kg^{-1}.K^{-1}$)
U	= heat transfer coefficient	($W.m^{-2}.K^{-1}$)
A	= heating surface	(m^2)
T_w	= temperature of the wall	(°C)
T_0	= initial temperature	(°C)

U is calculated from the next equation:

$$\frac{1}{U} = \frac{1}{\alpha_{glycerol}} + \frac{d_w}{\lambda_w} + \frac{1}{\alpha_{melk}} \qquad (2.2)$$

α	= heat transfer coefficient	($W.m^{-2}.K^{-1}$)
d_w	= thickness of container wall	(m)
λ_w	= heat conductivity of the container wall	($W.m^{-1}.K^{-1}$)

As $\alpha_{glycerol}$ was unknown, α_{water} was used. U is calculated to be about 400 Wm^{-2}. The results are shown in Table 2.1 and Figure 2.1 for a bath temperature of 120°C. The temperature in the large tubes was measured with a thermocouple; temperature versus heating time is shown in Figure 2.1. The measured temperatures are reasonably similar to the calculated temperatures, especially after 1 minute. The samples were cooled immediately after heating. The small tubes were rotated in the glycerol bath, so the heating-up period was in fact shorter.

Time s	Small tubes °C	Large tubes °C
0	20	20
20	88	77
40	110	101
60	117	112
80	119	117
100	119.7	119
120	119.91	119.4
140		119.7
160		119.88
180		119.95

Table 2.1 Calculation of temperature in the tubes during heating in a glycerol bath of 120 °C

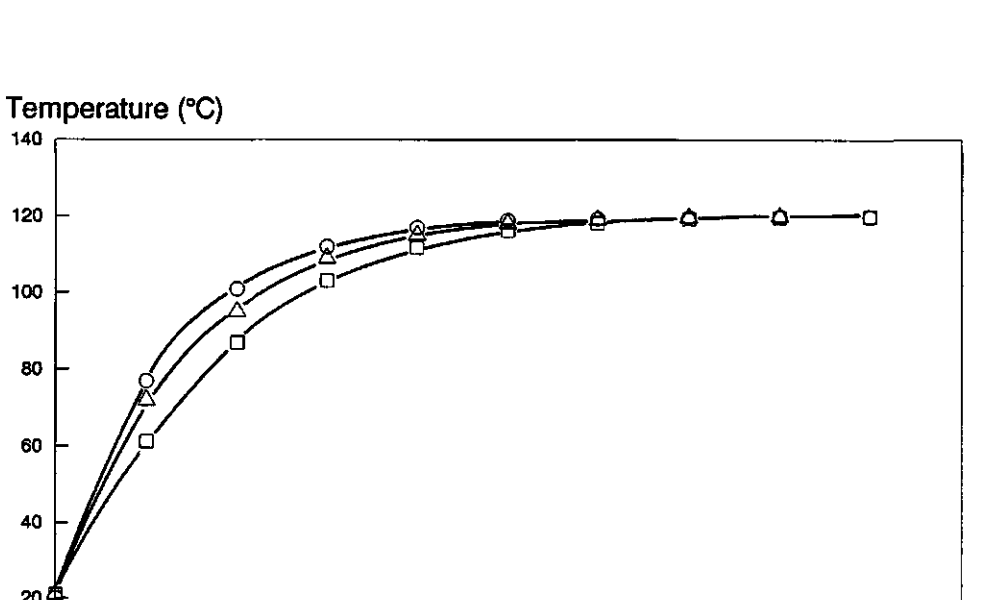


Figure 2.1 Temperature in the tubes versus heating time. □ = half-way the tubes, △ = in the top of the tubes, ○ = temperature calculated according to Eq. (2.1)

2.2.4.2 UHT

Continuous heating was performed using a pilot plant UHT apparatus, capacity 100 l/hr, suitable for direct and indirect heating. The apparatus consists of a preheater, steam injection (direct UHT) or indirect heat exchanger (using steam as the heating medium), holding tubes of varying lengths (1-8 m, internal diameter 10 mm), a flash cooler (direct UHT) or an indirect cooler (using water as the cooling medium). The preheater was usually warmed to 70°C, and the final temperature after cooling was about 20°C. The pressure was mostly adjusted to 4 or 5 bar.

3 REACTION PRODUCTS OF LACTOSE DURING STERILIZATION

In this chapter experiments are described mimicking conventional sterilization. The samples (milk or model solutions) were heated in stainless steel tubes (small and large) in a glycerol bath in the temperature range 90-150°C.

3.1 Heating of milk

3.1.1 Identification of reaction products

Sugars

To study the reactions of lactose during heat treatment it is necessary to know the degradation products formed. First of all sugars are formed; lactose can be isomerized into lactulose or epilactose and it can be hydrolysed into galactose and glucose. From literature it is known that only small amounts of epilactose are formed; only 1.5 % of total sugar after 2 hours heating of milk at 120°C against 9.1% lactulose (Martinez-Castro and Olano, 1980). Olano et al. (1989) also found the epilactose formation during heating processes of milk to be about one tenth of the lactulose formation. Tagatose, an isomerization product of galactose, was also detected, but only in very small amounts (Richards, 1963). Recently, Troyano et al. (1992a) determined tagatose in heated milk; from their data can be derived that tagatose formation is about 1% of galactose formation. Therefore, it was decided to follow the degradation of lactose and the formation of lactulose and galactose. An HPLC chromatogram of heat-treated milk showing the peaks of those compounds is shown in Figure 3.1. It may be possible that epilactose is eluted on the carbohydrate column used at about the same retention time as lactose and lactulose; unfortunately, the retention time of epilactose could not be determined because it is not available as a standard. Glucose is eluted at about the same retention time as lactulose. Olano et al. (1989) reported that the glucose concentration after heating (30 min 120°C) remained almost unaltered. From the mass balance determined after an experiment, it may be deduced whether compounds are missing or not.

The reproducibility of the sugar determination was studied by injecting the standard mixture 9 times, by determining the sugar concentration of ten samples of raw skim milk and by injecting one sample 9 times. The results are shown in

Table 3.1. The difference between A and B in Table 3.1 indicates the variability due to HPLC analysis and the variability due to the sample preparation. As these were samples of raw skim milk, the peak with the retention time of lactulose was probably glucose, so not lactulose but glucose was measured.

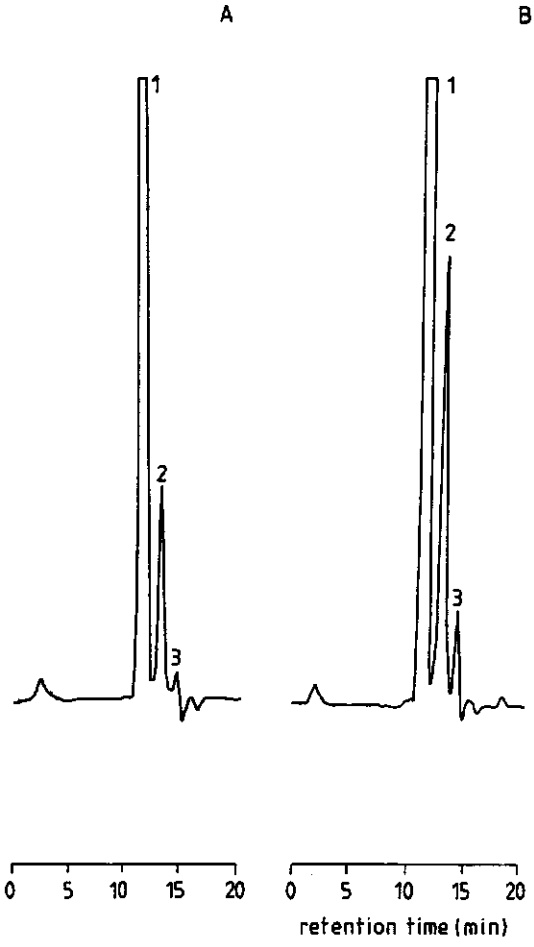


Figure 3.1 Chromatograms of a sugar standard solution (A) and skim milk heated for 5 min at 140°C (B). 1 = lactose, 2 = lactulose, 3 = galactose

Sample	A			B		
	Lactose mmol/kg	Glucose (?) mmol/kg	Galactose mmol/kg	Lactose mmol/kg	Glucose (?) mmol/kg	Galactose mmol/kg
1	139.07	0.82	1.40	140.67	0.57	1.14
2	139.01	0.81	1.43	141.81	0.53	1.11
3	140.45	0.78	1.26	141.39	0.57	1.11
4	140.54	0.78	1.40	141.07	0.56	1.18
5	134.96	0.76	1.36	142.05	0.56	1.14
6	139.35	0.78	1.40	142.09	0.55	1.06
7	138.75	0.77	1.40	141.31	0.56	1.14
8	137.86	0.77	1.40	142.27	0.56	1.14
9	139.22	0.76	1.36	141.12	0.56	1.11
10	139.76	0.78	1.40			
mean	138.90	0.78	1.38	141.53	0.56	1.13
SD	1.59	0.02	0.05	0.55	0.01	0.03
CV	1.15%	2.90%	3.45%	0.39%	2.12%	2.92%

Table 3.1 Reproducibility of the sugar determination. A = ten replicates of a raw skim milk sample; B = one raw skim milk sample, 9 times injected. SD = standard deviation; CV = coefficient of variation

Skim milk was heated several times at 120°C, from these results the standard deviation as a result of the variability due to heat treatment can be calculated. As the initial raw skim milk was not of one batch and thus varied in initial lactose concentration, the standard deviations of the lactose decrease and the lactulose and galactose formation were calculated. The standard deviation of each heating time as well as the pooled standard deviation of these standard deviations were calculated (Table 3.2).

Experi-ment	Heating time				
	0 min	6.5 min	11.5 min	16.5 min	21.5 min
Lactose decrease (mmol/kg)					
1	0.00	3.77	7.06	8.17	10.76
2	0.00	2.80	5.45	8.71	10.40
3	0.00	2.87	5.71		
4	0.00	-1.70	0.82	4.19	5.16
5	0.00		5.70	11.05	13.10
mean		1.94	4.95	8.03	9.86
		(3.15)	(5.98)	(9.31)	(11.42)
SD		2.46	2.39	2.84	3.35
		(0.54)	(0.73)	(1.53)	(1.47)
CV		127.30%	48.40%	35.50%	34.00%
		(17.2%)	(12.2%)	(16.4%)	(12.8%)
PSD					2.76
					(1.11)
Lactulose formation (mmol/kg)					
1	0.00	1.27	3.14	4.55	5.67
2	0.00	1.96	3.34	4.97	6.34
3	0.00	1.81	3.33		
4	0.00	2.01	3.85	5.32	7.06
5	0.00		3.28	4.83	5.81
mean		1.88	3.39	4.92	6.22
SD		0.13	0.27	0.32	0.63
CV		7.14%	7.98%	6.51%	10.13%
PSD					0.38

Table 3.2 continued

Galactose formation (mmol/kg)					
1	0.00	0.27	0.75	1.23	1.81
2	0.00	0.41	0.95	1.61	2.29
3	0.00	0.39	0.86		
4	0.00	0.38	0.85	1.51	2.29
5	0.00		0.81	1.48	1.98
mean		0.36	0.84	1.46	2.09
SD		0.06	0.07	0.16	0.24
CV		17.36%	8.69%	11.08%	11.39%
PSD					0.15

Table 3.2 *Reproducibility of the sugar determination after heat treatment at 120°C.* SD = Standard deviation; CV = Coefficient of variation; PSD = Pooled standard deviation. Between brackets: results after omitting experiment 4

HMF, furfural and lysine

A second reaction path is the Maillard reaction. Products that may be formed during the early Maillard reaction are HMF and furfural; these compounds can also be determined by HPLC; a chromatogram of heat-treated milk is shown in Figure 3.2. The reproducibility of the HMF determination was determined by heating whole milk 15 min at 100°C from which then 9 samples were prepared and the reproducibility of the HMF formation was determined by analysing 7 samples of indirectly UHT heated skim milk during 64 s at 140°C. The results are given in Table 3.3.

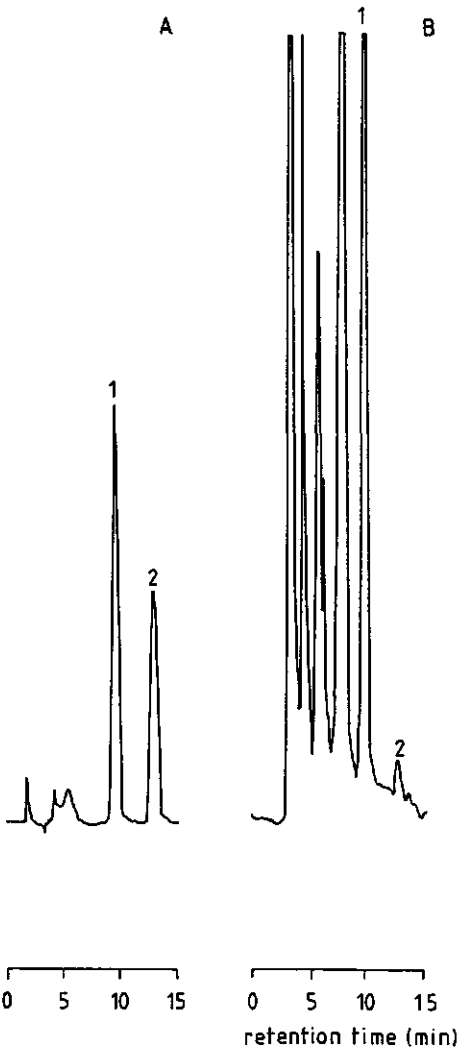


Figure 3.2 Chromatograms of a HMF and furfural standard solution (A) and skim milk heated for 13 min at 140°C (B). 1 = HMF, 2 = furfural

Sample	HMF concentration μmol/l (15 min 100°C)	HMF concentration μmol/l (64 s 140°C)
1	17.07	33.34
2	16.73	32.82
3	19.16	32.74
4	16.42	31.13
5	17.00	30.92
6	15.83	31.27
7	17.57	31.90
8	15.09	
9	16.18	
mean	16.78	32.02
SD	1.15	0.96
CV	6.88%	2.98%

Table 3.3 Reproducibility of the HMF determination. SD = standard deviation; CV = coefficient of variation

The Maillard reaction is a reaction between a reducing sugar and an amino group, in the case of milk, primarily of lysine residues. The lysine concentration was determined by a colorimetric method. In order to measure the reproducibility of the lysine determination ten samples of the same raw skim milk were used. The results are given in Table 3.4. The variation in lysine concentration including the heating process was estimated to be about 2% as coefficient of variation (van Boekel, 1992, unpublished data). The lysine concentration decreased during heating, as the lysine residue reacts with lactose to form the Amadori compound. This means that the decrease of lysine content is a measure for the formation of the Amadori compound. According to Henle et al. (1991b) an estimation of the extent of the initial Maillard reaction can be made by measurement of modified, i.e. unavailable, lysine.

Sample	Lysine concentration mmol/l
1	18.68
2	18.72
3	18.79
4	18.05
5	18.58
6	18.47
7	18.82
8	18.65
9	18.97
10	18.30
mean	18.61
SD	0.27
CV (%)	1.45

Table 3.4 Reproducibility of the lysine determination. SD = standard deviation; CV = coefficient of variation

Organic acids

In both degradation pathways (sugar degradation and Maillard reaction), organic acids can be formed, as discussed in section 1.2. Therefore, an extract of heat-treated skim milk was injected onto the HPLC organic acids column and the retention times of the peaks in the chromatogram were compared to retention times of several organic acids. Acids, which could be present in heated milk according to literature, were injected, such as levulinic, hippuric, formic, uric, propionic, ascorbic, oxalic, orotic, phosphoric, butyric, pyruvic, acetic, citric and lactic acid. The many peaks in the chromatogram of heat-treated milk were rather difficult to identify (Figure 3.3).

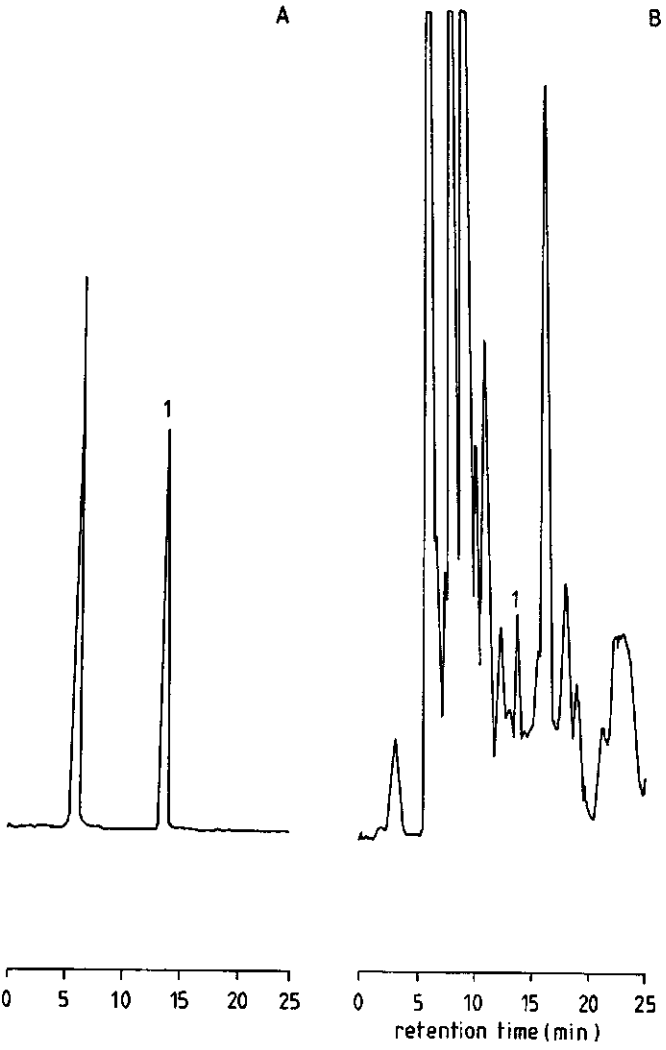


Figure 3.3 Chromatograms of a formic acid standard solution (A) and skim milk heated for 13 min at 140°C (B). 1 = formic acid

Corbett and Kenner (1953) described the degradation of carbohydrates by alkali and reported the formation of isosaccharinic acid in the case of lactose (Figure 3.4). As isosaccharinic acid was not available, it was tried to synthesize it (Corbett and Kenner, 1953).

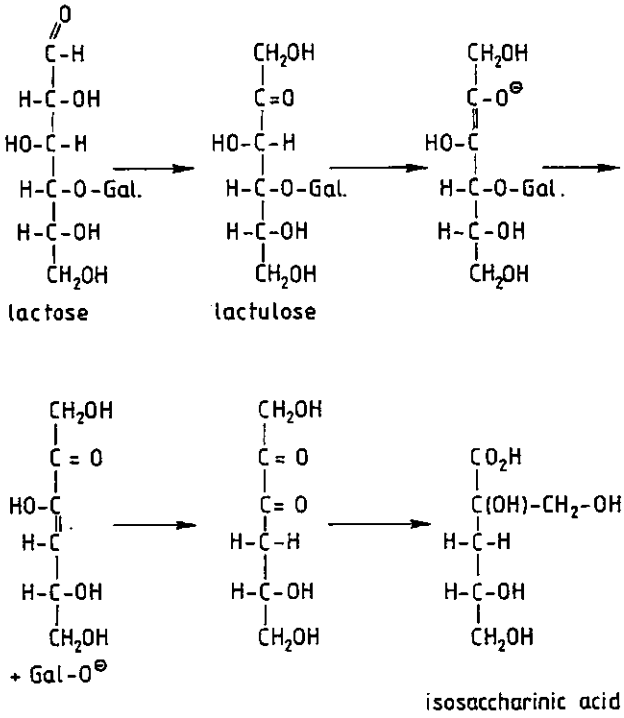


Figure 3.4 Formation of isosaccharinic acid from lactose according to Corbett and Kenner (1953). Gal. = galactose

However, it appeared to be difficult to obtain a pure compound and the peaks found in the HPLC chromatogram (Figure 3.5) did not correspond with any of the peaks found in the chromatogram of heat-treated skim milk. The capacity factors for organic acids on the same type of column found by De Bruijn (1986) were compared to those found in our solutions. However, it appeared to be difficult to compare them. In heated milk, the peaks of citric, acetic and glycolic acid could be determined according to the capacity factors determined by De Bruijn; those peaks seemed to increase somewhat with increasing heating time and temperature, but not as much as the peak of formic acid did. In model solutions, sometimes malic and lactic acid could be determined; these peaks also increased with heating time.

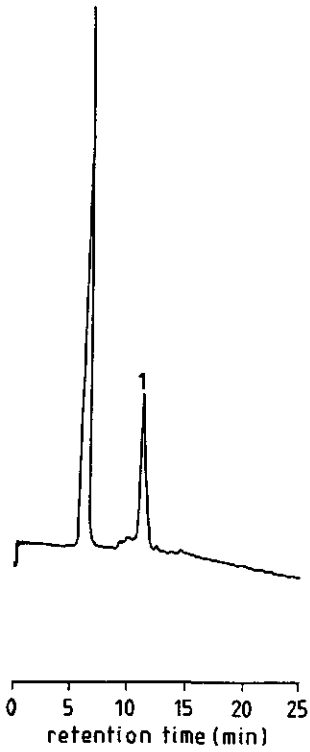


Figure 3.5 Chromatogram of synthesized isosaccharinic acid (1)

The formation of formic acid in the heat-treated milk was very clear, so, the formic acid concentration of several heated milk samples was determined. The formic acid concentration appeared to be rather high, so it was also tried to determine formic acid with another method to check that the HPLC-peak of formic acid did not coincide with another peak. As a first approach, RI detection was used instead of UV detection; although the chromatogram as a whole became more complex, the formic acid peak appeared to be the same, qualitatively as well as quantitatively. Marsili et al. (1981) described an HPLC-method for organic acid analysis and found uric acid to coincide with formic acid. For that reason both uric acid and formic acid were injected at the HPLC-column, but they had clearly different retention times. Furthermore, it was decided to try to analyse formic acid by gas-liquid chromatography (GLC). Heat-treated milk was analysed for formic acid with both HPLC and GLC, and also unheated milk with added formic acid was analysed. The GLC-method was not very reliable, as the duplicates showed a rather

large difference. Probably, the formic acid remained partly in the needle or septum. However, the results were of the same order of magnitude as the HPLC results. So we concluded that the amount of formic acid estimated by HPLC with UV detection is realistic. In order to calculate the reproducibility of the formic acid determination and the heat treatment, nine samples of milk were heated 18 min at 140°C. The formic acid concentrations found are given in Table 3.5.

Sample	Formic acid concentration mmol/kg
1	4.16
2	4.12
3	3.31
4	3.53
5	3.19
6	3.00
8	2.77
9	3.64
mean	3.47
SD	0.50
CV	14.41

Table 3.5 Reproducibility of the formic acid determination and heat treatment. SD = standard deviation; CV = coefficient of variation

According to Isbell (1976), sugars are degraded by heating in the presence of O_2. D-glucose, D-mannose and D-fructose are degraded under influence of O_2 forming arabinonic acid and formic acid. Since the headspace of the tubes in which the milk was heated contained air, O_2 could have an effect on sugar degradation in heated milk. As arabinonic acid was not commercially available, it was synthesized according to the method of Kiliani and Kleeman (1884) described by Green (1948). After oxidation of D-arabinose with bromide, three new peaks were found in the organic acids chromatogram, but none of them corresponded to a peak found in heated milk or model solutions. From this result it can be concluded that no arabinonic acid was formed in heat-treated milk (assuming that bromine oxidation

of arabinose did indeed result in arabinonic acid). Therefore, O_2 had probably not a large effect on sugar degradation in our experiments, at least not according to the scheme of Isbell (1976).

Deoxyribose

Finally, it could be concluded that if lactose is degraded into galactose and formic acid, a compound with 5 C-atoms or less must be formed, at least as an intermediate. From the scheme of the alkaline degradation of glucose given by De Wit (1979), it appeared that this is probably 2-deoxy-D-ribose. So, the formation of this compound was also studied; it was analysed by HPLC on the carbohydrate column and appeared to have a retention time of 22.6 minutes.

3.1.2 Formation of reaction products in skim milk

Sugars

Milk was heated 0-20 min at 110-150°C in the small tubes (of 4.5 ml). The concentrations of lactose, lactulose and galactose were determined by HPLC. The results are shown in Figure 3.6. Tagatose was not found in sterilized milk. The lactulose concentrations are in agreement with the results of Martinez-Castro and Olano (1978), who found 2.5 to 5.8 mM lactulose in commercial sterilized milks. Geier and Klostermeyer (1983) found 2.5 to 4.0 mM lactulose in in-bottle sterilized milk samples and Andrews (1984) reported 2.0 to 3.5 mM lactulose in sterilized milk (Andrews, 1986). Calvo and Olano (1989) found 3.5 mM lactulose in milk heated 20 min at 120°C and 6.2 mM lactulose in milk heated 30 min at 120°C. For the same milk they found 1.5 and 2.4 mM galactose, respectively.

Formic acid

Skim milk was heated 0-40 minutes at 110-140°C in the larger tubes of 13 ml. The concentration of formic acid was determined by HPLC (Figure 3.6). The amount of formic acid found by Kometiani (1931) after heating milk 30 min at 120°C was with 9.1 mM about four times higher. The results of Gould (1945b) and Gould and Frantz (1946) are in the same order of magnitude as the results found in the present study.

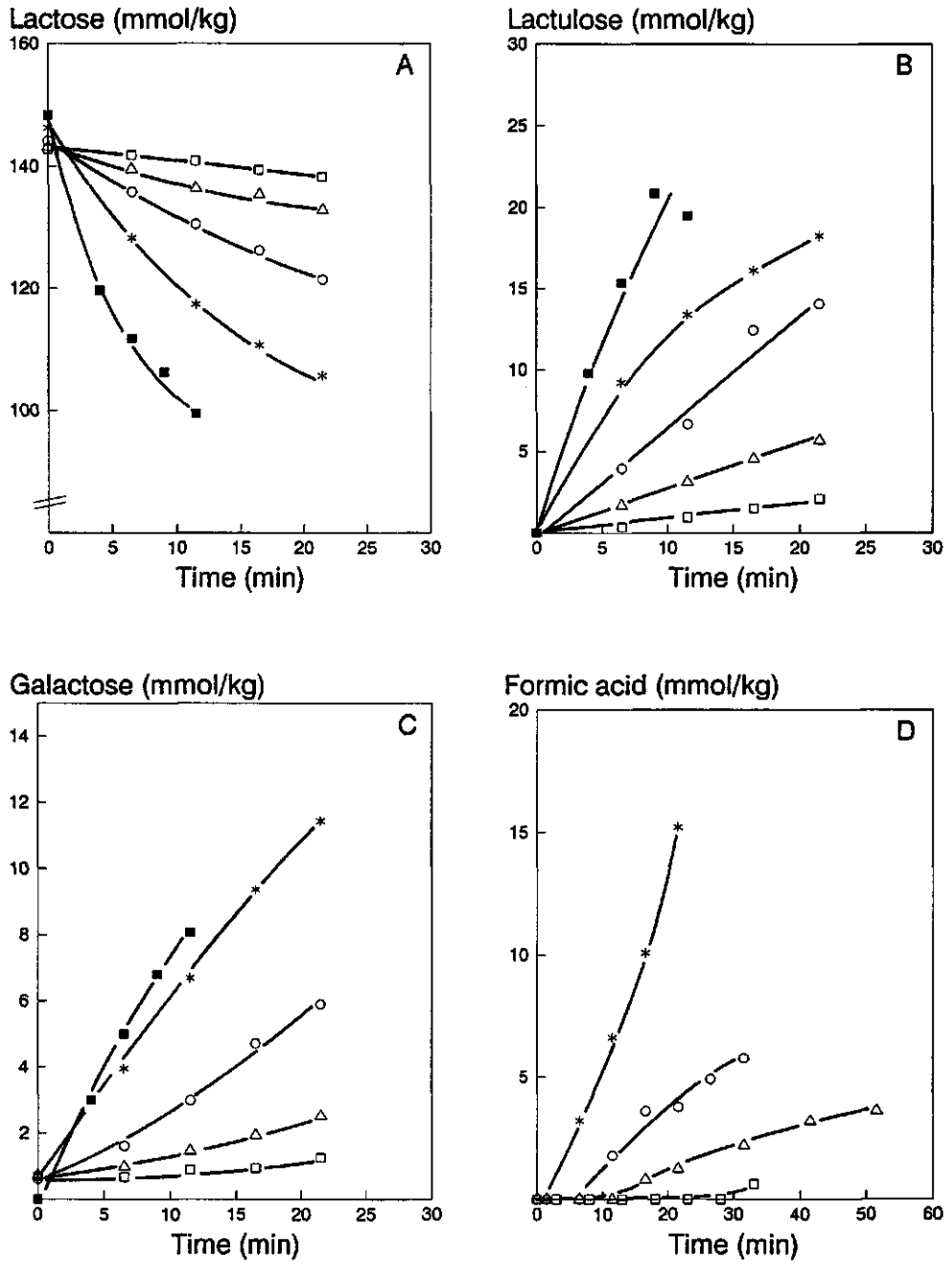


Figure 3.6 Degradation of lactose (A) and formation of lactulose (B), galactose (C) and formic acid (D) in heated skim milk. □ = 110°C, ▵ = 120°C, ○ = 130°C, * = 140°C, ■ = 150°C

pH

After heat treatment the samples were cooled in ice-water. About 30 min after heating, the pH of the samples was measured at 20°C. The pH of the samples lowered with increased heating temperature and heating time (Table 3.6).

Time min	110°C	120°C	130°C	140°C	150°C
0	6.68	6.68	6.68	6.69	6.67
1.5	6.58	6.63	6.62	6.58	6.45
4					6.22
6.5	6.57	6.58	6.51	6.41	5.91
9					5.75
11.5	6.55	6.55	6.43	6.19	5.61
16.5	6.53	6.50	6.28	5.79	
21.5	6.53	6.43	6.23	5.86	
26.5	6.52		6.15		
31.5	6.50	6.40	6.05		
41.5		6.31			
51.5		6.24			

Table 3.6 pH of skim milk after heat treatment, measured at 20°C, 30 min after heating at various temperatures

The drop in pH is mainly caused by the formation of acids. To determine the relation between pH drop and formation of formic acid, raw and heat-treated milk were titrated with 0.1 N NaOH till the pH was 8.3. From the difference in used NaOH between raw and heated milk the total amount of acid formed was calculated. The formic acid concentration was determined by HPLC. The results are shown in Table 3.7.

Heating time min	pH	Acid formation mmol/l*	Formic acid formation mmol/l
0	6.71	0	0
3	6.64	1.14 (0.08)	0.69
13	6.19	7.75 (0.12)	6.84
23	5.91	12.06 (0.12)	11.62

Table 3.7 Total acid formation and formic acid formation after heating at 140°C (*the results are the mean of three determinations; the standard deviation is given between brackets)

From these results it can be concluded that the drop in pH due to heat treatment is mainly explained by formic acid formation. The remainder is probably due to H^+ formation because of changes in salt equilibria. As the difference between acid formation and formic acid formation is constant, it is likely that this fast initial drop in pH is due to the precipitation of tertiary calcium phosphate with concomitant release of H^+, which at high temperature occurs in less than 5 min (van Boekel et al., 1989). Unpublished data of van Boekel (1986) indicated that also at 120 and 130°C formic acid formation almost completely accounts for total acid formation.

HMF, furfural and furfuryl alcohol

Free HMF and furfural as well as total (potential) HMF and furfural were determined in heat-treated skim milk. For determination of total (potential) HMF or furfural the samples were heated again at 100°C in acetic acid during sample pretreatment, to induce the formation of HMF or furfural from the precursors (early Maillard products, Amadori compound). Thus, total HMF is the sum of free HMF and the precursors of HMF, the same goes for furfural. The results are shown in Figure 3.7. Compared to the formation of lactulose, galactose and formic acid, HMF and furfural formation is very low, only in the order of micromoles. These results are in agreement with those of Fink (1984), who determined total HMF in sterilized whole milk and of Fink and Kessler (1986), who measured HMF in UHT-treated milks, heated for rather long times. The concentration of free HMF is about ten times that found by Horak (1980), which may be due to the difference in

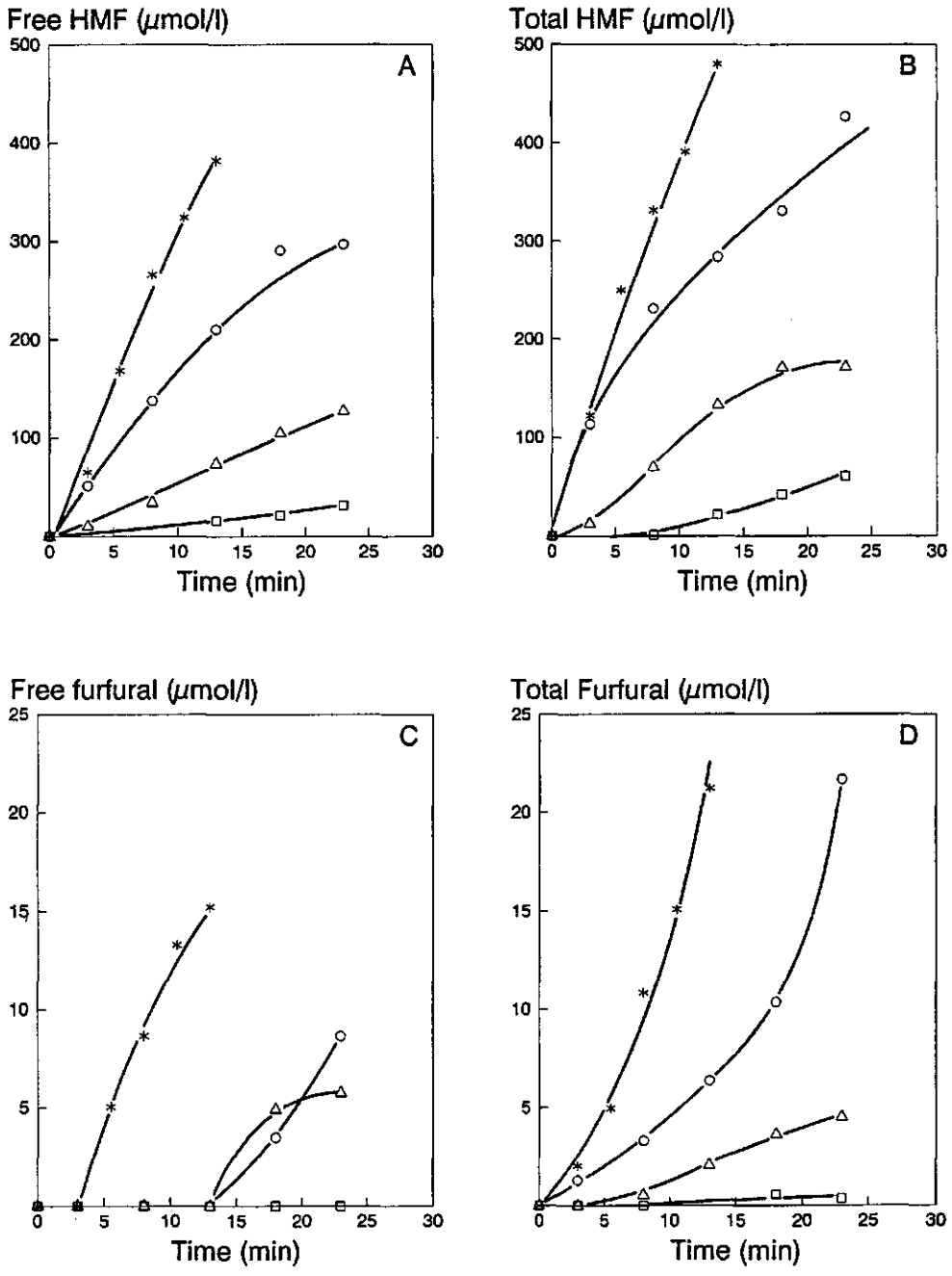


Figure 3.7 Formation of free and total HMF and furfural in heated skim milk. □ = 120°C, △ = 130°C, ○ = 140°C, * = 150°C

analysis technique (Horak used a colorimetric method).

Furfuryl alcohol formation was determined in skim milk heated at 140°C. The maximum concentration furfuryl alcohol found was 380 μmol/kg after 23 min heating at 140°C. This is about 10 to 50 times lower as compared to the formic acid formation. Furfuryl alcohol was also added to raw skim milk; after heating for 8 min at 140°C no furfuryl alcohol was degraded, so, apparently, furfuryl alcohol is rather stable in heated milk. Patton (1950b) also determined furfuryl alcohol in heated condensed skim milk (30 per cent total solids) and found 2.5 mmol furfuryl alcohol per kg condensed milk after autoclaving 2.5 hr at 127°C. From our results it may be concluded that formation of formic acid by this route (see Figure 1.9, Patton, 1950b) is not the main one.

Lysine

Lysine degradation was followed in heat-treated skim milk; the milk was heated for 0-20 minutes at 120-150°C. The results are shown in Figure 3.8. They are in agreement with the results of Horak (1980), who found a degradation of lysine in the same order of magnitude. The results are also in agreement with those of Horak if plotted according to a second order reaction: the reciprocal of lysine concentration versus heating time. The fact that this plot holds for the disappearance of lysine (supposedly due to reaction with lactose) means that the activity of lactose must be of the same order of magnitude as the activity of lysine. However, the initial concentration of lactose in milk is about ten times the initial concentration of lysine. In other words, this suggests that about 10% of the lactose is in the open chain form during heating. This can be concluded if the activity coefficient of lysine is about 1, but probably it is lower. The results of Henle et al. (1991a) and Turner et al. (1978) suggest that the reaction between lactose and lysine depends on the accessibility of the reactive lysyl groups, so not all lysyl groups are reactive, meaning that the activity coefficient of lysine is lower than 1.

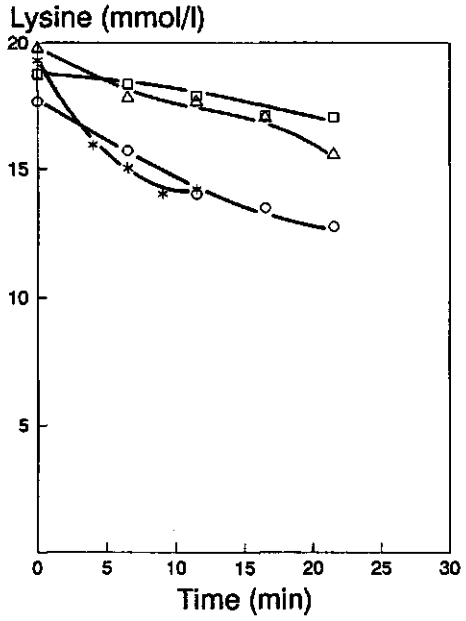


Figure 3.8 Lysine degradation in skim milk. □ = 120°C, △ = 130°C, ○ = 140°C, * = 150°C

The lysine degradation can be used to the estimate the initial Maillard reaction and as the total HMF formation is also supposed to be a result of the Maillard reaction, the correlation between lysine degradation and HMF formation was determined. Linear regression analysis of the data obtained at 120 and 130°C yielded Eq. (3.1) and those at 140 and 150°C yielded Eq. (3.2). The results are also shown in Figure 3.9A.

$$\Delta Lys = 17.4 * HMF + 375 \qquad r^2 = 0.89 \qquad (3.1)$$

$$\Delta Lys = 11.8 * HMF + 47 \qquad r^2 = 0.95 \qquad (3.2)$$

The same was calculated for the correlation between lysine and free HMF, resulting in Eq. (3.3) for 120 and 130°C and Eq. (3.4) for 140 and 150°C. These results are shown in Figure 3.9B.

$$\Delta Lys = 27.0 * HMF + 476 \qquad r^2 = 0.87 \qquad (3.3)$$

$$\Delta Lys = 14.7 * HMF + 212 \qquad r^2 = 0.95 \qquad (3.4)$$

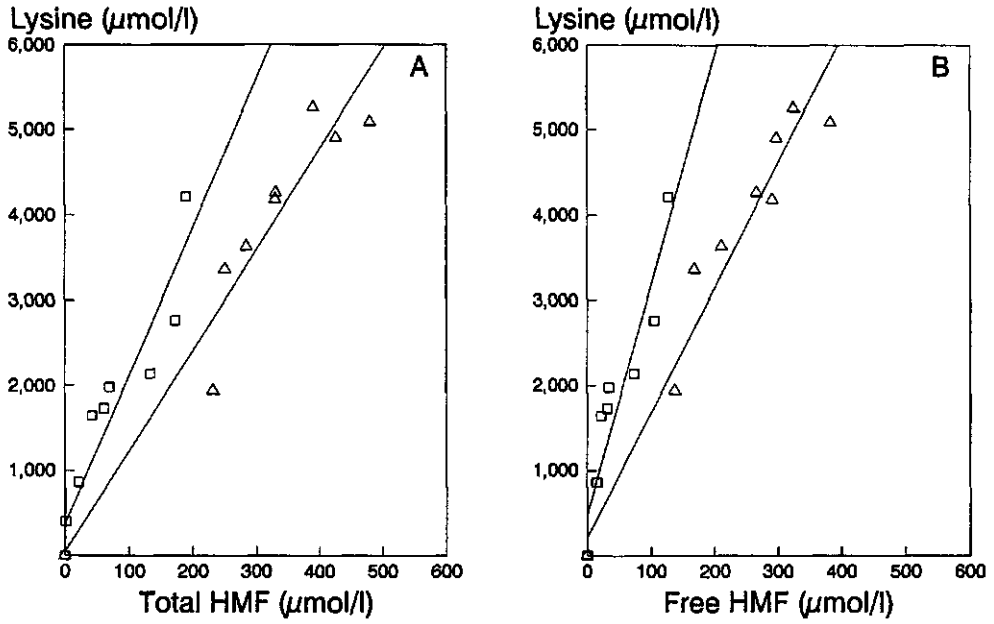


Figure 3.9 Linear correlation between total and free HMF formation and lysine degradation. □ = 120 and 130°C, △ = 140 and 150°C

Mass balance

A mass balance of the degradation of lactose is given in Table 3.8. For the mass balance, the number of moles lactose which are degraded should be equal to the sum of the number of moles of lactulose and galactose formed and the number of moles lysine lost, as these lysine residues are still bound to lactose; formic acid was not taken into account because it was assumed to be formed synchronously with galactose. Missing material (deficit) must be ascribed to neglection of not-determined compounds, such as epilactose and advanced Maillard reaction products. The total standard deviation of the calculation of the deficit can be calculated according to:

$$\sigma^2_{deficit} = \sigma^2_{lactose} + \sigma^2_{lactulose} + \sigma^2_{galactose} + \sigma^2_{lysine} \qquad (3.5)$$

This equation results in a total standard deviation of 1.2, meaning that a deficit of 1.2 or less is within the standard deviation. It is seen that up until 140°C deficits are not very high, indicating that we have covered the main degradation products. At 140°C and especially at 150°C, the deficits become higher, which is most

probably due to development of advanced Maillard reaction products, since these milks were extremely dark coloured. Olano et al. (1989) also determined the loss of carbohydrates after heating for 20 min at 120°C; they found a loss of about 1 mmol/l.

T °C	6.5 min mmol/kg	%	11.5 min mmol/kg	%	16.5 min mmol/kg	%	21.5 min mmol/kg	%
110*	0.6**	-**	0.7**	-**	1.6	47.9	1.9	41.5
120	1.2**	-**	2.0	29.4	0.4**	-**	1.0**	-**
130	1.3	15.6	2.3	17.2	-1.5	-8.2	-0.9**	-**
140	3.4	19.1	5.8	20.1	6.6	18.4	6.6	16.3
	4.0 min		6.5 min		9.0 min		11.5 min	
150	12.4	43.4	11.8	32.5	9.1	21.6	16.2	33.2

Table 3.8 Mass balance of the degradation reactions of lactose in milk; the molar deficit in mmol/kg and in % of degraded lactose is given

* = deficit calculated by subtracting galactose and lactulose formation from the lactose degradation, as lysine was not determined

** = within measurement error for deficit

3.1.3 Heating of dialysed and diafiltered skim milk

To study the effect of lactose concentration on the formation of degradation products, the lactose content of the milk must be varied. By dialysis against JK-buffer lactose can be removed from milk and, after that, varying concentrations of lactose can be added. After dialysis and lactose addition, the milk was heated and the formation of the degradation products was studied for various initial lactose concentrations. Raw skim milk was dialysed against JK-buffer during 6 days. After that, no lactose could be detected anymore by HPLC, and the pH was still 6.64.

131.5 mmol/l or 65.7 mmol/l lactose was added to the dialysed skim milk. The milk was then heated for 10 min at 120°C and the sugar concentration was determined (Figure 3.10). Obviously, in milk without lactose no lactulose and galactose were detected. The milk containing 131.5 mmol lactose/l developed a more intense brown colour after heat treatment than the milk containing 65.7 mmol lactose per l did. Lactose was degraded to the same extent as in skim milk, the amount of lactulose formed was also the same as in skim milk, and the amount of galactose formed was somewhat lower. The mass balance showed that the molar deficit (calculated by subtracting galactose and lactulose formation from the lactose degradation) which could not be explained by formation of known degradation products (further degradation of lactose), was higher for a higher lactose concentration (Table 3.9). However, formic acid, lysine, HMF and furfural were not determined.

Sample	pH after heating	Deficit mmol/l
Skim milk (142 mmol/l)	6.49	1.61
+ 65.7 mmol/l	6.53	0.21
		1.02
+ 131.5 mmol/l	6.51	5.17
		5.20

Table 3.9 Mass balance of the degradation of lactose in skim milk and in dialysed milk with added lactose after heating 10 min at 120°C (duplicates)

In the next dialysis experiment, skim milk was dialysed against JK-buffer, with addition of 200 mg/l sodiumazide; after one week the skim milk was lactose-free. Milk with varying concentrations of lactose was heated at 140°C and formation

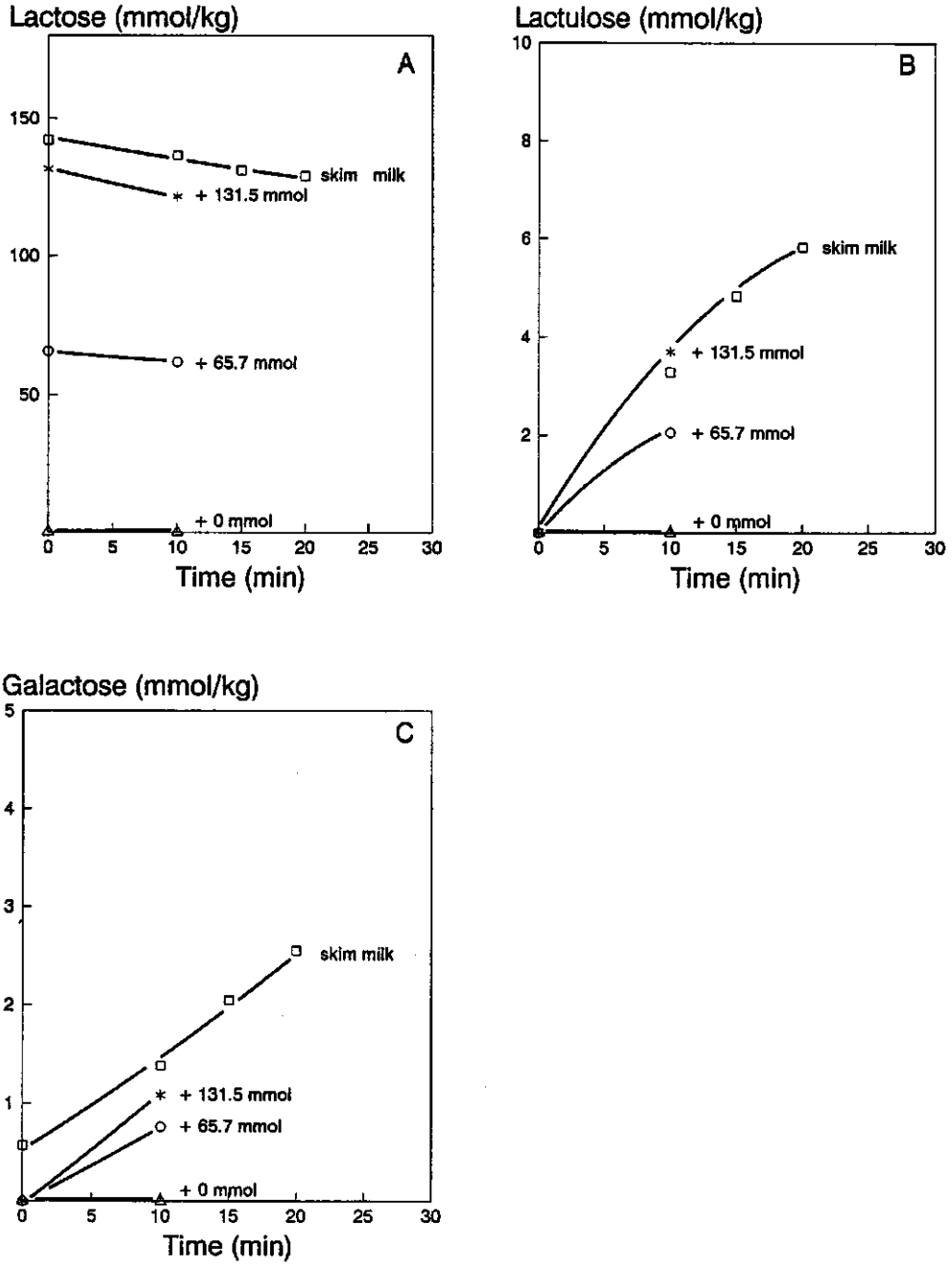


Figure 3.10 Lactose degradation (A) and lactulose (B) and galactose (C) formation during heating at 120°C. □ = normal skim milk, ▵ = dialysed skim milk, ○ = dialysed skim milk heated after addition of 65.7 mmol lactose/l, • = dialysed skim milk heated after addition of 131.5 mmol lactose/l

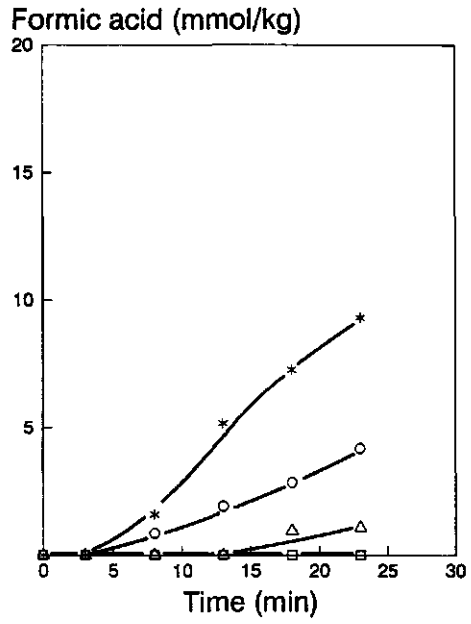


Figure 3.11 Formation of formic acid in dialysed skim milk heated at 140°C after addition of different amounts of lactose. □ = 0 mmol lactose, △ = 13.73 mmol lactose/l, ○ = 68.6 mmol lactose/l, * = 137.3 mmol lactose/l

of formic acid was studied; the results are shown in Figure 3.11. The pH of the milk after heating is given in Table 3.10. The higher the lactose concentration, the more formic acid was formed. In the absence of lactose no formic acid was formed, so the formation of formic acid clearly is a result of the degradation of lactose. This is in line with experiments of Patton and Flipse (1957), who concluded from experiments with C^{14} labelled lactose that formic acid was derived from C_1 of the glucose moiety of lactose. Also, no formation of new peaks was found on the organic acids chromatogram of heated milk with no lactose. In the absence of lactose, however, the pH decreased from 6.64 to 6.13 (Table 3.10), this is probably due to precipitation of calcium phosphate (van Boekel et al., 1989) or, perhaps, degradation of sodiumazide. In the presence of lactose several peaks were seen, for most of them peak height increased with heating time and concentration of lactose.

Heating time min	+ 0 mmol/l lactose	+ 13.73 mmol/l lactose	+ 68.6 mmol/l lactose	+ 137.3 mmol/l lactose
0	6.64	6.64	6.64	6.64
3	6.60	6.58	6.54	6.58
8	6.47	6.43	6.32	6.32
13	6.31	6.28	6.03	6.32
18	6.20	6.16	5.94	5.74
23	6.13	6.10	5.83	5.66

Table 3.10 pH of dialysed milk heated at 140°C after addition of lactose, measured at 20°C 30 min after heating

Since dialysis took such a long time, it was tried to reduce the lactose content in a faster way by diafiltration. The filtrate, containing lactose, was thrown away and the retained solute was filled up with JK-buffer. Lactose (183.5 mmol/l) was added to the retentate and it was heated at 130°C. Sugar degradation and formation was followed and the results are shown in Figure 3.12. Retentate containing no lactose was also heated, but no lactulose and galactose were formed. The pH after heat treatment and the molar deficit (calculated by subtracting lactulose and galactose formation from the lactose degradation) of the heated retentate are given in Table 3.11. The degradation of lactose and the formation of galactose in the milk after addition of lactose, were comparable to the results on skim milk (Figure 3.6); only the formation of lactulose was slightly less than that in normal skim milk.

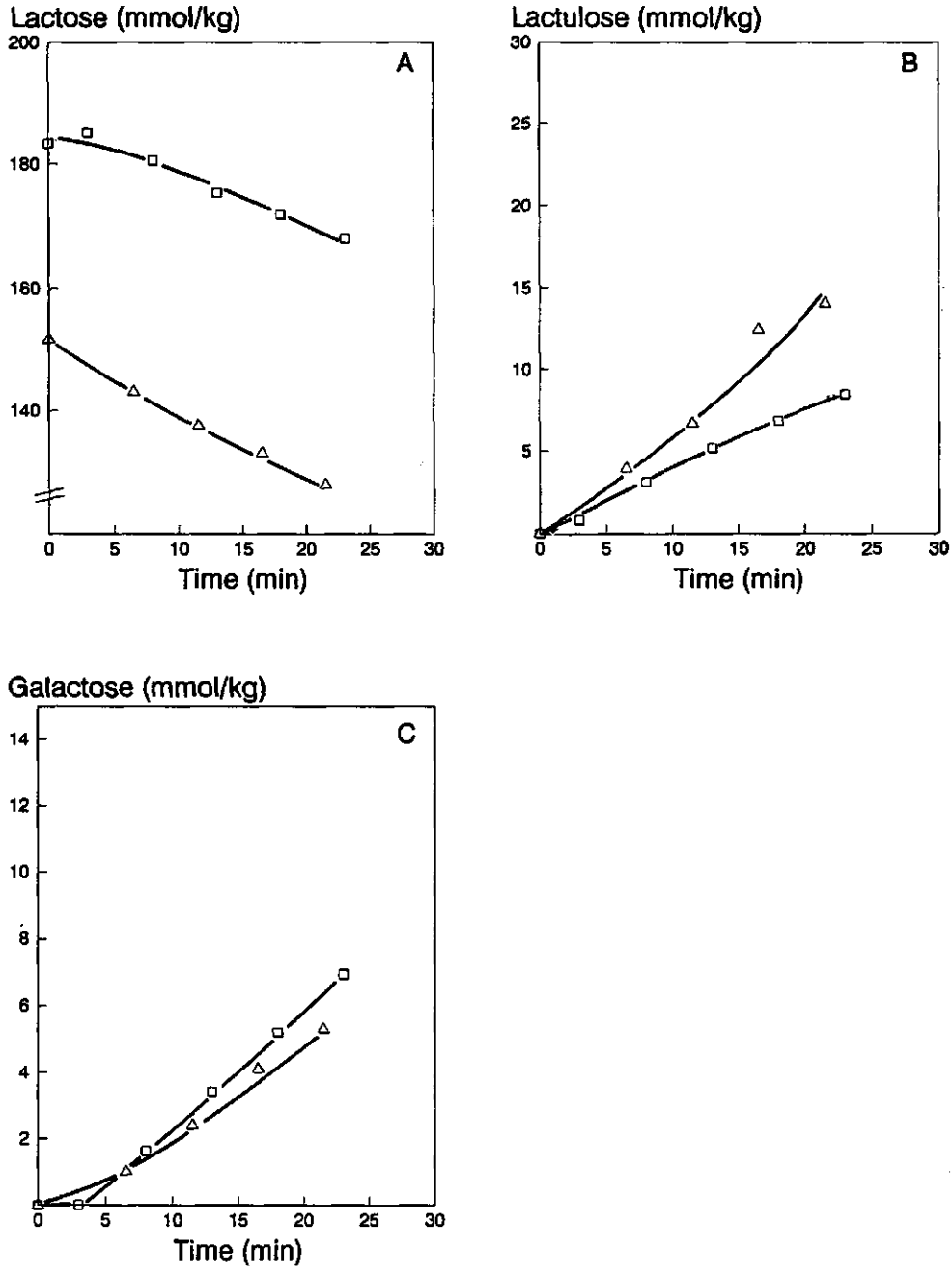


Figure 3.12 Lactose degradation (A) and lactulose (B) and galactose (C) formation in skim milk (△) and diafiltered skim milk with 199 mmol/l lactose added (□) after heating at 130°C

Heating time min	pH + lactose	pH - lactose	Deficit mmol/kg
0	6.39	6.39	0
3	6.20	6.30	0
8	6.10	6.26	0.6
13	6.03	6.24	1.9
18	5.90	6.17	2.0
23	5.83	6.15	2.5

Table 3.11 pH of diafiltered milk heated at 130°C after addition of lactose, pH of diafiltered milk heated at 130°C without addition of lactose and the molar deficit after heat treatment of retentate with added lactose

3.2 Model solutions

As dialysis took a long time and diafiltration was very slow because of fouling of the membrane, and because the milk system is very complicated and contains several compounds that can affect the reactions, it was decided to study model solutions also. In a model solution containing casein and lactose dissolved in JK-buffer, it is quite easy to vary the lactose or protein concentration. Also model solutions containing casein and lactulose, casein and galactose, casein and formic acid, casein and HMF and casein and deoxyribose were studied, as well as the same model solutions but now without protein.

3.2.1 Model solutions containing lactose and casein or lactose

A model solution containing water (hence, without milk salts), casein (2.6%) and lactose (about 140 mM), pH 6.7, was heated at 120, 130 and 140°C; formic acid and sugars were determined (Figure 3.13) as well as pH (Table 3.12). The molar deficit, this means the amount of lactose degraded minus the formation of lactulose and galactose (hence, without lysine degradation), is also given in Table 3.12. The results of the sugar determinations are comparable with the results of the sugar degradation/formation in normal skim milk. In these preliminary experiments no JK-buffer was used.

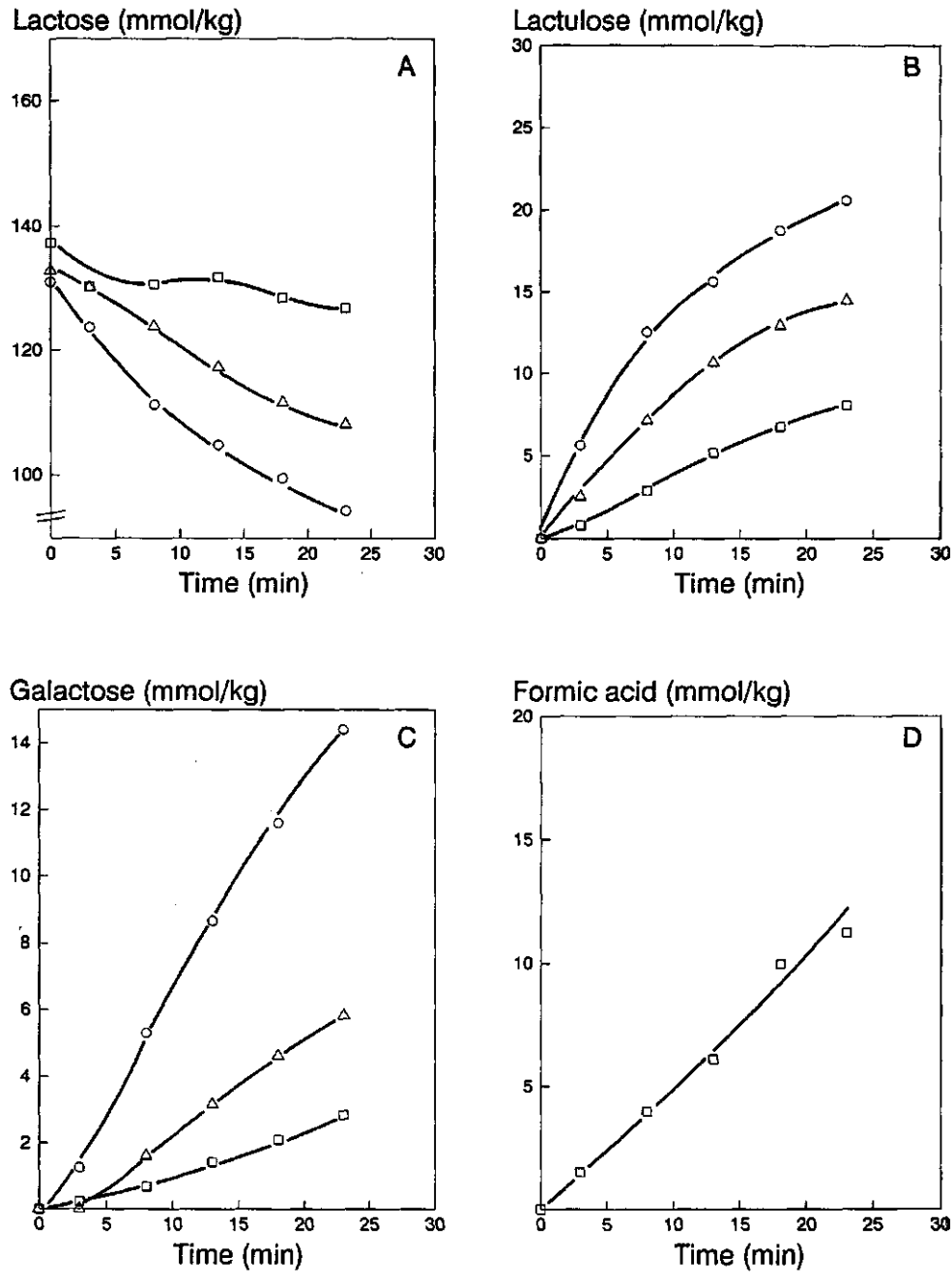


Figure 3.13 Sugar degradation and formation in a lactose-casein model solution in water. A = lactose degradation, B = lactulose formation, C = galactose formation. □ = 120°C, △ = 130°C, ○ = 140°C. D = formic acid formation at 140°C (□)

Time	120°C		130°C		140°C	
min	pH	deficit	pH	deficit	pH	deficit
0	6.70	-	6.73	-	6.70	-
3	6.72	6.0	6.72	0.2*	6.57	0.4*
8	6.64	3.1	6.56	0.2*	6.19	2.0
13	6.57	-1.1*	6.34	1.8	5.87	1.9
18	6.50	0.0	6.15	3.7	5.66	1.3
23	6.40	-0.4*	6.05	4.5	5.41	1.8

Table 3.12 pH and molar deficit (mmol/kg) after heat treatment of model solutions containing lactose (140, 134 and 140 mM) and casein (2.6%) dissolved in water. * = within measurement error

Model solutions containing water, casein and varying concentrations of lactose were heated at 130°C. The higher the concentration of lactose the higher the formation of lactulose and galactose and the larger the degradation of lactose (Figure 3.14) and the decrease of pH (Table 3.13). The decreasing pH must have been a result of the formation of organic acids; unfortunately, formic acid and HMF formation and lysine degradation were not determined. The low pH of the unheated model solution containing only lactose was probably due to the CO_2 content; the CO_2 disappeared after heating and the pH increased. In Table 3.14 the deficit is given: this is the decrease of lactose in mmol/kg minus the formation of lactulose and galactose in mmol/kg as % of the lactose degradation (without accounting for the Maillard reaction). In the case of the model solution containing only lactose, the lactose concentration increased during heat treatment; of course, this is impossible. Probably a compound was formed with the same retention time as lactose. Also indicated in Figure 3.14 is the change in heated skim milk. It is seen that the results are quite comparable to the model solutions containing the same amount of lactose.

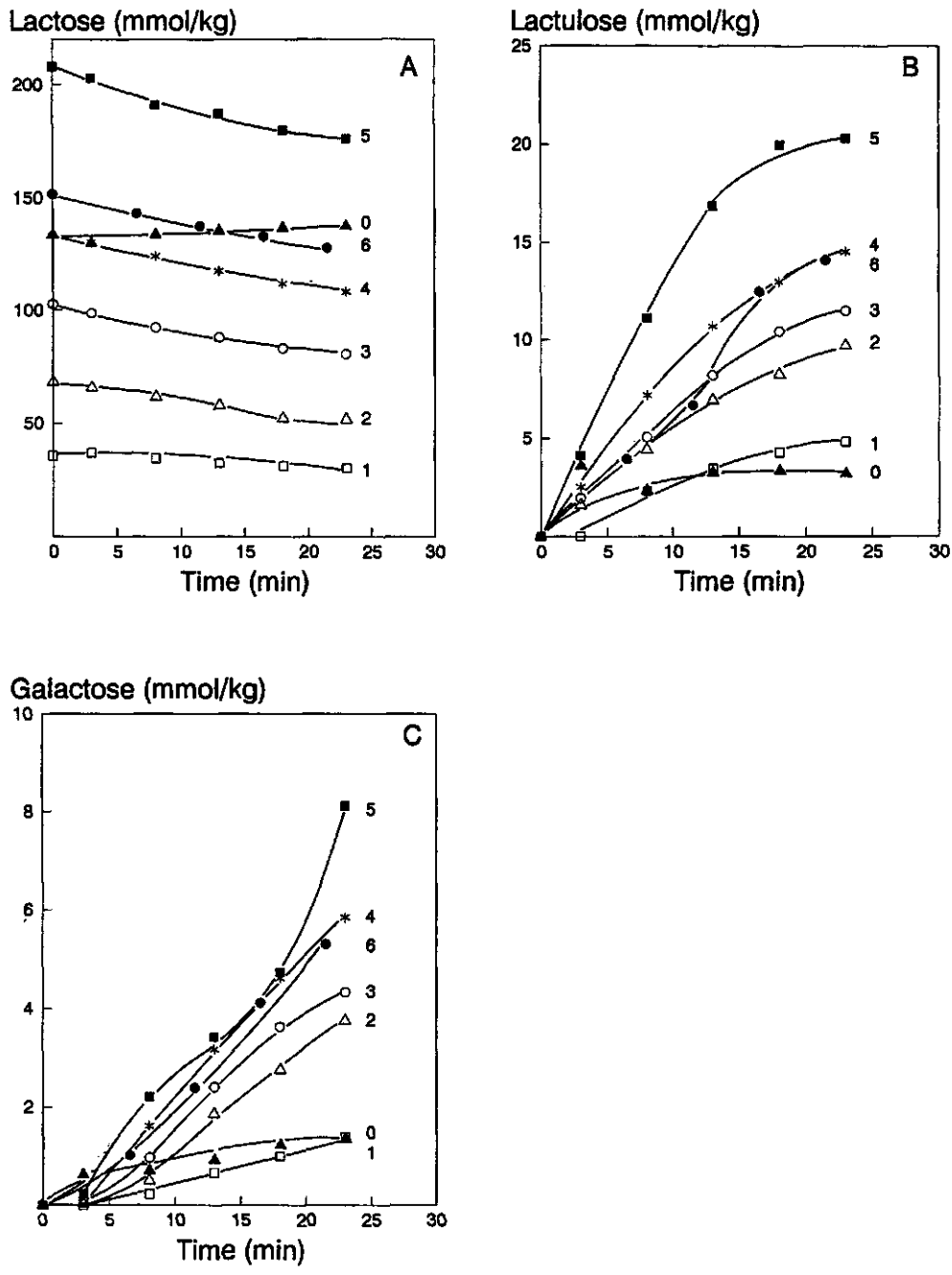


Figure 3.14 Lactose degradation (A), lactulose (B) and galactose (C) formation after heating at 130°C of lactose-casein model solutions in water with varying concentrations of lactose. □ = 35 mM, △ = 70 mM, ○ = 105 mM, * = 134 mM, ■ = 210 mM, ▲ 134 mM without casein, ● = skim milk

Time min	35 mM	70 mM	105 mM	134 mM	210 mM	134* mM
0	6.75	6.83	6.77	6.73	6.75	4.55
3	6.71	6.81	6.73	6.72	6.70	6.28
8	6.65	6.68	6.61	6.56	6.46	5.83
13	6.57	6.51	6.50	6.34	6.23	5.60
18	6.47	6.37	6.31	6.15	6.00	5.38
23	6.41	6.27	6.19	6.05	5.86	5.30

Table 3.13 pH in model solutions containing variable concentrations of lactose and casein (2.6%) dissolved in water; after heat treatment at 130°C

* model solution containing only lactose

Time min	35 mM	70 mM	105 mM	134 mM	210 mM	134* mM
3	-	33.0	46.5	0.7**	-**	-
8	1.3	25.6	42.2	2.4	21.5	-
13	10.2	17.4	28.4	11.4	1.9	-
18	10.5	32.0	28.4	17.4	12.5	-
23	10.5	19.2	28.8	18.0	11.2	-

Table 3.14 Deficit as % of lactose degradation in model solutions containing varying concentrations of lactose after heating at 130°C

* model solution containing only lactose. ** = within measurement error

Model solutions containing casein (2.6%) and lactose (134 mM) or only lactose (134 mM) dissolved in JK-buffer were heated at 140°C. The concentrations of sugars, formic acid and HMF were determined (Figures 3.15 and 3.16). The lactulose and galactose formation was much less in the absence than in the presence of casein. This is contrary to the results of Greig and Payne (1985), who heated solutions of lactose and of lactose and lysine for varying times 0-1800 s at 113, 119 or 125°C, and found the rate of production of lactulose to be greater

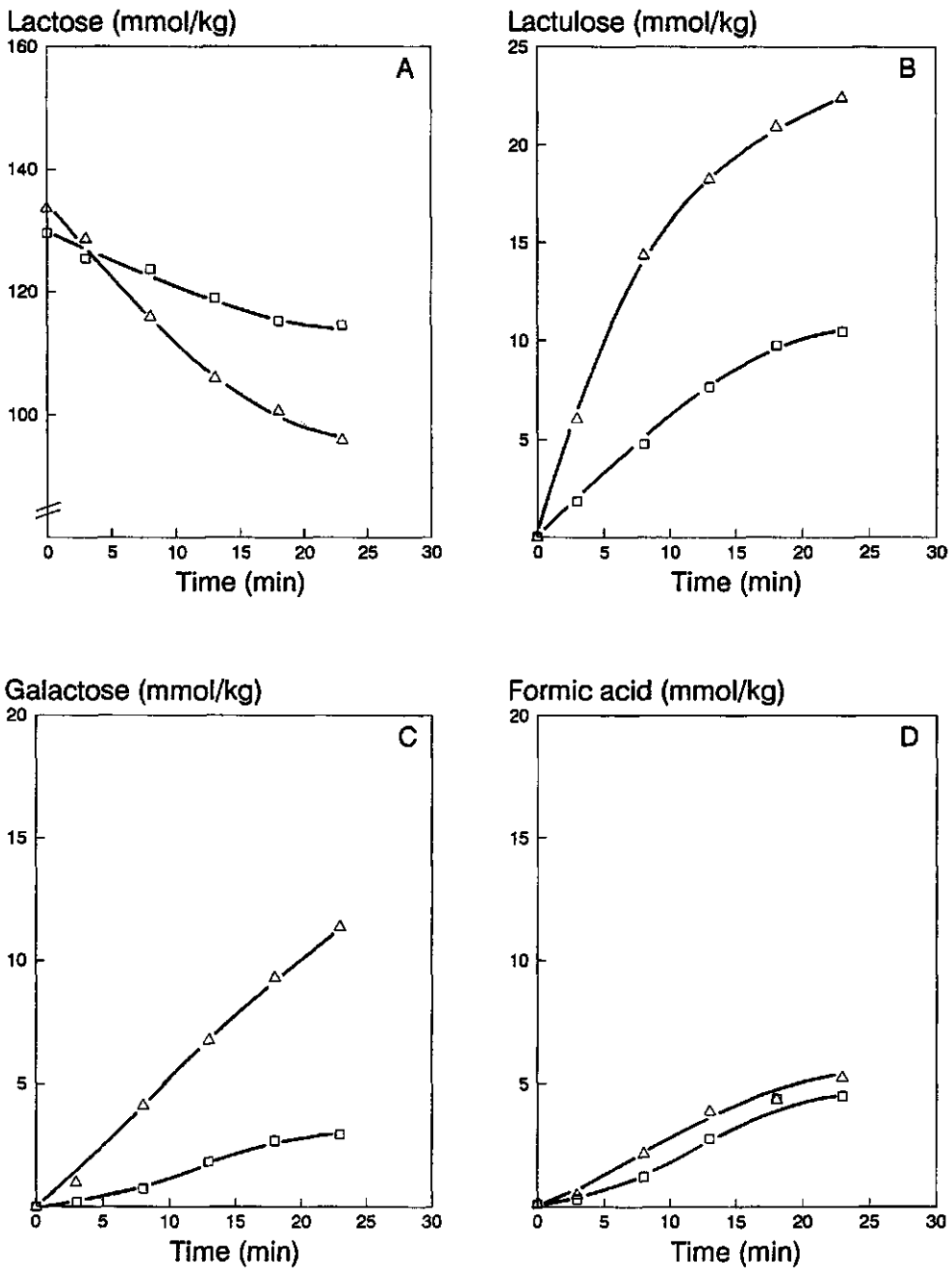


Figure 3.15 Degradation of lactose (A), formation of lactulose (B), galactose (C) and formic acid (D) in lactose model solutions (□) and lactose-casein model solutions (△) after heating at 140 °C

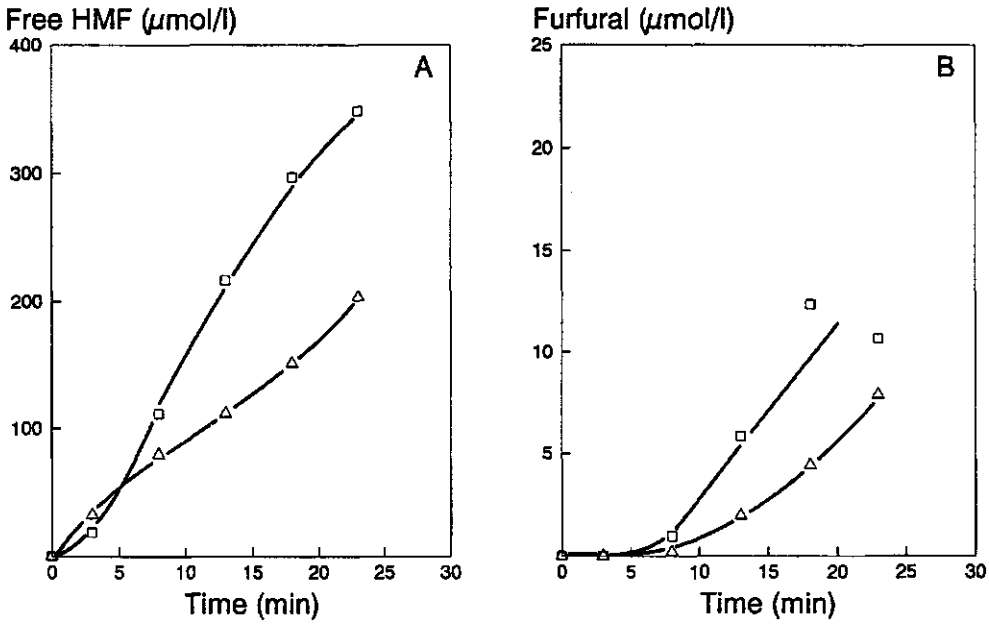


Figure 3.16 Free HMF (A) en furfural (B) formation in lactose model solutions (□) and lactose-casein model solutions (▵) after heating at 140°C

in the lactose solution. They also compared heated whole milk and heated ultrafiltrate (300-3600 s at 125°C) and found that lactulose was formed more quickly in the ultrafiltrate, so they concluded that the presence of protein reduced the rate at which lactulose was formed. However, Andrews and Prasad (1987) found the lactulose content of heated ultrafiltrate to be much lower than that of milk, but increasing amounts of protein in milk reduced the lactulose formation.

The formic acid formation was about the same for both solutions, however, it was less than in heat-treated skim milk. HMF-formation, on the contrary, was slower in the presence of casein. As a result of these experiments, it was decided to also investigate model solutions containing casein and lactulose, casein and galactose, casein and formic acid and casein and HMF; this will be discussed later in this chapter. It was also decided to vary the concentration of casein.

Variation of casein concentration

Model solutions containing lactose (about 130 mM), 0, 2.6 or 5.2% casein dissolved in JK-buffer were heated at 95, 120 and 140°C. Sugars, formic acid and HMF were determined (Figures 3.17 to 3.20). The change in pH after heat treatment is given in Table 3.15.

Time min	Temp. °C	Cala 2.6 in water	Cala 2.6 in JK-buffer	Cala 5.2 in JK-buffer	La in JK-buffer	JK-buffer
0	95		6.57		6.60	6.60
60	95		6.51		5.92	5.93
120	95		6.44		5.85	5.83
180	95		6.39		5.85	5.86
240	95		6.36		5.87	5.84
0	120		6.60	6.60	6.60	
3	120		6.54	6.56	6.19	
18	120		6.40	6.38	5.83	
33	120		6.24	6.14	5.77	
48	120		6.10	6.00	5.70	
63	120		5.99	5.81	5.66	
0	140	6.70	6.60	6.60	6.60	
3	140	6.57	6.49	6.53	5.89	
8	140	6.19	6.22	6.22	5.73	
13	140	5.87	5.95	5.92	5.49	
18	140	5.66	5.75	5.75	5.51	
23	140	5.41	5.55	5.57	5.53	

Table 3.15 pH in model solutions after heat treatment at 95, 120 and 140°C. Cala 2.6: casein(2.6%)-lactose(4.5%) model solution; Cala 5.2: casein (5.2%)-lactose(4.5%) model solution; La: lactose model solution (about 130 mM lactose)

The amount of Amadori compound still present in the solution was estimated from HMF using Eqs. 3.1, 3.2 and 3.4 given in 3.1.2. According to Henle et al. (1991b) the lactulosyllysine concentration is equal to the lysine degradation. The mass balance for all these model solutions was calculated by subtracting the formation of lactulose and galactose and the calculated lysine degradation from the decrease in lactose concentration. The molar deficit stands for further degradation products like Maillard products. The mass balance is given in Table 3.16. The standard

deviation is calculated according to Eq. 3.5, both with and without the standard deviation of lysine the total standard deviation of the deficit is 1.2.

Time	Temp.	Cala 2.6		Cala 5.2		La	
min	°C	mmol/kg	%	mmol/kg	%	mmol/kg	%
0	95	0	-			0.0	-
60	95	-0.9*	-*			0.5*	-*
120	95	-0.7*	-*			-0.1*	-*
180	95	0.3*	-*			-0.3*	-*
240	95	-0.2*	-*			1.4	36.9
0	120	0	-	0	-	0.0	-
3	120	-1.1*	-*	0.8*	-*	-0.8*	-*
18	120	-0.1*	-*	4.7	26.5	-0.9*	-*
33	120	1.3	7.5	6.7	23.6	-0.9*	-*
48	120	1.6	7.5	7.0	21.2	-0.4*	-*
63	120	4.9	17.0	10.3	26.8	-1.0*	-*
0	140	0	-	0	-	0.0	-
3	140	-2.7	-53.0	1.1*	-*	2.1	51.4
8	140	-2.0	-11.5	3.6	12.4	0.4*	-*
13	140	0.8*	-*	6.1	15.4	1.0*	-*
18	140	0.4*	-*	7.5	16.6	1.9	13.1
23	140	0.8*	-*	9.5	18.4	1.6	10.5

Table 3.16 Molar deficit in model solutions in mmol/kg and as % of lactose decrease, after heat treatment at 95, 120 and 140°C. Cala 2.6: casein(2.6%)-lactose(4.5%) model solution in JK-buffer; Cala 5.2: casein (5.2%)-lactose(4.5%) model solution in JK-buffer; La: lactose model solution in JK-buffer. * = within measurement error

With increasing casein concentration more lactose was degraded and more lactulose and galactose were formed. This is different from results by Olano et al.

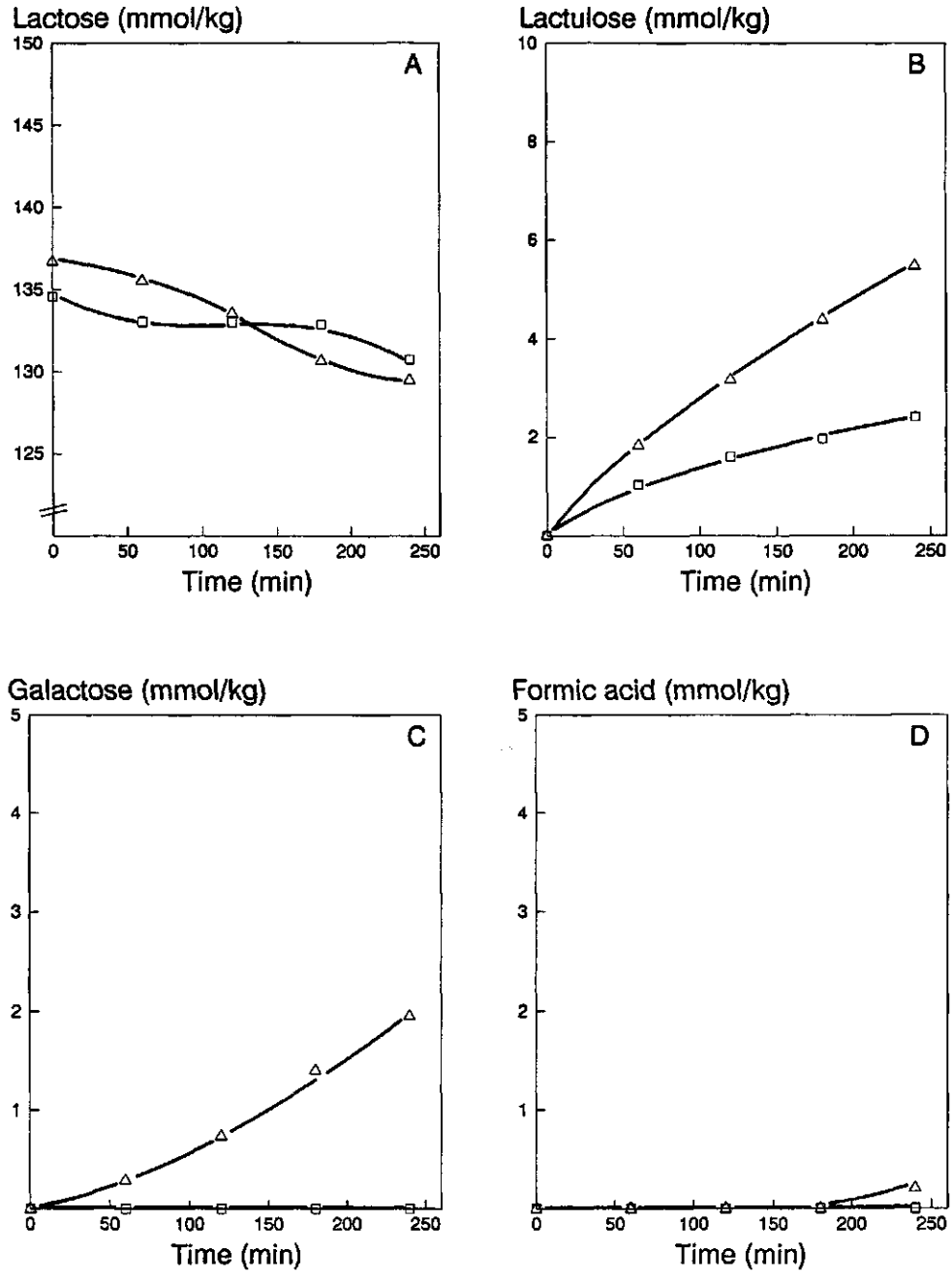


Figure 3.17 Degradation of lactose (A), formation of lactulose (B), galactose (C) and formic acid (D) in lactose model solutions (□) and lactose-casein(2.6%) model solutions (△) after heating at 95°C

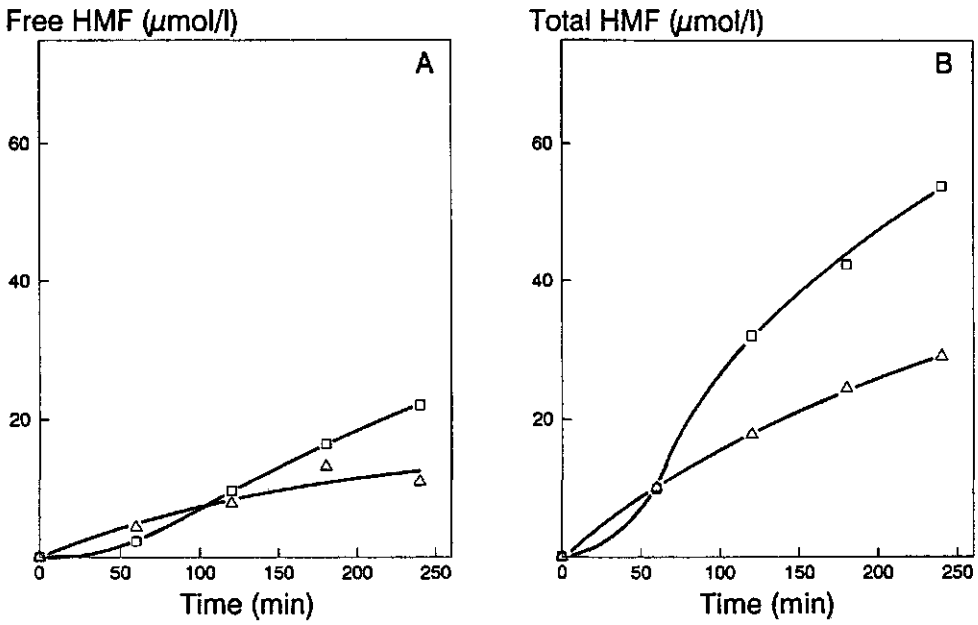


Figure 3.18 Free HMF (A) en total HMF (B) formation in lactose model solutions (□) and lactose-casein(2.6%) model solutions (△) after heating at 95°C

(1989) who heated a 5% lactose-in- buffer solution with varying amounts of N-α-acetyl-L-lysine for 20 min at 120°C. They found an increase in galactose and epilactose formation after addition of N-α-acetyl-L-lysine, but further addition decreased the amount of galactose and epilactose found. The lactulose formation decreased after addition of N-α-acetyl-L-lysine and with further addition. Andrews and Prasad (1987) found that a small amount of milk protein increased the lactulose formation above that formed in ultrafiltrate, but further addition of protein reduced the formation of lactulose. They suggested that this was either a result of increased reaction between lactose and protein, reducing the substrate for lactulose formation, or, a result of reaction of lactulose with protein reducing the amount of lactulose formed. Possibly both reactions take place. Calvo and Olano (1989) heated ultrafiltrate, milk and concentrate. They found that galactose and epilactose contents increased with increasing protein concentration; however, lactulose content decreased. They found that galactose formation increased in the presence of casein, but not in the presence of α-acetyl-lactulosyl-lysine and it was not liberated from what they call glycoproteins, by which they probably meant serum proteins. They suggested that the increased galactose formation was a

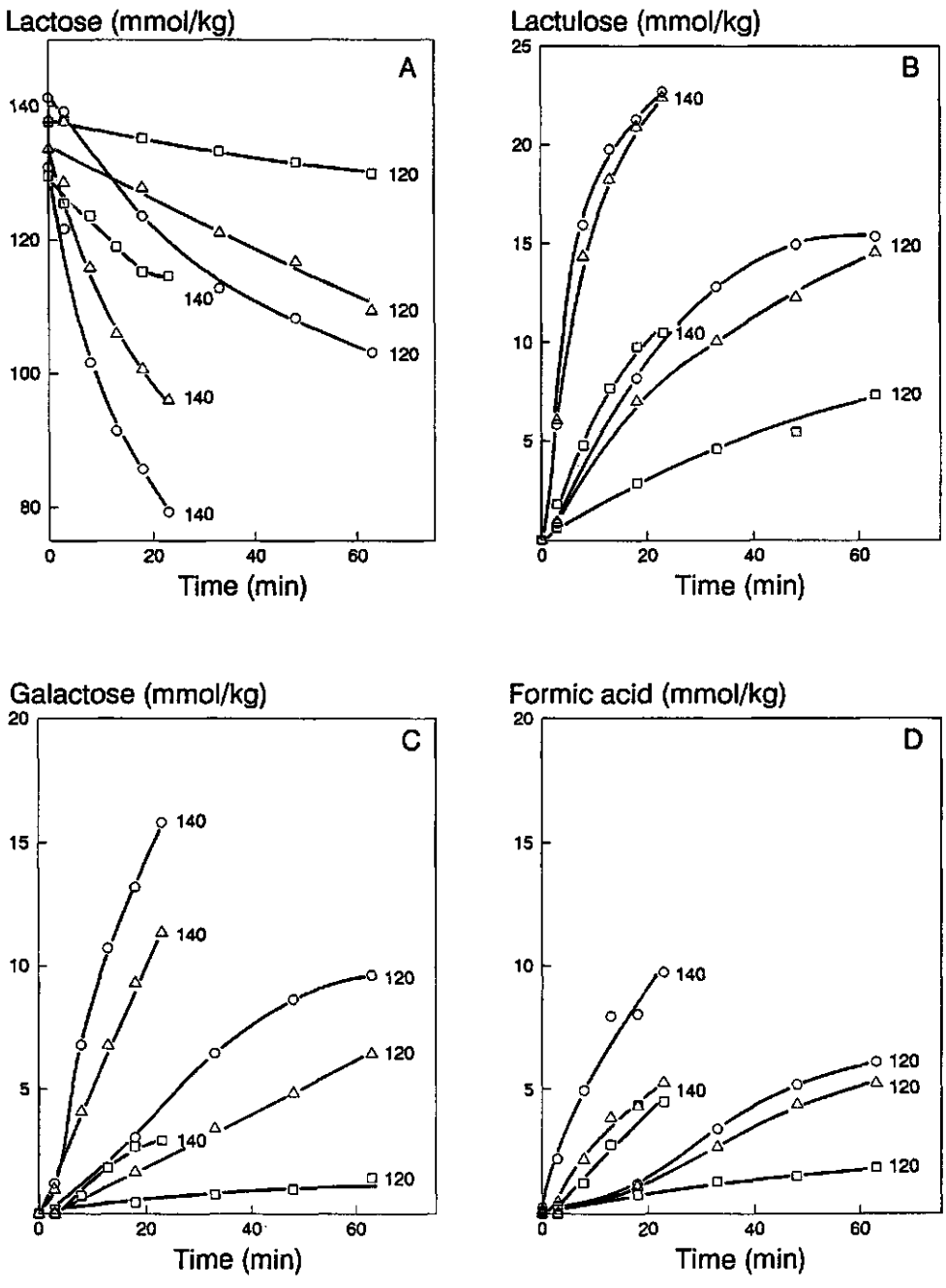


Figure 3.19 Degradation of lactose (A), formation of lactulose (B), galactose (C) and formic acid (D) in lactose model solutions (□), lactose-casein(2.6%) model solutions (△) and lactose-casein(5.2%) model solutions (○) after heating at 120 and 140°C

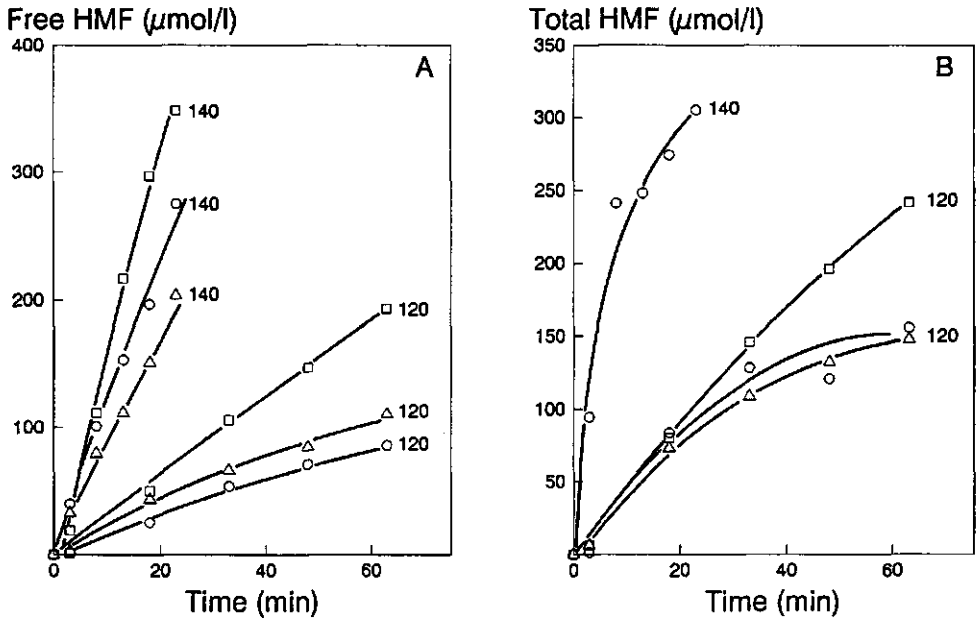


Figure 3.20 Free HMF (A) en total HMF (B) formation in lactose model solutions (□), lactose-casein(2.6%) model solutions (△) and lactose-casein(5.2%) model solutions (○) after heating at 120 and 140°C

result of degradation of the lactulose-lysine formed from lactose and lysine.

Also formic acid formation increased with increasing casein concentration. In contrast, HMF formation was less at a higher casein concentration. However, at 140°C, the results of HMF formation are not in line with the earlier results, as in the model solution containing 5.2% casein free HMF formation was higher than in the model solution containing 2.6% casein (Figures 3.18 and 3.20).

Early Maillard reaction

Studying the Amadori complex without the possible interference of sugars and caramelization would give more insight into its behaviour. In order to do so, milk, or the model solution has to be heated mildly and dialysed after that. Erlenmeyer flasks with 100 ml model solution containing lactose (134 mM) and casein (2.6%) dissolved in JK-buffer were put in an oven at 55, 75 or 95°C. Afterwards colour, pH and lysine concentration were determined (Table 3.17).

T °C	Time h	Colour	pH	Lysine concentration mmol/l
unheated	48	creamy	6.70	12.23
55	24	creamy	6.71	12.51
	48	creamy	6.69	12.34
75	24	yellow	6.56	12.08
	48	dark yellow	6.40	11.95
95	7	orange/brown	6.46	11.73
	24	dark brown	5.71	10.21

Table 3.17 Changes in colour, pH and lysine concentration of model solutions after storage at 55, 75 or 95°C

At 75 and 95°C colour was already formed, indicating that the Maillard reaction was in an advanced stage. Lysine was degraded in detectable amounts. The same experiment was performed at lower temperatures: 55, 60 and 65°C. The results are shown in Table 3.18.

T °C	Time h	Colour	pH	Lysine concentration mmol/l
unheated	72	creamy, clear	6.64	12.97
55	72	creamy, opaque	6.65	12.64
60	72	creamy, opaque	6.62	12.89
65	72	creamy, more opaque	6.53	12.36

Table 3.18 Changes in colour, pH and lysine concentration of model solutions after storage at respectively 55, 60 or 65°C

There was hardly any difference in colour between 7, 55 and 60°C; at 65°C lysine was degraded, but no brown colour formed. This probably meant that lactose had

reacted with lysine residues of the casein. From this preliminary experiment it could be concluded that after 72 h at 65°C the Amadori compound had been formed, but no advanced Maillard products were formed yet. So, it was decided to store a model solution at 65°C for 72 hours, after that, about 600 μmol/l lysine was lost, meaning that about 600 μmol/l Amadori compound had been formed. After dialysis of this model solution against JK-buffer, the remaining lactose was removed but not the lysine-lactose complex, as it is bound to protein. After dialysis, the retentate was heated at 120 and 140°C and sugars, formic acid and HMF were determined (Figure 3.21). Lactose, galactose, formic acid and HMF were formed. There were at least two possible degradation pathways; hydrolysis of the sugar-amino complex resulting in lactose and amino acid residues or degradation of the complex resulting in galactose, formic acid and other degradation products. Especially galactose was formed, suggesting that the sugar-amino complex was mainly degraded and only in small amounts hydrolysed. At 140°C the galactose concentration decreased after 13 minutes heating, suggesting that the Amadori compound was totally degraded and now galactose was also degraded, resulting in an continuing increase in formic acid concentration. No lactulose could be observed; this means that the lysine-lactose complex is not hydrolysed into lysine and lactulose. A mass balance is given in Table 3.19; the amount of sugar-amino complex still present in solution was calculated by subtracting the lactose and galactose formation from the initial Amadori compound concentration (0.57 mmol/kg). However, the sum of lactose and galactose formation is more than 0.57 mmol at 140°C; this can only be explained by inaccuracy of the measurements. The amount of formic acid formed is also higher than the expected concentration, probably the degradation of reaction products of galactose results also in formic acid formation or this high concentration is also a result of an inaccuracy of the measurement. After 13 min at 140°C the galactose concentration decreased, implying that galactose was degraded and probably no sugar-amino complex was present anymore. These results are not in agreement with the results of Patton and Flipse (1953) who did not find lactose; perhaps their lactose determination was not sensitive enough for such small amounts. From their results they only concluded that some form of carbohydrate was involved, but they did not know the precise chemical nature of the lactose-protein complex and its heat decomposition products.

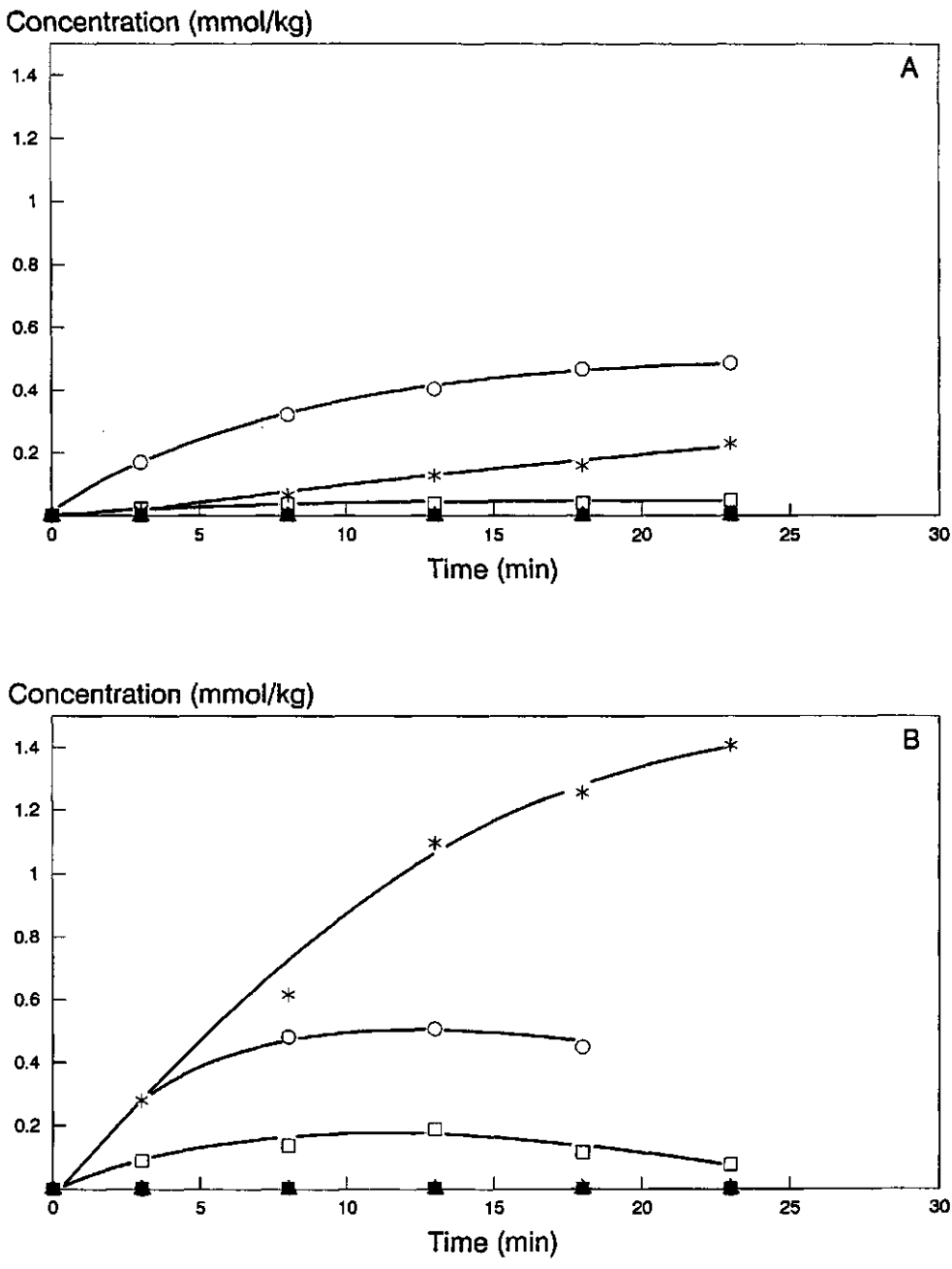


Figure 3.21 Degradation of the Amadori-compound during heating at 120°C (A) and 140°C (B) of a dialysate of a model system heated 72 hours at 65°C. □ = lactose, △ = lactulose, ○ = galactose, * = formic acid, ■ = HMF

T °C	Time min	pH	Lactose mmol/kg	Galac- tose mmol/kg	Formic acid mmol/kg	Sugar-amino complex mmol/kg
120	0	7.08	0.00	0.00	0.00	0.57
120	3	7.01	0.02	0.17	0.02	0.38
120	8	6.95	0.04	0.32	0.07	0.21
120	13	6.90	0.04	0.40	0.13	0.13
120	18	6.83	0.04	0.47	0.16	0.06
120	23	6.79	0.05	0.49	0.23	0.03
140	0	6.63	0.00	0.00	0.00	0.57
140	3	6.51	0.09	0.00	0.28	0.48
140	8	6.29	0.14	0.48	0.62	-0.05
140	13	6.15	0.19	0.51	1.10	-0.13
140	18	6.11	0.12	0.45	1.26	0.00
140	23	6.07	0.08	0.00	1.41	0.00

Table 3.19 Mass balance of degradation of the Amadori compound during heating at 120 and 140°C of a dialysate of a model solution heated 72 hours at 65°C

From these results it can be concluded that lactulose is not formed as a result of the Maillard reaction. However, in the presence of casein more lactulose was formed (Figure 3.15B), so this must either be due to protein acting as a catalyst, or to a pH effect: in the presence of casein the pH drop is less than in the absence of casein, most probably because of the buffering capacity of the casein. Lactulose formation rate increases with pH, as is now well documented. Much less HMF is formed from the lactose-protein complex; only 1% as compared to normal skim milk heated at 140°C. The HMF concentration also decreased after 8 min heating at 140°C. Furfural could not be detected in these solutions.

3.2.2 Model solutions containing lactulose and casein or lactulose

Model solutions containing lactulose (8.8 mM) and casein (0 and 2.6%) dissolved in JK-buffer with a pH of 6.6 were heated at 120°C and 140°C. The pH

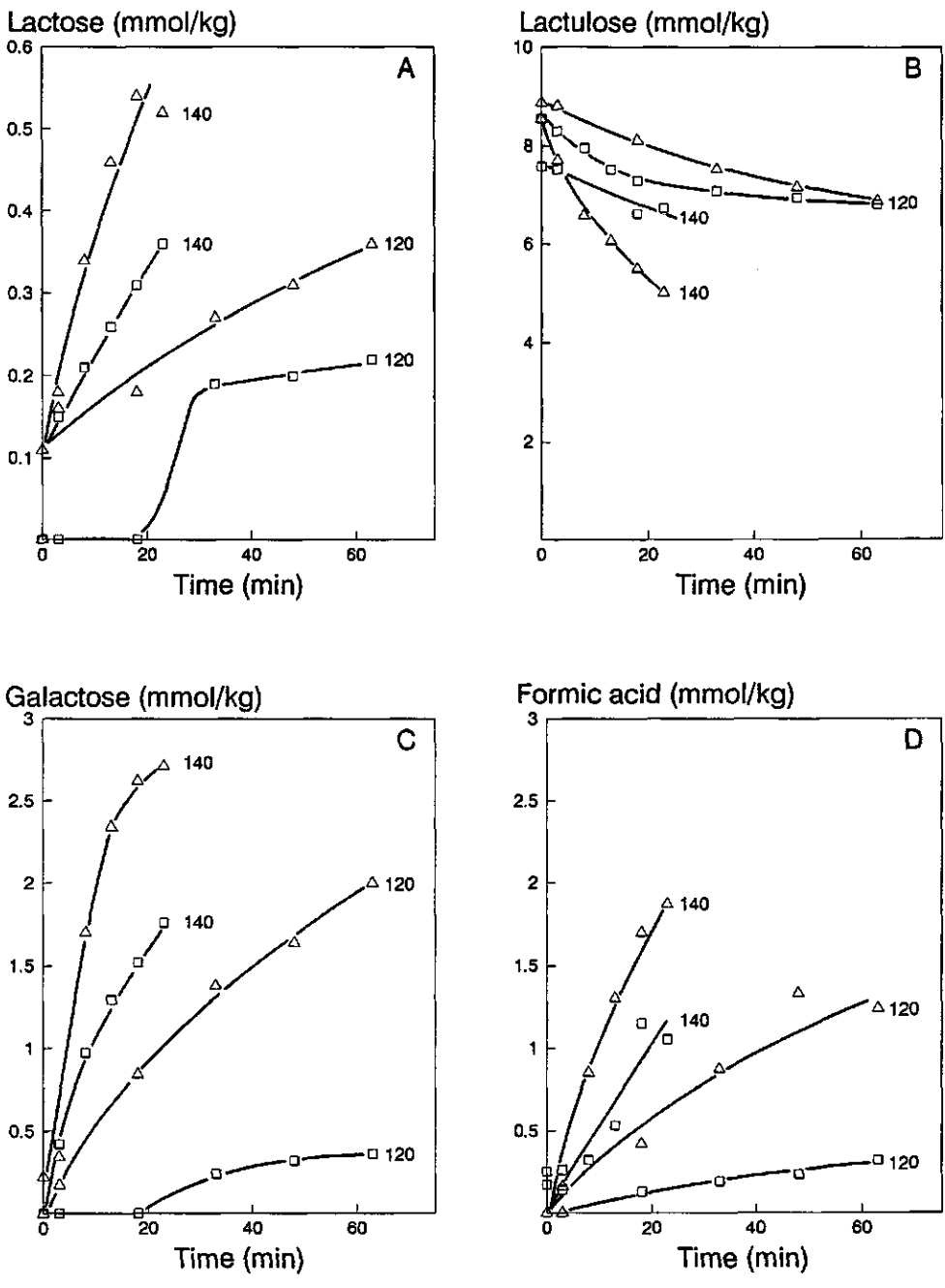


Figure 3.22 Formation of lactose (A), galactose (C) and formic acid (D) and degradation of lactulose (B) in lactulose model solutions (□) and lactulose-casein(2.6%) model solutions (△) after heating at 120 and 140°C

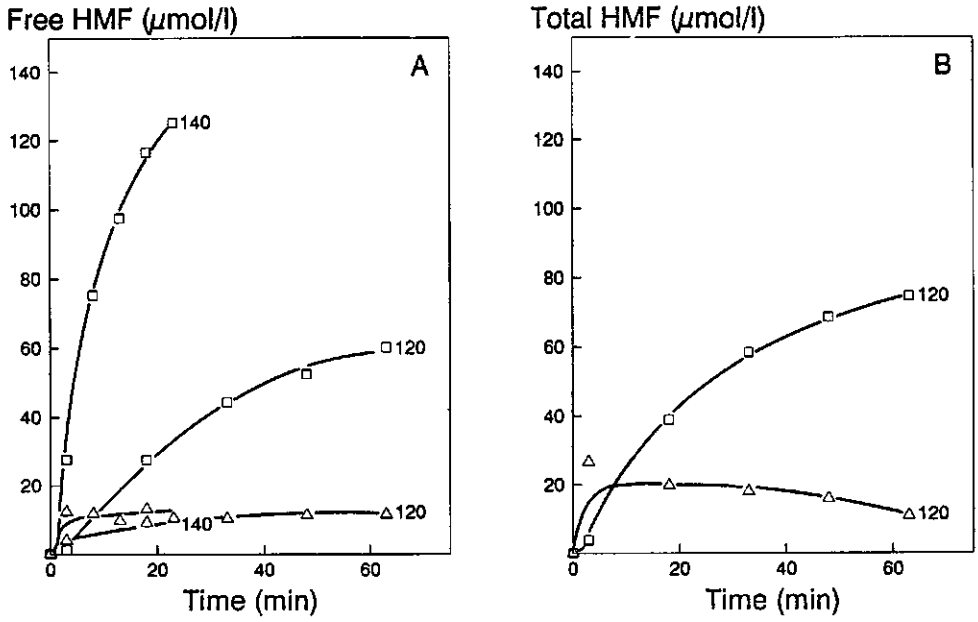


Figure 3.23 Free HMF (A) en total HMF (B) formation in lactulose model solutions (□) and lactulose-casein(2.6%) model solutions (△) after heating at 120 and 140°C

Heating time min	Calu 2.6 120°C	Lu 120°C	Calu 2.6 140°C	Lu 140°C
0	6.60	6.60	6.58	6.60
3	6.56	6.33	6.52	6.49
8			6.34	6.22
13			6.17	5.95
18	6.50	5.89	6.07	5.75
23			6.00	5.55
33	6.41	5.87		
48	6.32	5.81		
63	6.24	5.77		

Table 3.20 pH of model solutions in JK-buffer after heat treatment. Calu 2.6: Lactulose (8.8 mM) and casein (2.6%); Lu: Lactulose (8.8 mM)

was measured 30 min after heat treatment (Table 3.20).

Sugars, formic acid, HMF and furfural were determined (Figures 3.22 and 3.23). Lactose, galactose and formic acid formation were greater in the presence of casein, probably because of buffering, whereas both free and total HMF formation in the presence of casein was less than in the absence of casein. From this it can be concluded either that free HMF reacts with casein to further degradation products, or that less HMF is formed. But, as the initial HMF formation in the presence of casein is greater than in the absence of casein at 120°C, and formation of a brown colour is more intense in the presence of casein, the former conclusion is more likely. A mass balance of the degradation of lactulose is given in Table 3.21; the deficit is calculated by subtracting the amounts of lactose and galactose formed from that of lactulose degraded (formation of Maillard type products was not taken into account, as no lysine was determined). Sometimes the mass balance results in a negative deficit; this means an increase in the total amount of moles and is, of course, impossible. Probably this is a result of experimental inaccuracy. In this case the standard deviation of the deficit was calculated according to Eq. 3.6:

$$\sigma^2_{deficit} = \sigma^2_{lactulose} + \sigma^2_{lactose} + \sigma^2_{galactose} \qquad (3.6)$$

As the lactose formation is only very low, the standard deviation found for lactulose is also used for lactose. Thus, $\sigma_{deficit} = 0.6$, from this it can be concluded that almost all deficits reported in Table 3.21 are within the measurement error.

Heating time min	Calu 2.6 120°C mmol/kg	%	Lu 120°C mmol/kg	%	Calu 2.6 140°C mmol/kg	%	Lu 140°C mmol/kg	%
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	-0.3*	-*	0.1*	-*	0.7	78.1	-0.3*	-*
8					0.3*	-*	-0.6*	-*
13					0.0*	-*	-0.5*	-*
18	-0.3*	-*	0.3*	-*	0.2*	-*	0.1*	-*
23					0.6*	-*	-0.3*	-*
33	-0.3*	-*	0.1*	-*				
48	-0.2*	-*	0.1*	-*				
63	-0.4*	-*	0.2*	-*				

Table 3.21 Mass balance of lactulose model solutions in JK-buffer after heat treatment. Deficit in mmol/kg and as % of lactulose degradation. Calu 2.6: Lactulose (8.8 mM) and casein (2.6%); Lu: Lactulose (8.8 mM). * = within measurement error

3.2.3 Model solutions containing galactose and casein or galactose

To study the behaviour of galactose during heat treatment, a model solution containing galactose (11.1 mM) or galactose and casein (2.6%) in JK-buffer was heated at 140°C. Sugars, formic acid, free HMF and furfural were determined (Figure 3.24). The change of pH is given in Table 3.22. The mass balance is also given in Table 3.22; the deficit is calculated by subtracting the formic acid formed from the galactose degraded in the case of the galactose model ($\sigma_{deficit}$ = 0.5) and by subtracting the formic acid and tagatose formed from the galactose degraded ($\sigma_{deficit}$ = 0.5) in the case of the galactose-casein model (formation of Maillard type products was not taken into account, as no lysine was determined).

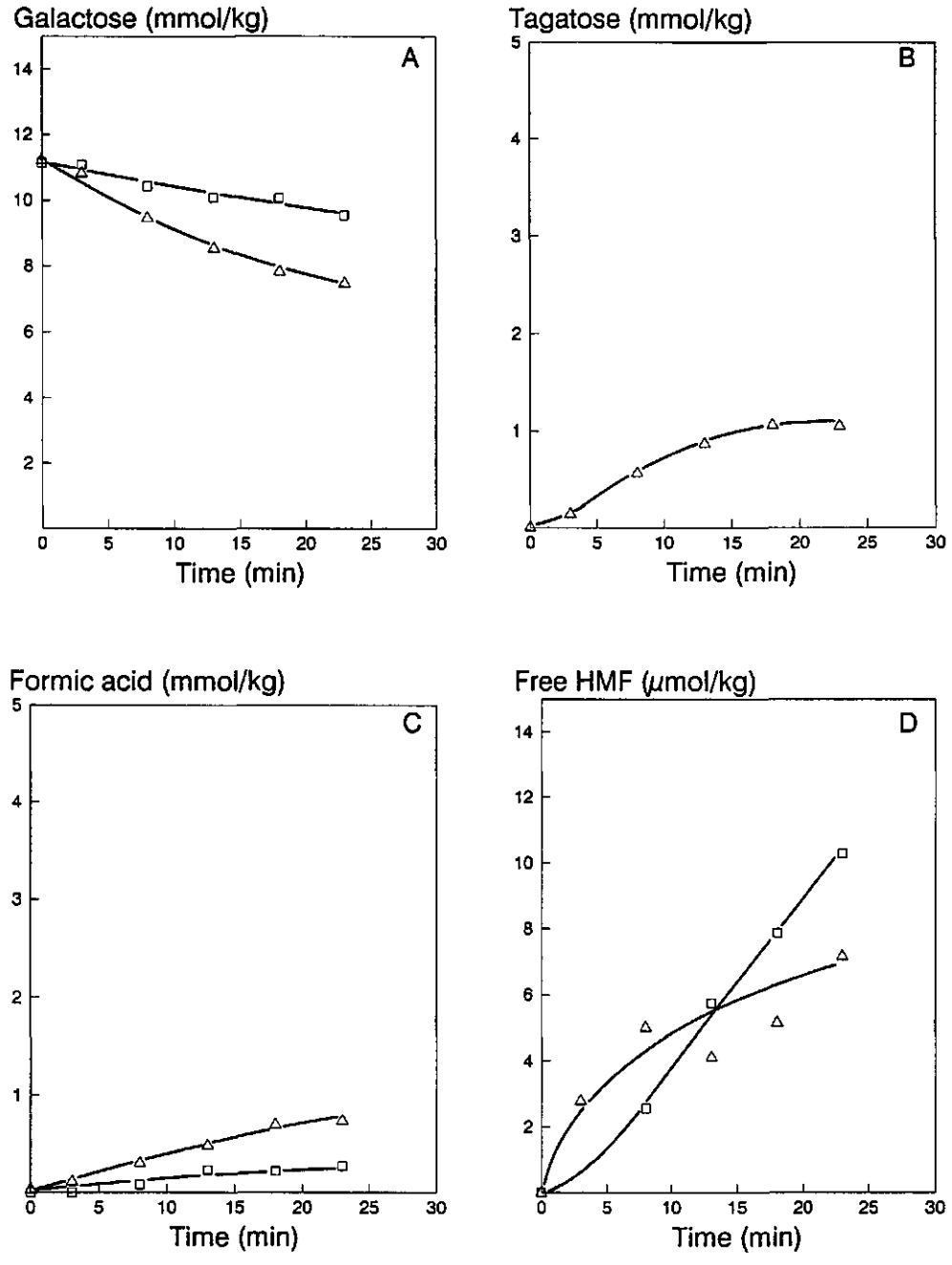


Figure 3.24 Galactose degradation (A) and formation of tagatose (B), formic acid (C) and free HMF (D) in galactose (□) and galactose-casein(2.6%) (△) model solutions after heating at 140°C

Time	Ga			Caga		
min	pH	deficit		pH	deficit	
		mmol/kg	%		mmol/kg	%
0	6.72	0.0	0.0	6.62	0.0	0.0
3	5.93	0.1*	-*	6.52	0.2*	-*
8	5.80	0.6	87.9	6.37	0.9	51.2
13	5.75	0.8	78.3	6.22	1.3	49.8
18	5.71	0.9	79.6	6.12	1.6	48.1
23	5.72	1.3	83.0	6.04	2.0	52.1

Table 3.22 pH and mass balance of the galactose model solution in JK-buffer (Ga) and galactose-casein model solution in JK-buffer (Caga) after heat treatment at 140°C. Deficit in mmol/kg and in % of galactose degradation. * = within the measurement error

Galactose was degraded in both solutions, but more so in the presence of casein. In that case also more formic acid was formed (5% of galactose present, after 20 min at 140°C). Tagatose was only determined in the galactose-casein model: the retention time of tagatose appeared to be 30.15 min; unfortunately, the HPLC chromatograms of the galactose model were only run for 25 min, so, tagatose formation was not determined. From these results it can be concluded that galactose is also degraded at such high temperatures. Calvo and Olano (1989) also heated a solution of galactose in SMUF for 20 or 30 min at 120°C in the presence or absence of protein (serum proteins and casein); they found that the degradation of galactose was reduced in the presence of casein as well as whey proteins.

The formation of HMF was higher for the first 13 minutes in the presence of casein and after 13 minutes higher in the absence of casein; this is probably due to degradation of HMF in the presence of casein. To check this, HMF was also heated in the presence and absence of casein.

3.2.4 Model solutions containing HMF and casein or HMF

Model solutions containing HMF (323 μM) and furfural (34 μM) in JK-buffer and HMF (357 μM), furfural (34 μM) and casein (2.6%) in JK-buffer were heated

Time min	HMF and furfural in JK-buffer	HMF, furfural and 2.6% casein in JK-buffer
0	6.60	6.60
3	5.88	6.55
8	5.76	6.51
13	5.69	6.37
18	5.66	6.28
23	5.69	6.23

Table 3.23 pH in a model solution containing HMF and furfural in JK-buffer and in a model solution containing HMF, furfural and casein in JK-buffer after heat treatment at 140°C

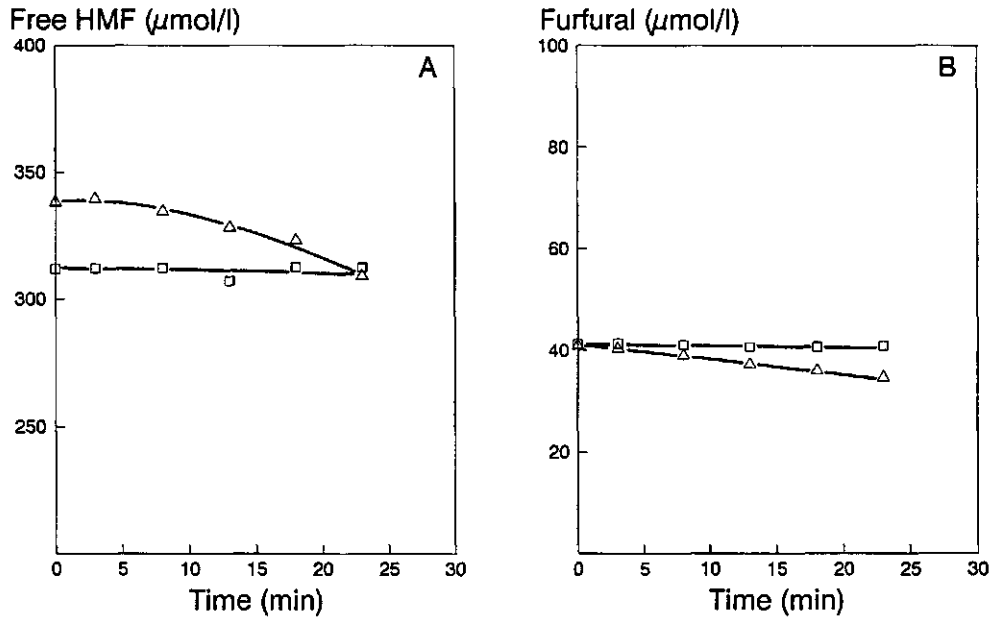


Figure 3.25 Free HMF (A) and furfural (B) degradation in HMF/furfural (□) and HMF/furfural-casein(2.6%) (△) model solutions after heating at 140°C

at 140°C. The change of pH is given in Table 3.23.
The HMF and furfural concentration are shown in Figure 3.25; in the presence of casein some HMF and furfural were slowly degraded, in the absence of casein the concentrations did not change. This was somewhat unexpected, because our impression from the literature is that these compounds are very reactive and would readily polymerize. Apparently, they need other components like protein or amino acids to do so. No formic acid formation could be detected. The decrease in pH as shown in Table 3.23 was thus likely caused by changes in salt equilibria (see also Table 3.15 for heating JK-buffer).

3.2.5 *Model solutions containing formic acid and casein*

To study the behaviour of formic acid during heat treatment, formic acid (14 mM) was added to a solution containing casein (2.6%) in JK-buffer, the pH was readjusted to 6.6 with KOH and the solution was stirred until the casein was dissolved. The change of pH after heat treatment at 140°C is shown in Table 3.24, this presumably being a result of salt changes (see also Table 3.23). The level of formic acid did not change during heat treatment (results not shown), so formic acid appears to be stable during heating.

Time min	pH
0	6.61
3	6.50
8	6.39
13	6.26
18	6.15
23	6.09

Table 3.24 pH in formic acid-casein model solution in JK-buffer after heating at 140°C

3.2.6 Model solutions containing deoxyribose and casein or deoxyribose

If lactose is degraded into lactulose, galactose, formic acid and HMF, there still is a missing compound. Formic acid is, according to Patton and Flipse (1957), derived from carbon atom 1 in the glucose moiety of lactose. Hence, a compound of five carbon atoms would remain, and from the results is shown that furfural and furfuryl alcohol are not nearly formed in such quantities. In the scheme of De Wit (1979), a C_5-structure is mentioned which appears to be deoxyribose, so it was tried to analyse for deoxyribose in heated milk and model solutions. However, deoxyribose was not found in the HPLC-chromatogram of heated milk and model solutions. This may be caused by either of two effects: it is not formed at all or it is degraded very rapidly. To study the degradation of 2-deoxy-D-ribose, it was added to skim milk and the milk was heated at 140°C. After heating for 15 min, 60% of the deoxyribose added was left. To study its degradation products, deoxyribose (2.89 mM) was dissolved in JK-buffer with and without casein (2.6%) and heated at 140°C. The change in pH is shown in Table 3.25.

Time min	Deoxyribose in JK-buffer		Deoxyribose and casein (2.6%) in JK-buffer	
	pH	colour	pH	colour
0	6.78	clear	6.65	opaque
3			6.55	light creamy
8			6.42	creamy
13	6.09	opaque	6.32	creamy-orange
18			6.29	light orange
23	5.96	light creamy	6.23	orange

Table 3.25 pH of a deoxyribose model solution without casein in JK-buffer and with casein in JK-buffer after heat treatment at 140°C

From the results (Figure 3.26) it can be concluded that deoxyribose was degraded during heating. The degradation was much more intensive in the presence of casein. A new peak was found in the chromatogram which was also found in the chromatograms of the degradation of lactose, lactulose and galactose in the model

solution in JK-buffer and in heated milk. The peak first increased and then decreased after prolonged heating. In an attempt to identify this peak, D(-)ribose and D(+)xylose were injected, but the retention times of these sugars did not correspond to the retention time of the new peak. From this experiment it may be concluded that deoxyribose is formed during heat treatment of model solutions and is degraded rapidly to other, as yet unknown, degradation products.

Upon completion of this work, Troyano et al. (1992b) reported the presence of 3-deoxypentulose in heated milk. It was also found when lactose was heated under alkaline conditions. A mechanism of its formation via the 1,2-enediol and followed by the loss of formic acid was proposed. They found 1.5 mmol/l after heating milk 20 min at 135°C, the isolated 3-deoxypentulose was also rather unstable in alkaline solutions and probably it is also unstable in heated milk. From these results it can be concluded that when lactulose is degraded, several C5 compounds, galactose and formic acid are formed. Deoxyribose, 3-deoxypentulose, furfural and furfuryl alcohol are part of these C5 compounds.

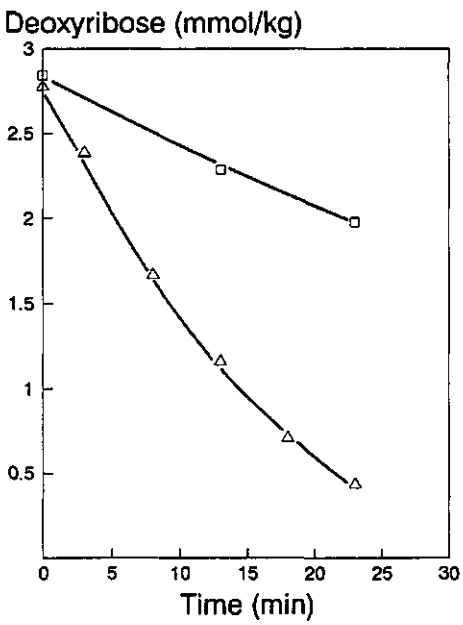


Figure 3.26 Degradation of deoxyribose in deoxyribose (□) and deoxyribose-casein(2.6%) (△) model solution after heating at 140°C

3.3 Conclusion

From the experiments described in this chapter the main degradation routes of lactose can be described. Heating lactose resulted in the formation of lactulose, galactose, formic acid, HMF, furfural and further degradation products, both in the presence and absence of casein and lactulosyllysine in the presence of casein. If lactulose was heated it was partially isomerized into lactose (very small amounts) and degraded into galactose, formic acid, HMF, furfural, deoxyribose and further degradation products, in the presence and absence of casein. Galactose was degraded into tagatose, formic acid, HMF, furfural, deoxyribose and further degradation products. In the presence of casein, HMF and furfural are degraded into advanced Maillard products, though in smaller quantities than expected from literature. Formic acid is stable during heat treatment and deoxyribose is very unstable and degraded into unknown compounds.

If the Amadori compound from lactose and protein-bound lysine was heated in the absence of lactose at 120°C, lactose, galactose, formic acid and HMF were formed. After heating at 140°C the same compounds were formed, but now the formation of galactose, HMF and lactose showed a maximum (Figure 3.21B). Probably the Amadori compound was fully degraded (about 600 μmol/l) and then the lactose and galactose formed were also degraded, resulting in formation of formic acid and other (unknown) reaction products.

From these results we derived a reaction network model for the degradation pathways of lactose: see Figure 3.27. In this network, compound X denotes an unstable C6-intermediate that is quickly degraded into a C5-compound (amongst others deoxyribose) and formic acid. In chapter 4 the degradation of lactose in UHT-treated milk is studied and compared with the results of sterilized milk to see whether the results fit in the same model. Finally, in chapter 5, an attempt is made to establish kinetic parameters for the model depicted in Figure 3.27; we tried to model the degradation of lactose by computer simulations to be able to predict the degradation of lactose during heat treatment of milk. The results of the experiments described in chapter 3 and 4 will then be compared to the results of the simulation.

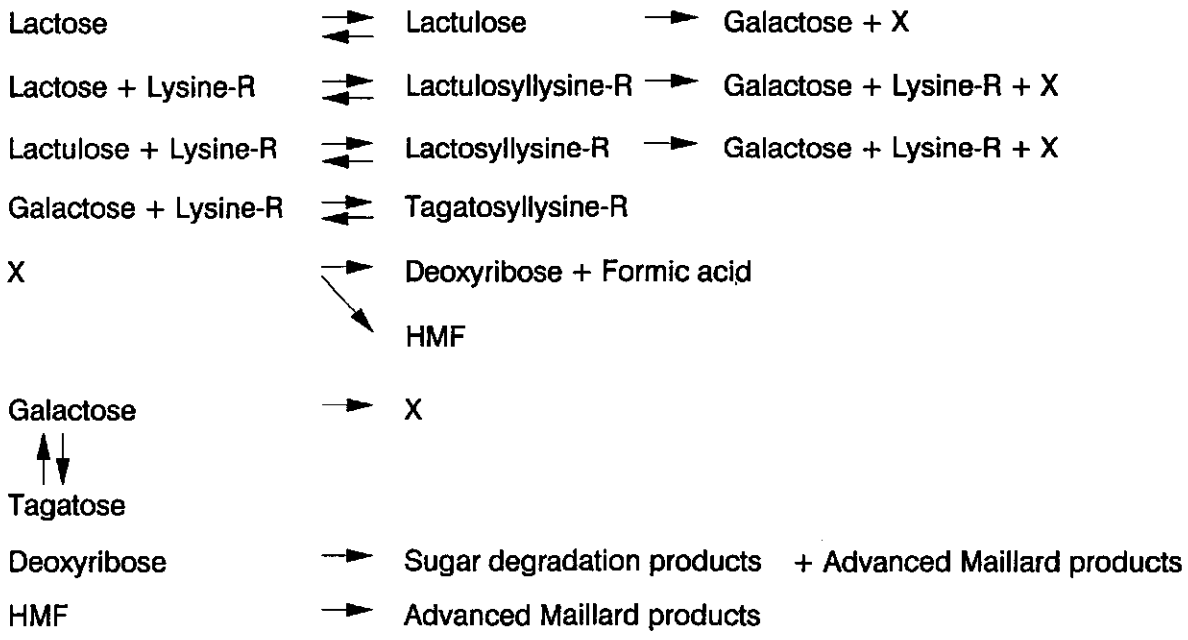


Figure 3.27 Postulated model for the degradation pathways of lactose during heat treatment of milk. X: unknown C-6 compound

4 REACTION PRODUCTS OF LACTOSE DURING UHT TREATMENT

In this chapter experiments are described using UHT treatment, i.e. relatively short times at high temperatures. The milk was heated in a pilot plant UHT apparatus in the temperature range 110-150°C. The milk samples were analysed for the same reaction products as described in 3.1.1. The influence of the fat and protein contents of the milk on the formation of reaction products was studied.

4.1 UHT treated milk

4.1.1 Heat intensity

During UHT treatment, the milk is heated either directly (by steam injection) or indirectly (by a heat exchanger) to a certain temperature in the heater; after that the milk is held at that temperature for some time in holding tubes of varying lengths inducing varying heating times; and then the milk is cooled directly (by flash evaporation) or indirectly, respectively. In our experiments, the temperature before and after the holding tubes appeared to be not the same: the temperature decreased by about 3°C (somewhat depending on the length of the holding tube). This means that in the case the temperature was measured before the holding tube, the real mean temperature of the milk was lower than measured and in the case the temperature was measured after the holding tube, the real mean temperature of the milk was higher. A second effect on heat intensity in the case of indirectly heated UHT-milk is the warming-up and cooling-down periods; they are mostly neglected, but during these periods chemical reactions will take place. So, the calculated heating-time is shorter than the total heating time including warming-up and cooling-down period. It can thus be concluded that the time-temperature combinations used for kinetic calculations are mostly not the actual times and temperatures occurring in the UHT apparatus. In literature, these effects are not usually mentioned. Swartzel and coworkers (Nunes and Swartzel, 1990) developed a calculation method to include heating-up and cooling down periods, resulting in so-called equivalent times and temperatures. Application of this method showed that the contribution of heating-up and cooling-down was almost negligible for chemical reactions in the pilot plant we have used (van Boekel, 1992, unpublished data). In addition, residence time distribution can play a part especially

if the flow is laminar. In our case, however, Reynolds numbers of about 10 000 were realized, so that an almost flat flow profile may be assumed.

4.1.2 Sugar isomerization in UHT treated milk

The first experiments with the UHT pilot plant apparatus were performed at a single heating time with varying temperature; this means using only one holding tube. For every heating time the flow rate was different. It appeared difficult to plot these results; if the concentration of sugars was plotted against heating time, the temperature was not exactly (e.g.) 140°C for all the heating times and it was neither the same batch of milk. Consequently, the data in a concentration-time plot differed in temperature and originated from different milks. In subsequent experiments, the milk was heated at one temperature for different times, by changing the holding tubes (while holding the flow constant). These results were markedly better to use for kinetic interpretation. The results of the sugar determinations and total HMF of direct and indirect heat-treated UHT milks are shown in Figures 4.1 and 4.2, respectively. The protein, fat and lactose content and the pH of the original milks are given in Table 4.1. In Figure 4.1 the temperature is the mean of the temperature before and after the holding tube. The same holds in Figure 4.2, except for the measurements at 135, 145 and 155°C, in which cases the temperature given is that measured before the holding tube.

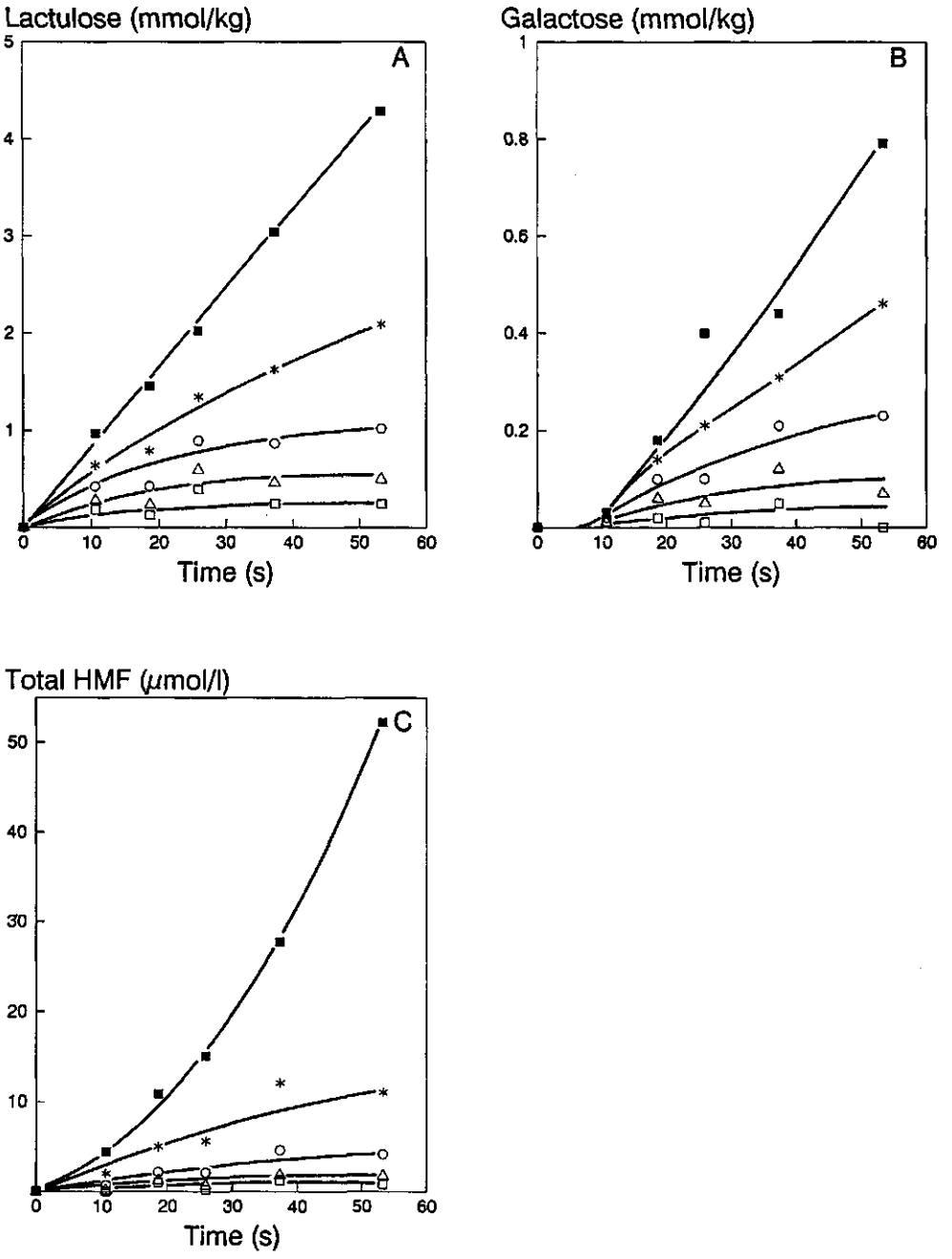


Figure 4.1 Formation of lactulose (A), galactose (B) and total HMF (C) in direct UHT heated skim milk. □ = 110°C, △ = 120°C, ○ = 130°C, * = 140°C, ■ = 150°C

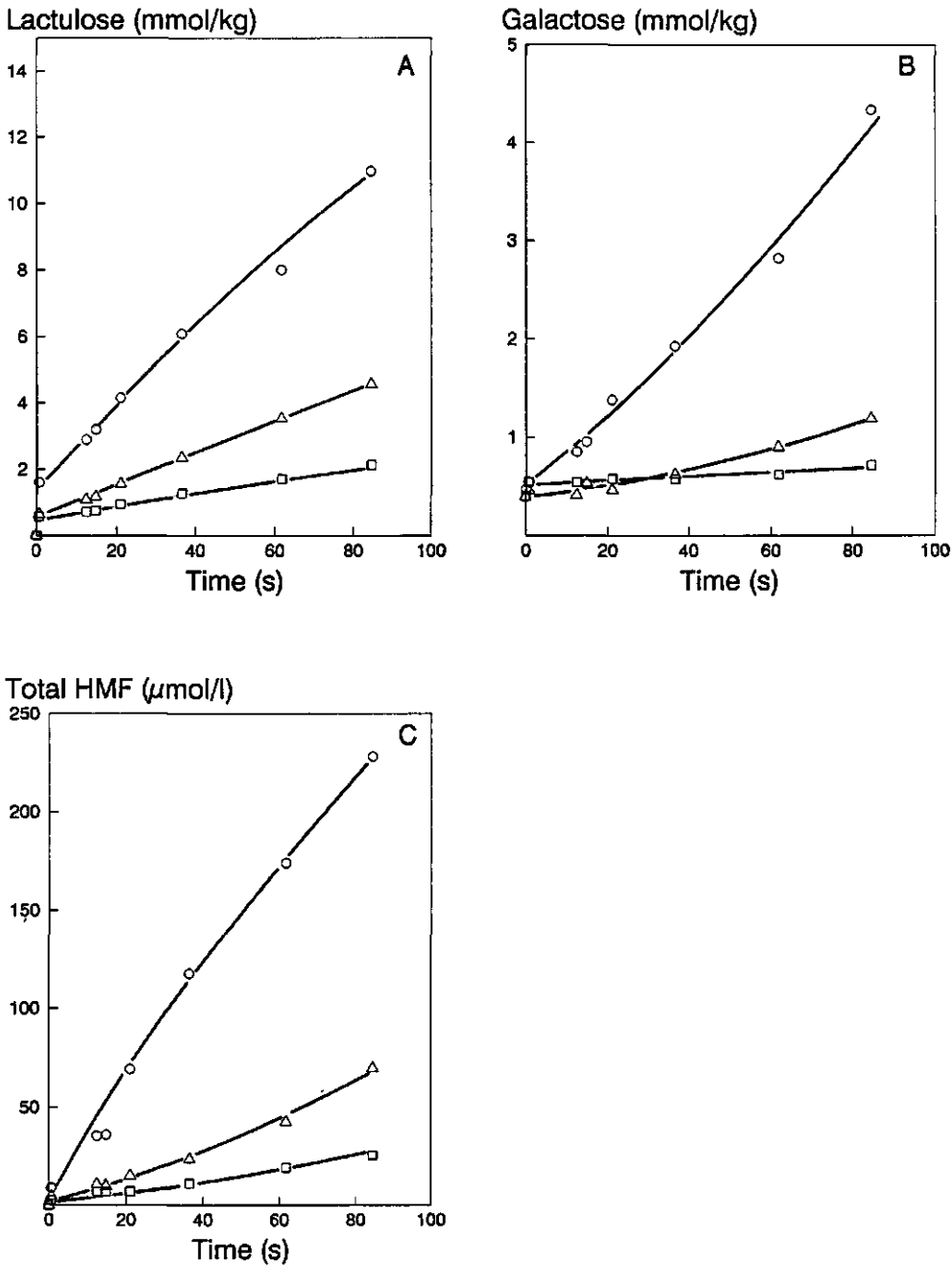


Figure 4.2 Formation of lactulose (A), galactose (B) and total HMF (C) in indirect UHT heated skim milk. □ = 135°C, △ = 145°C, ○ = 155°C

Experiment	Fat content %	Protein content %	Lactose content %	pH
10.6 s direct	0.08	3.26	4.94	6.69
12.7 s indirect	0.07	3.35	4.79	6.66
18.6 s direct	0.11	3.49	4.82	6.72
19.1 s indirect	0.09	3.28	4.86	6.70
25.9 s direct	0.08	3.39	4.84	6.62
27.1 s indirect	0.09	3.29	4.92	6.64
37.3 s direct	0.22	3.61	4.68	6.67
36.6 s indirect	0.18	3.56	4.70	6.63
53.3 s direct	0.06	3.38	4.83	6.68
55.7 s indirect	0.07	3.42	4.89	6.66

Table 4.1 pH and composition of original milks, as measured by Milko scan

Lactulose formation during UHT treatment has been determined by many authors. Nangpal (1988), for instance, determined lactulose formation in direct and indirect heated UHT milk (pilot plant UHT apparatus by Alfa-Laval). The results of Nangpal were of the same order of magnitude as ours; the lactulose concentrations ranged from 0.02 mmol/l lactulose after 7.26 s at 120°C to 4.1 mmol/l lactulose after 131.5 s at 150°C for direct heated milk and from 0.04 mmol/l lactulose after 7.54 s at 120°C to 3.4 mmol/l after 36.8 s at 150°C for indirect heated milk; he described the lactulose formation as a zero-order reaction. Geier and Klostermeyer (1983) found lactulose contents of 0.3 to 1.5 mmol/l in commercial UHT samples. Olano et al. (1989) found lactulose contents of 0.3 to 1.0 mmol/l in the case of commercial UHT milk samples from a direct-heating UHT plant and 1.2 to 2.2 in UHT milk from an indirect-heating UHT plant. The galactose contents of the same UHT samples were in the range of 0.5 to 0.9 mmol/l. Andrews (1984) distinguished pasteurized, UHT and sterilized milks by their lactulose content. A milk sample was considered direct UHT heat-treated when it contained less than 0.3 mmol/l lactulose while an indirectly heated sample contained more than 0.6 mmol/l.

The lactulose contents of the UHT milk samples in our experiments were 0.1 to 2.1 mmol/l. According to Fink and Kessler (1988), the UHT region is in the range 10 to 30 s 130°C or 1 to 10 s 150°C . The lactulose formation in this region was about 0.5 to 2 mmol/l in the case of indirect UHT milk (Figure 4.2) and 0.4 to 0.7 mmol/l in the case of direct UHT milk (Figure 4.1). Galactose contents were found between 0.5 and 0.8 mmol/l in this region (Figures 4.1 and 4.2). These results are thus seen to be in agreement with those found in literature.

4.1.3 HMF and furfural formation

The formation of HMF and furfural in UHT treated milk was also determined. However, the formation of furfural was very low (0.7 μmol was formed after heating 87.6 s at 145°C), and it was decided not to analyse the furfural contents any more.

The total HMF formed was also in the order of micromoles, though much higher than furfural (Figures 4.1 and 4.2). The formation of free HMF was very low; it was determined in skim milk after heating at 140°C, and the results are given in Table 4.2. The amount of total HMF increased with increasing time and temperature.

Heating time s	Free HMF μmol/l	Total HMF μmol/l
1.5	0.05	4.0
12.8	0.6	7.8
21.8	1.8	11.8
37.8	4.1	19.4
64.0	10.3	33.5

Table 4.2 Free and total HMF formation after indirect heating of skim milk at 140°C

Mottar (1983) measured total HMF in indirect heat-treated milk and found values varying from 2.9 μmol/l after about 3 s 130°C till 22.3 μmol/l after 20 s 150°C.

Fink and Kessler (1988) found a total HMF value of 4-11 μmol in commercial UHT milk samples. Fink and Kessler (1986) also heated milk in a pilot plant apparatus with holding times from 10 to 2400 s; they also found rather high HMF concentrations (130 - 275 μmol/l) in indirectly heated milk after 100 s at 150 and 160°C. They concluded that the HMF value could be used to distinguish UHT milk from sterilized milk. Konietzko and Reuter (1986) also found a total HMF value of 1.5 to 14.8 μmol/l in UHT milk from different commercial UHT plants. They concluded that the concentration of total HMF can be used as a chemical index for the thermal deterioration of milk during UHT treatment, if the values are in the linear range: they found the HMF formation to be linear with time till 18 s 130° or 6 s 150°C. Dehn-Müller (1989) determined total HMF in directly UHT treated milks and found HMF values in the range 0.5-28 μmol/l after heating 2-128 s at 100-150°C. Dehn-Müller performed linear regression of furosine values on total HMF values and found the following relation:

$$\text{Furosine} = 2.34 * \text{HMF} + 1.76 \qquad r^2 = 0.92 \qquad (4.1)$$

where the furosine concentration is in mg/l and the HMF concentration in μmol/l.

Dehn-Müller also determined furosine and HMF concentrations in commercial directly and indirectly heated UHT-milks and found total HMF varying from 0 to 21.3 μmol/l. In this case also a linear regression of furosine on HMF values was performed:

$$\text{Furosine} = 4.016 * \text{HMF} + 8.530 \qquad r^2 = 0.72 \qquad (4.2)$$

where furosine concentration is in mg/l and HMF concentration in μmol/l.

However, these equations do not correspond very well with each other. Erbersdobler and Dehn-Müller (1989) also described the relation between HMF and furosine found by Dehn-Müller, but they used not exactly the same values:

$$HMF = 0.247 * Furosine + 4.547 \qquad r = 0.846 \qquad (4.3)$$

where furosine is in mg/l and HMF in μmol/l.

Furosine can in principle be related to the lactulosyllysine content. Assuming that the furosine yield is 40% of the lactulosyllysine content (Erbersdobler, 1986) and converting mg/l into mmol/l (M = 254 for furosine), yields:

$$Lactulosyllysine = \frac{Furosine * 2.5}{254} \qquad (4.4)$$

where furosine is in mg/l and lactulosyllysine in mmol/l.

Using relation 4.3, we can convert total HMF content to furosine, and from relation 4.4 the lactulosyllysine content then can be calculated:

$$Lactulosyllysine = 0.0398 * HMF - 0.1812 \qquad (4.5)$$

where HMF is in μmol/l and lactulosyllysine in mmol/l.

It is clear, however, that this conversion induces considerable error, but it is the best estimate we can give for the lactulosyllysine content in the cases where lysine content was not determined.

Kind and Reuter (1990) found that the HMF value not only increased with increasing heating temperature and time, but also with initial lactose concentration. They concluded that the suitability of the HMF value for detecting heat treatment is limited, as also in raw milk an HMF value is found, which is moreover not constant (this is found by most research workers).

HMF is a result of both the Maillard reaction and the isomerization reaction (Chapter 3). However, the formation of total HMF is very small, only μmoles, and HMF as such is not a good measure for occurrence of the early Maillard reaction. Unfortunately, it was for us not possible to measure the formation of lactulosyllysine. According to Dehn-Müller (1989) and Erbersdobler and Dehn-

Müller (1989) it is possible to estimate the formation of lactulosyllysine using Equation (4.5). However, the total HMF formation includes also the formation of HMF as a result of the isomerization reaction, as a result of which, especially at high temperatures (over 140°C) the lactulosyllysine concentration will be overestimated. On the other hand, it is very likely that this formation due to isomerization reactions is included in their equation.

4.1.4 Formation of formic acid

The formation of formic acid was determined in milk that was indirectly heated 0-87.6 s at 150°C. The results are shown in Table 4.3. From these results it becomes clear that during normal UHT treatment (only a few seconds at 140 or 150°C) no formation of formic acid takes place, or the level of formic acid is below the detection limit. Unfortunately, the pH after UHT treatment of this experiment was not determined. In another experiment, the pH of milk heated 0-87.6 s at 145°C did not change with heat treatment and no formic acid formation could be detected.

Heating time s	Formic acid mmol/kg
0	0
1.5	0
12.8	0
15.4	0
21.8	0
37.8	0.92
64.0	1.76
87.6	2.66

Table 4.3 Formic acid contents after indirect UHT heat treatment at 150°C

4.1.5 Mass balance

The mass balance of the degradation of lactose during UHT treatment was calculated by subtracting the lactulose and galactose and the lactulosyllysine formation from the lactose degradation. However, the loss of lysine was not measured, so the amount of Amadori product present in the heat treated milk was calculated (Equation 4.5). In preliminary experiments, the lysine degradation during UHT heat treatment was determined, but the degradation level was mostly within the measurement error. The maximum lysine loss was 2 mmol/l after heating 72 s at 150°C. In order to calculate the Amadori product, Eq. (4.5) was used.

Time s	135°C mmol/kg	%	145°C mmol/kg	%	155°C mmol/kg	%
0	0	0	0	0	0	0
0.66	-0.34*	-*	0.84*	-*	6.17	43.8
12.4	-0.65*	-*	8.16	85.8	9.11	67.0
14.8	-1.55	263	4.99	77.0	11.35	69.8
21.0	-1.51	525	7.99	78.4	14.43	65.4
36.5	-0.06*	-*	2.82	45.5	7.50	38.5
61.7	-0.16*	-*	5.80	51.4	10.94	38.9
84.5	-0.24*	-*	4.61	36.6	5.35	18.4

Table 4.4 Mass balance of the degradation reactions of lactose in indirect UHT treated milk; the deficit in mmol/kg and % of the lactose degradation. * = within measurement error

The maximum loss of lysine in sterilized milk was about 4 mmol/l (see Figure 3.8), so, the maximum amount of lactulosyllysine formed calculated with Eq. (4.5) after heating 21.0-84.5 s at 155°C was rather high (2.57-8.89 mmol/l). So, probably, at this temperature Equation (4.5) is not reliable. The negative values at 135°C reflect the inaccuracy of the determinations. The total standard deviation of the calculation of the mass balance can be calculated according to:

$$\sigma^2_{total} = \sigma^2_{lactose} + \sigma^2_{lactulose} + \sigma^2_{galactose} + \sigma^2_{HMF} \quad (4.6)$$

This results in a total standard deviation of 1.5, meaning that the values at 135° are almost all within the standard deviation. The impossible values at 14.8 and 21.0 s at 135°C are due to unexplained variations in the lactose concentrations that sometimes occurred in these experiments.

The amount of missing moles in UHT treated milks is, especially at 155°C, large. This means that further degradation products, like advanced Maillard products, were formed.

4.2 Influence of fat content

To study the influence of fat content on the formation of lactulose and HMF, milk with varying fat contents was indirectly UHT heated. Milks with 0.1, 1.5, 3.0, 4.2 or 4.6% fat were heated. Also recombined milk with a fat content of 4.6% was heated. The latter experiment was done to determine any effect of the composition of the fat globule membrane on the degradation reactions (in recombined milk the constituents of the original milk fat globule membrane are not present). The fat, protein and lactose contents as determined by Milko scan, and the pH are given in Table 4.5. The flow in the UHT apparatus was 21.8 ml/s.

Milk	Fat content %	Protein content %	Lactose content %	pH
skimmed	0.10	3.46	4.80	6.66
semi skimmed	1.51	3.42	4.81	6.68
whole	2.98	3.37	4.71	6.68
whole	4.23	3.25	4.20	6.66
skimmed	0.16	3.49	4.73	6.74
whole	4.57	3.33	4.47	6.69
recombined	4.62	3.26	4.47	6.67

Table 4.5 Composition of original milks determined by Milko scan and pH

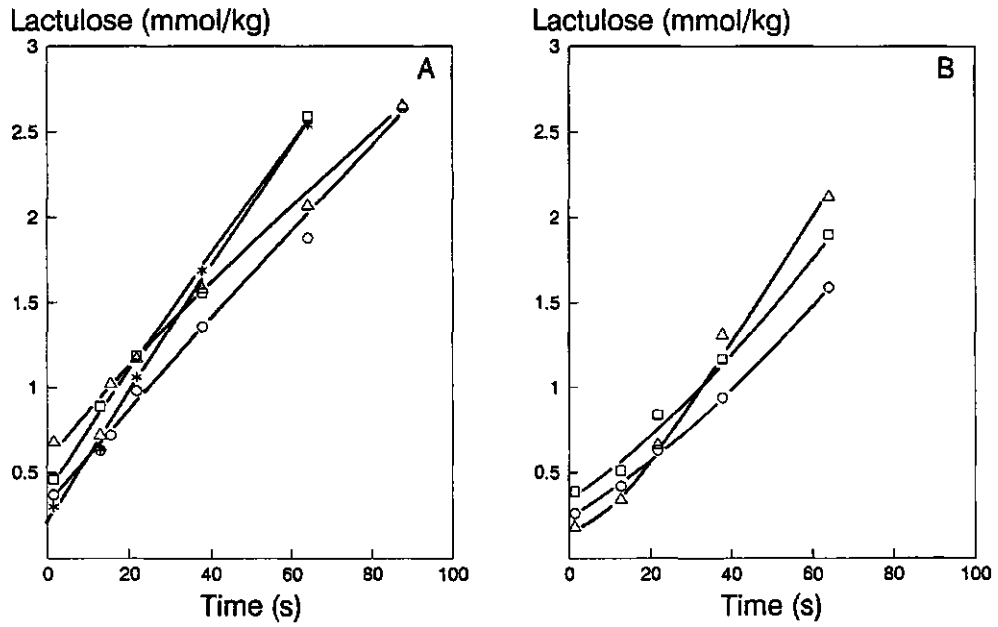


Figure 4.3 Formation of lactulose in indirect UHT heated milk with varying fat contents. Figure A: □ = skim milk with 0.1% fat, △ = semi skimmed milk with 1.51% fat, ○ = whole milk with 2.98% fat, * = whole milk with 4.23% fat. Figure B: □ = skim milk with 0.16% fat, △ = whole milk with 4.57% fat, ○ = recombined milk with 4.62% fat

4.2.1 Influence of fat content on formation of lactulose

The results of the formation of lactulose in indirect UHT milk with varying fat contents are shown in Figure 4.3. The milk with 1.51 and 2.98% fat was from one batch, the milk with 0.1 and 4.23 % was from another batch and the milk with 0.16 and 4.57% fat was from a third batch. From these results, no significant influence of the fat content on the lactulose formation can be found. This is contrary to the results of De Koning et al. (1990) who found a 40-50% increase in lactulose formation in milk with 3% fat as compared to milk with 1.5% fat. However, Andrews (1984) and Geier and Klostermeyer (1983) also concluded that the fat content of the heated milk did not have any influence on lactulose formation. In recent studies it appeared that UHT heating of milk products with increasing fat contents seems to result in somewhat lower lactulose contents (van Boekel, 1992, private communication), maybe because the heating intensity is less with increasing fat contents, for reasons yet unknown.

4.2.2 Influence of fat content on the HMF formation

The HMF formation in indirectly UHT heated milk with varying fat contents is shown in Figure 4.4. When the results of the milk with 1.51% fat are compared to the results of the milk with 2.98% fat, the HMF formation was somewhat higher in the milk with 2.98% fat, but, when the HMF formation in milk with 0.16 is compared to the milk with 4.23% fat, the HMF formation was somewhat higher in the case of the lowest fat content. In Figure 4.4B the HMF formation is almost the same for the three kinds of milk. It may be concluded that the presence of fat has no significant effect on degradation reactions of lactose, at least not in the range 0-4.5% fat. So, the results of De Koning et al. (1990) are rather doubtful, especially because in recent studies a slight opposite effect of fat content is shown (van Boekel, 1992, private communication).

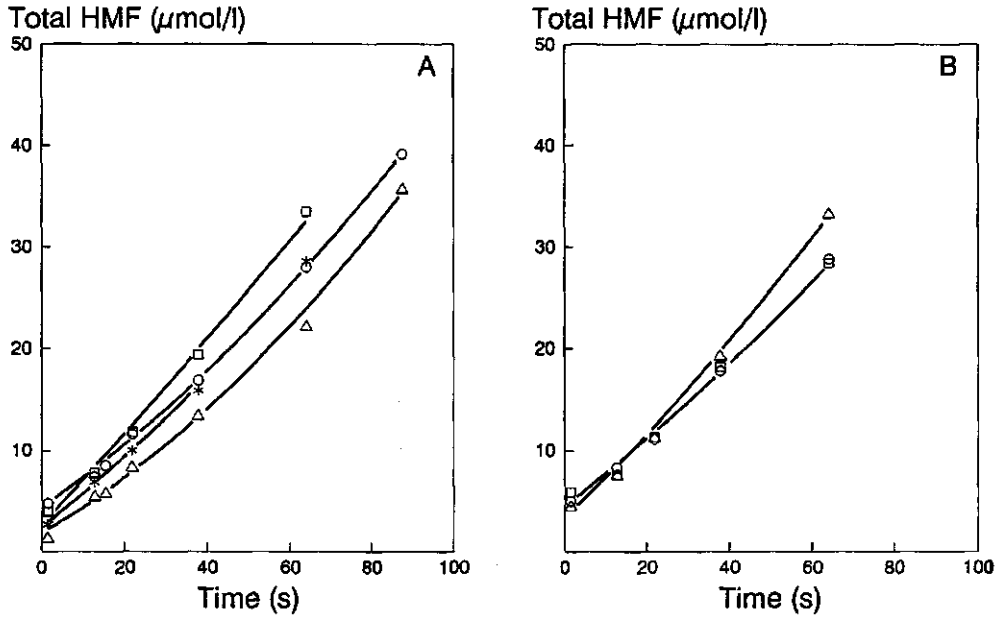


Figure 4.4 Formation of total HMF in indirect UHT heated milk with varying fat contents. Figure A: □ = skim milk with 0.1% fat, ▵ = semi skimmed milk with 1.51% fat, ○ = whole milk with 2.98% fat, * = whole milk with 4.23% fat. Figure B: □ = skim milk with 0.16% fat, ▵ = whole milk with 4.57% fat, ○ = recombined milk with 4.62% fat

4.3 Influence of protein content

To study the influence of the protein content of the milk on the formation of degradation products of lactose, milks with varying protein contents were indirectly heated in the UHT apparatus at 120, 130, 140 or 145°C; the temperature was measured after the holding tube. The flow was adjusted to 21.8 ml/s. The protein concentration was altered by ultrafiltration. Normal skim milk, permeate, retentate and a mixture of 50% skim milk and 50% permeate were heated. The fat percentages as measured with the Milko scan, the protein percentage as determined with Kjeldahl as well as the initial pH are given in Table 4.6. Clearly, the lactose content as measured by the Milko scan is incorrect due to the fact that this apparatus was calibrated on milk of normal composition; milks having an abnormal composition cause deviations.

Sample	pH	Fat %	Protein %	Lactose %	Lactose* %
skim milk	6.65	0.03	3.74	4.59	4.52
mixture	6.66	0.03	1.92	4.45	3.93
permeate	6.55	-	0.18	4.01	3.92
retentate	6.65	-	4.52	5.26	4.72

Table 4.6 The pH, fat and protein content of milk fractions. The fat content was determined by Milko scan, the protein content by Kjeldahl analyses (6.38*N), the lactose content by Milko scan and the lactose* content by HPLC

The results of the determinations of lactulose, galactose and HMF concentrations after heating at 120, 130, 140 and 145 °C are given in Figures 4.5 to 4.8. The formation of formic acid was only determined at 145°C and is shown in Figure 4.9. From the figures it can be concluded that at 120 and 130°C no influence of protein concentration on lactulose formation is found; at 140 and 145°C a slight effect on lactulose formation is visible. The lactulose formation is higher at a lower protein concentration; this is the opposite effect as shown in sterilized model solutions (3.2.1). An explanation may be that further degradation did not occur and pH buffering due to protein is not important here.

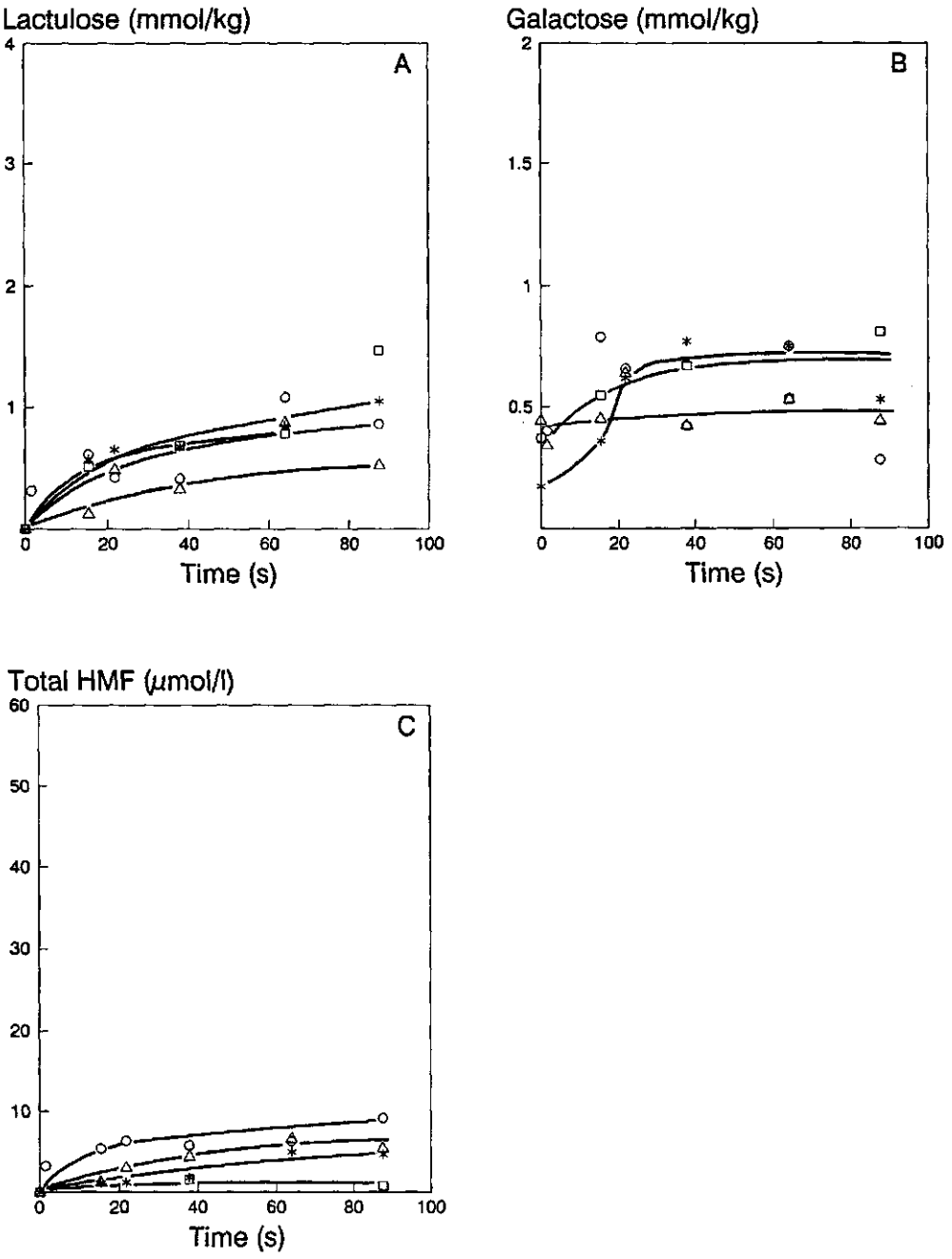


Figure 4.5 Formation of lactulose (A), galactose (B) and total HMF (C) in indirect UHT heated milks with varying protein concentrations at 120°C. □ = retentate containing 4.52% protein, △ = skim milk containing 3.74% protein, ○ = mixture of skim milk and permeate containing 1.92%, * = permeate containing 0.18% protein

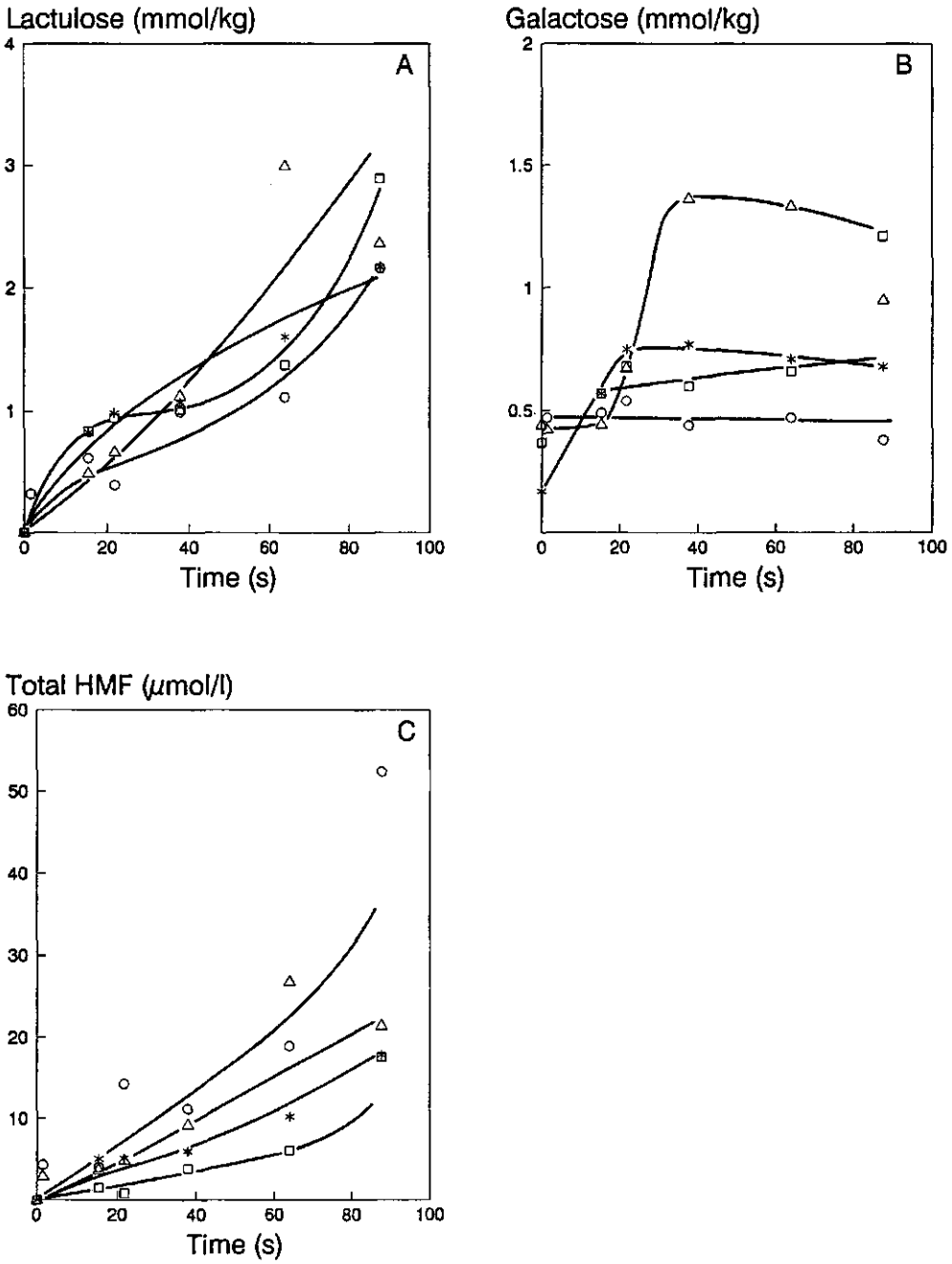


Figure 4.6 Formation of lactulose (A), galactose (B) and total HMF (C) in indirect UHT heated milks with varying protein concentrations at 130°C. □ = retentate containing 4.52% protein, △ = skim milk containing 3.74% protein, ○ = mixture of skim milk and permeate containing 1.92%, * = permeate containing 0.18% protein

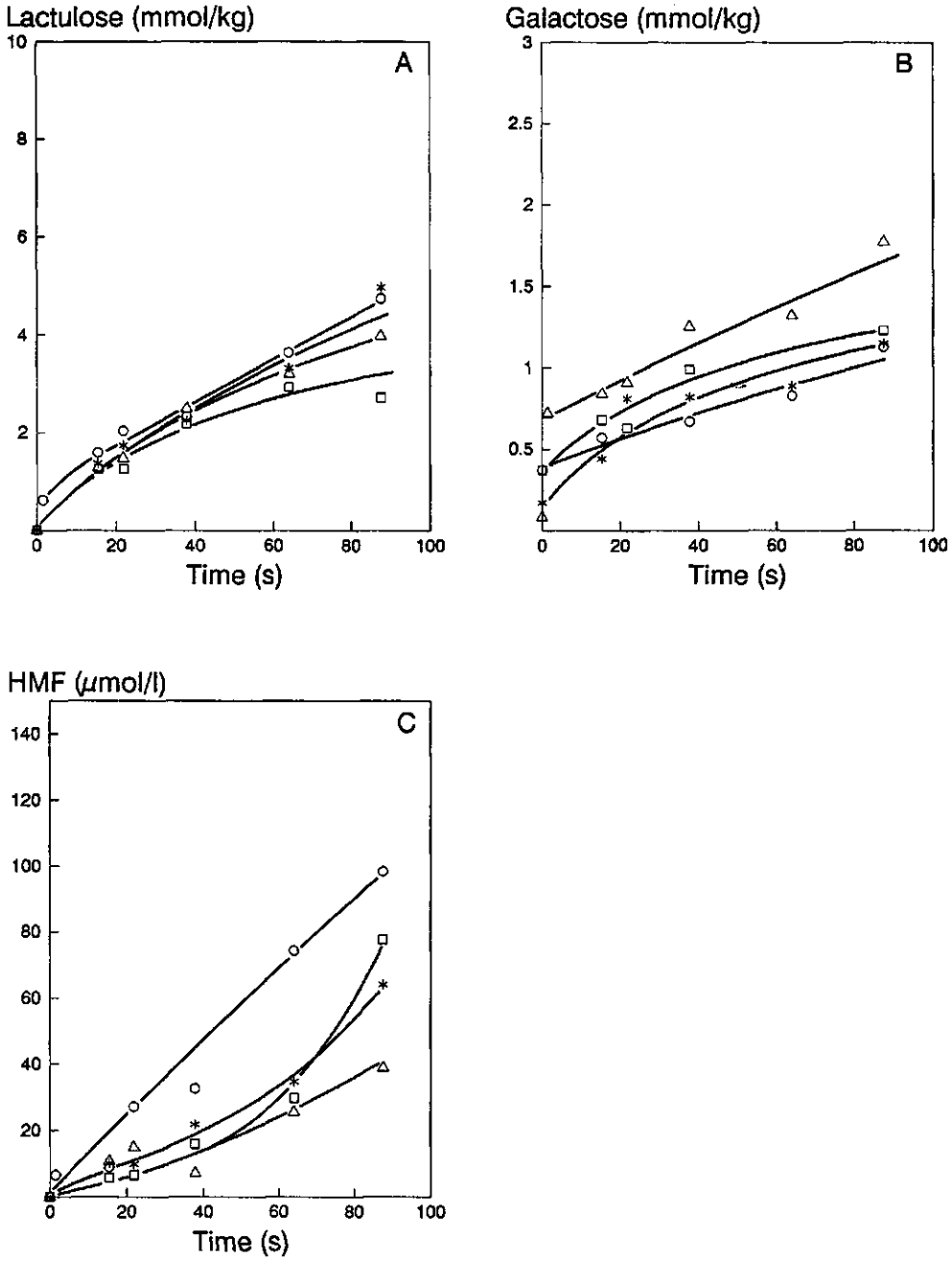


Figure 4.7 Formation of lactulose (A), galactose (B) and total HMF (C) in indirect UHT heated milks with varying protein concentrations at 140°C. □ = retentate containing 4.52% protein, △ = skim milk containing 3.74% protein, ○ = mixture of skim milk and permeate containing 1.92%, * = permeate containing 0.18% protein

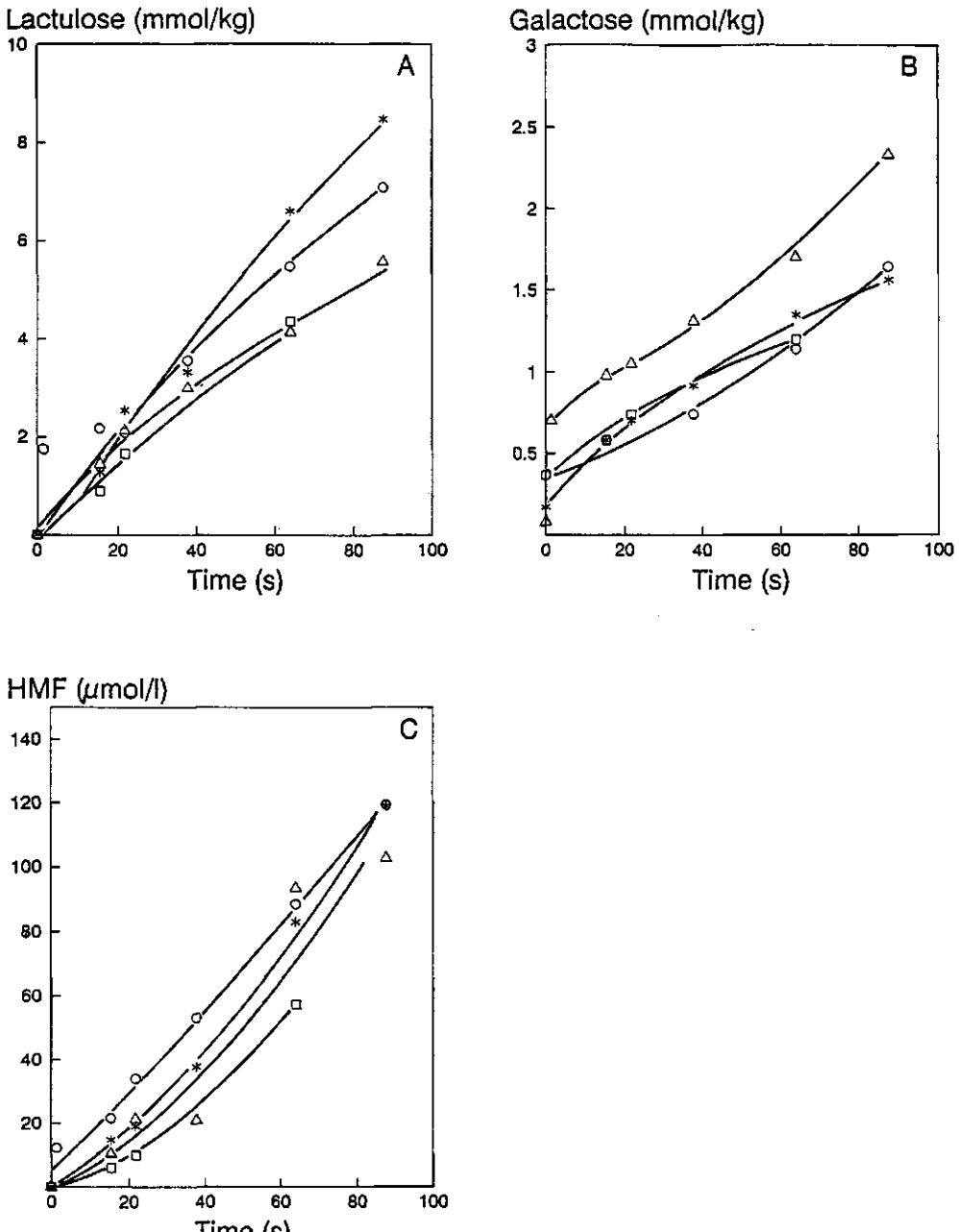


Figure 4.8 Formation of lactulose (A), galactose (B) and total HMF (C) in indirect UHT heated milks with varying protein concentrations at 145°C. □ = retentate containing 4.52% protein, △ = skim milk containing 3.74% protein, ○ = mixture of skim milk and permeate containing 1.92%, * = permeate containing 0.18% protein

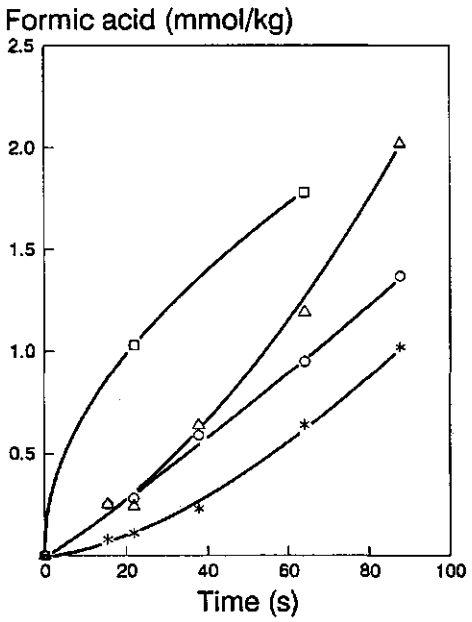


Figure 4.9 Formation of formic acid in indirect UHT heated milks with varying protein concentrations at 145°C. □ = retentate containing 4.52% protein, △ = skim milk containing 3.74% protein, ○ = mixture of skim milk and permeate containing 1.92%, * = permeate containing 0.18% protein

Greig and Payne (1985) also heated milk and ultrafiltrate (0.006% casein) 300-3600 s at 125°C and found the lactulose formation to be higher in the ultrafiltrate. They concluded that the lower lactulose content in the presence of protein is probably due to the binding of lactulose in an amino-sugar complex. Andrews and Prasad (1987) found two effects of protein on lactulose formation: a small amount of protein increased the formation of lactulose, but increasing amounts of protein reduced the formation of lactulose. The authors suggested that the latter was due to increased condensation of lactulose with protein, and/or increased condensation of lactose with protein resulting in a reduction of the substrate concentration for lactulose formation. After heating (2 h at 90°C, 3 or 15 min at 110°C and 2 and 4 min at 130°C), Andrews and Prasad (1987) found that the pH of the unconcentrated milk samples was still above 6.55, but the pH of the ultrafiltrate (initially 6.66) fell to a pH of about 6.19, dependent on the severity of heat treatment; this may account for the lactulose content of the ultrafiltrate being much lower than that of the milk heated in the same way.

Unfortunately, in our experiment, the pH of the heated permeate was not determined.

Calvo and Olano (1989) also studied the influence of protein content on the sugar formation. They heated milk, concentrate and ultrafiltrate and found more lactulose formation upon lowering the protein content. However, compared to normal milk the galactose content was higher in the concentrate and lower in the ultrafiltrate. In Figures 4.5 to 4.8 no clear relationship between protein content and galactose formation can be found.

Neither was a correlation found between HMF formation and protein content. Lee and Nagy (1990) studied the reactivities of sugars as judged by the formation of HMF in sugar-catalyst model solutions at pH 3.5. They found the rate of HMF formation from glucose and sucrose to be slightly enhanced in the presence of amino acids, whereas no enhancement occurred when fructose was the substrate. In the case of lactose degradation, glucose is supposed to be the reactive part and in the case of lactulose, fructose is the reactive part. Free HMF was also formed in the permeate; this means either that HMF is formed as a result of the isomerization or caramelization of lactose or that there is still enough protein present or a small amount of small peptides and amino acids to react in the Maillard reaction.

The formation of formic acid was only determined in the milk systems heated at 145°C. With increasing protein concentration more formic acid is formed. The pH of the heated milk systems decreased hardly (Table 4.7), but slightly more in the heated retentate and mixture.

Sample	pH before heat treatment	pH after heat treatment
skim milk	6.65	6.61
mixture	6.66	6.50
permeate	6.55	-
retentate	6.65	6.55

Table 4.7 pH of the milk systems before heat treatment and after maximum heat treatment

Compared to the sterilized milk (Figure 3.6D) only small amounts of formic acid were formed. Increasing formation of formic acid with protein concentration seems to indicate that degradation reactions increase with protein content, as was already suggested in the previous chapter (see Figure 3.27).

4.4 Conclusions

From the results of the experiments with UHT treated milks it can be concluded that the same reaction products are formed as in sterilized milks and model solutions, only in much lower concentrations because the heating times are much shorter. The initial pH of the milk was the same for sterilization and UHT treatment, but during sterilization the pH decreased remarkably, whereas it hardly decreased during UHT treatment. Very likely, the pH decrease during sterilization will have an effect on the reaction rate. The reaction products formed during both heat treatments are thus the same, but the amounts formed are quite different. From the mass balance of UHT treated milks it can be concluded that rather large amounts of advanced degradation products were formed during very intensive UHT treatment.

5 REACTION KINETICS OF LACTOSE DEGRADATION

In Chapters 3 and 4 the degradation of lactose during sterilization and UHT treatment is described. In Figure 3.27 a model for the degradation pathways was proposed. In this chapter, an attempt is made to describe the kinetic parameters for the lactose degradation. In order to do this, we tried to model the degradation of lactose by computer simulations which predict the degradation of lactose during heat treatment of milk. After that, the results of the experiments described in chapter 3 and 4 are compared with the results of the simulation.

5.1 Introduction

As the degradation reactions of lactose comprise a very complex network involving both isomerization, degradation and Maillard reactions, a methodology is needed to gain insight in the mechanisms. Antal et al. (1990) listed some useful steps to be considered in elucidating complex reaction networks as applied by them to acid-catalysed reactions of carbohydrates.

1 - *Identify all stable products and calculate the mass balance of the experiments.* This has been attempted in Chs. 3 and 4.

2 - *Identify species which are co-products of the same reaction pathway.* This has been described in Chs. 3 and 4.

3 - *Identify the early time-behaviour of the reaction products to distinguish primary reaction-pathways from secondary pathways.* The UHT results described in Ch. 4 give relevant information.

4 - *Identify the influence of pH on product formation.* This is known qualitatively from work of Geier and Klostermeyer (1983) and Martinez-Castro and Olano (1980). However, from a quantitative point of view there is very little information. We will pay attention to this further on.

5 - *Identify the influence of reactant concentration.* This has been done as described in Chs. 3 and 4.

6 - *Verify the roles of secondary reactions by experiment.* In Ch. 3 model solutions with lactulose, galactose, formic acid, HMF or deoxyribose with or without casein were heated to account for this.

7 - *Pose a model mechanism for the reaction network based on elementary reaction-steps. Use a non-linear least-squares algorithm to determine*

whether the model is quantitatively able to fit the experimental data. This will be the subject of this chapter.

8 - *Test the hypothesized mechanism using model compounds*. Partly, some results in Ch. 3 may be used for this.

Thus, most of these steps have been taken into account by us. This chapter is devoted mainly to steps number 7 and 8. We may add to this: The temperature dependence of each reaction step should be determined to check whether that is reasonable or not: it should more or less follow the Eyring relation (Eq. 1.1).

5.2 Evaluation of the model

In first instance, the pH dependency of lactose reactions was taken into account by assuming that the isomerization of lactose into lactulose is catalysed by OH^- ions. It may well be that other reactions are also influenced by OH^-, but it is only qualitatively well documented for the lactulose formation in milk (Adachi and Patton, 1961; Geier and Klostermeyer, 1983; Martinez-Castro and Olano, 1980), for the other reactions we don't know. So, in a first approximation, the pH dependency was accounted for in reaction step 1:

$$la + OH^- \rightleftarrows lu + OH^- \qquad (5.1)$$

Generally, the mechanism of alkaline isomerization is described according to Figure 5.1 (de Bruijn, 1986):

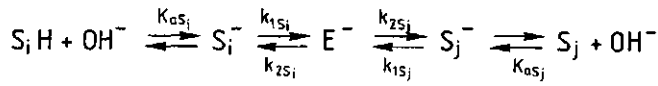


Figure 5.1 General mechanism of alkaline sugar isomerization

S_iH = sugar

S_i^- = sugar anion

E^- = enediol anion

K_{as_i} = dissociation constant

$$= \frac{S^-.H^+}{SH}$$

k = rate constant

The total sugar concentration, S_t, is:

$$S_t = SH + S^-$$

As the ionization of sugars in an alkaline medium is fast with respect to subsequent enediol anion formation, the decrease of S_t is equal to the decrease of S^-, so:

$$-\frac{dS_t}{dt} = -\frac{dS^-}{dt} \qquad (5.2)$$

As E^- is considered to be a very reactive intermediate, it will soon be in the steady-state, so

$$\frac{dE^-}{dt} = 0 \qquad (5.3)$$

The alkaline isomerization and degradation scheme of lactose is given in Figure 5.2. A similar scheme may be valid for milk.

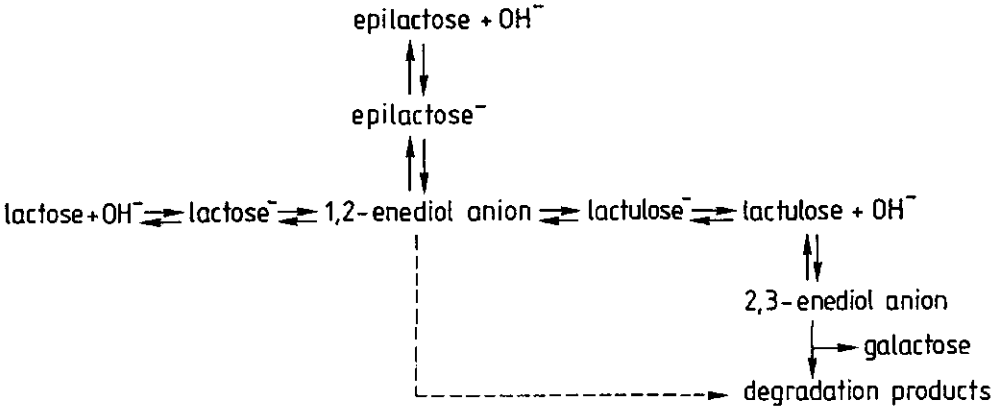


Figure 5.2 Isomerization and degradation of lactose at high pH

To simplify the kinetic model, the epilactose formation is not taken into account, as only small amounts of epilactose are found compared to lactulose formation

(Olano et al., 1989). According to de Bruijn (1986), who found that the degradation of glucose into acids occurred for 90% via the 2,3-enediol anion and only for 10% via the 1,2-enediol anion at high pH (0.01 M KOH), we assumed that the degradation of lactose also occurred mainly via the 2,3-enediol anion, hence via lactulose. So, a simplified scheme of the isomerization is given in Figure 5.3.

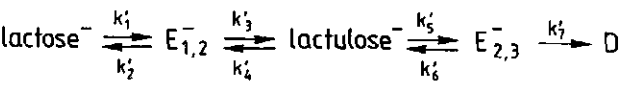


Figure 5.3 Simplified scheme of the isomerization of lactose

$E^-_{1,2}$ = 1,2-enediol anion

$E^-_{2,3}$ = 2,3-enediol anion

k = rate constant

D = degradation products

The following differential equations can be derived from Fig. 5.3:

$$\frac{dla^-}{dt} = -k'_1 la^- + k'_2 E^-_{1,2} \qquad (5.4)$$

$$\frac{dE^-_{1,2}}{dt} = k'_1 la^- - k'_2 E^-_{1,2} - k'_3 E^-_{1,2} + k'_4 lu^- = 0$$

$$\Rightarrow E^-_{1,2} = \frac{k'_1 la^- + k'_4 lu^-}{k'_2 + k'_3} \qquad (5.5)$$

$$\frac{dlu^-}{dt} = k'_3 E^-_{1,2} - k'_4 lu^- - k'_5 lu^- + k'_6 E^-_{2,3} \qquad (5.6)$$

$$\frac{dE^-_{2,3}}{dt} = k'_5 lu^- - k'_6 E^-_{2,3} - k'_7 E^-_{2,3} = 0$$

$$\Rightarrow E^{-}_{2,3} = \frac{k'_5 lu^-}{k'_6 + k'_7} \qquad (5.7)$$

$$\frac{dD}{dt} = k'_7 E^{-}_{2,3} \qquad (5.8)$$

Substitution of Eq. (5.5) into Eq. (5.4) gives

$$\frac{dla^-}{dt} = -k'_1 la^- + \frac{k'_2 k'_1 la^-}{k'_2 + k'_3} + \frac{k'_2 k'_4 lu^-}{k'_2 + k'_3}$$

$$= -la^-(k'_1 - \frac{k'_2 k'_1}{k'_2 + k'_3}) + (\frac{k'_2 k'_4}{k'_2 + k'_3}) lu^- \qquad (5.9)$$

And substitution of Eq. (5.7) into Eq. (5.6) gives

$$\frac{dlu^-}{dt} = \frac{k'_3 k'_1 la^-}{k'_2 + k'_3} + \frac{k'_3 k'_4 lu^-}{k'_2 + k'_3} - k'_4 lu^- - k'_5 lu^- + \frac{k'_6 k'_5 lu^-}{k'_6 + k'_7}$$

$$= (\frac{k'_3 k'_1}{k'_2 + k'_3}) la^- + (\frac{k'_3 k'_4}{k'_2 + k'_3} - k'_4) lu^-$$

$$- (k'_5 - \frac{k'_6 k'_5}{k'_6 + k'_7}) lu^- \qquad (5.10)$$

According to Eq. (5.2): $\frac{dla^-}{dt} = \frac{dla_t}{dt}$; it is also known that

$la_t = la + la^-$, and the ionisation of lactose can be described as an acid dissociation:

$$K_a = \frac{la^-.H^+}{la} = \frac{la^-.K_w}{la.OH^-} \qquad (5.11)$$

with $K_w = H^+.OH^-$. Since also $la = la_t - la^-$, we obtain:

$$la^- = \frac{OH^-}{\frac{K_w}{K_a} + OH^-} la_t = A\ la_t \qquad (5.12)$$

In the case of lu^- the same relation holds:

$$lu^- = \frac{OH^-}{\frac{K_w}{K_a} + OH^-} lu_t = A' lu_t \qquad (5.13)$$

Combination of Eqs. (5.9), (5.12) and (5.13) now results in:

$$\frac{dla_t}{dt} = -A\{k'_1 - \frac{k'_2 k'_1}{k'_2 + k'_3}\}la_t + A'\{\frac{k'_2 k'_4}{k'_2 + k'_3}\}lu_t \qquad (5.14)$$

And combination of Eqs. (5.10), (5.12) and (5.13) results in:

$$\frac{dlu_t}{dt} = A\{\frac{k'_3 k'_1}{k'_2 + k'_3}\}la_t + A'\{\frac{k'_3 k'_4}{k'_2 + k'_3} - k'_4\}lu_t$$

$$- A'\{k'_5 - \frac{k'_6 k'_5}{k'_6 + k'_7}\}lu_t \qquad (5.15)$$

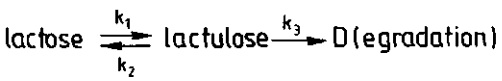


Figure 5.4 The overall reaction of lactose degradation

In our experiments, we have determined the total sugar concentration (la_t, lu_t) and not sugaranions, so we have to look for a relation between experimentally accessible rate constants and the elementary ones. The overall reaction with the experimentally accessible species is shown in Fig. 5.4. From this, the following differential equations can be derived:

$$\frac{dla_t}{dt} = -k_1 la_t + k_2 lu_t \qquad (5.16)$$

$$\frac{dlu_t}{dt} = k_1 la_t - k_2 lu_t - k_3 lu_t \qquad (5.17)$$

Comparing these equations with Eq. (5.13) results in the following equations describing k_1, k_2 and k_3:

$$k_1 = \frac{OH^-}{\frac{K_w}{K_a} + OH^-}\left(k'_1 - \frac{k'_2 k'_1}{k'_2 + k'_3}\right)$$

$$= \frac{OH^-}{\frac{K_w}{K_a} + OH^-}\left(\frac{k'_3 k'_1}{k'_2 + k'_3}\right) \qquad (5.18)$$

(K_a for lactose).

$$k_2 = \frac{OH^-}{\frac{K_w}{K_a} + OH^-}\left(\frac{k'_2 k'_4}{k'_2 + k'_3}\right) \qquad (5.19)$$

(K_a for lactulose).

$$k_3 = \frac{OH^-}{\frac{K_w}{K_a} + OH^-}\left(\frac{k'_7 k'_5}{k'_6 + k'_7}\right) \qquad (5.20)$$

(K_a for lactulose).

From these equations two conclusions can be drawn:

1 - At constant OH^- concentration the pseudo rate constants (k) are a function of the elementary rate constants (k') and can therefore indeed be considered as constants.

2 - If this mechanism is indeed correct, then it describes the pH-dependency of the degradation of lactose. The k' reaction constants are assumed to be pH independent, implying that for known OH^- concentration and known K_w and K_a the pH-dependency can be calculated for the pseudo rate constants. In the case of a low OH^- concentration (pH < 7), the k's are directly proportional to OH^-; K_a and K_w are both in the range of 10^{-12} to 10^{-13}. According to Honig (1963) the pK_w at 120 °C is about 11.8, the pH of milk at 120°C is about 6 (Walstra and Jenness, 1984), hence pOH is 5.8 (or OH^- is $1.6*10^{-6}$) and

$$\frac{OH^-}{\frac{K_w}{K_a} + OH^-} \approx \frac{OH^-}{\frac{K_w}{K_a}} .$$

However, it appears from this analysis that the influence of pH is the same in both directions of the isomerization. Hence, the levels of lactose and lactulose should not be influenced strongly by OH^-. This was indeed found experimentally by de Bruijn (1986) for alkaline isomerization of glucose; all rate constants increased with OH^- concentration at about the same proportion. In milk, this is apparently not so. Maybe, the relatively low activity of OH^- in milk, and the presence of other components (salts, amino groups) that may act like bases is the reason for this.

The Maillard reaction is generally believed to be promoted by OH^-, though this usually pertains to browning, not necessarily to the early stages (e.g. Nursten, 1986). A study by Olano et al. (1992) showed that the formation of a model Amadori compound, during 20 min heating at 120°C, was not pH dependent in the pH range 6-7.

The effect of OH^- on lactose degradation in milk is thus difficult to take into account in computer simulations, because we don't know by which mechanism; this aspect clearly needs further research. In actual fact, the OH^- level decreases during heating of milk, making things even more complicated. As a first approach, it was decided to leave the OH^- concentration out of the model. This implies that the (pseudo) rate constants we will derive may contain a pH dependency.

The model proposed to describe the degradation of lactose and its main reaction products is depicted again in Figure 5.5.

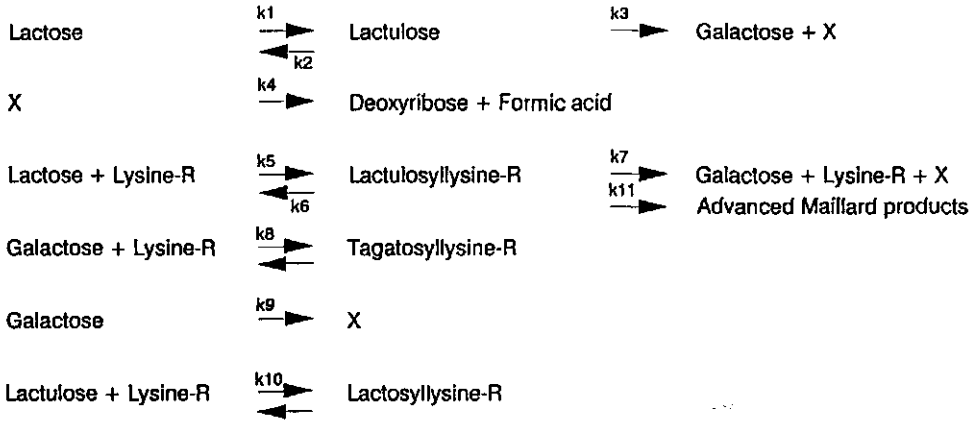


Figure 5.5 Steps in the proposed reaction network that describes the degradation of lactose during heating of milk, referred to as model 1. X: unknown C-6 compound

The following coupled, nonlinear, stiff ordinary differential equations (ODE's) can be derived from the network described in Figure 5.5:

$$\frac{dla}{dt} = -k_1 la + k_2 lu - k_5 la.lys + k_6 laly \qquad (5.21)$$

$$\frac{dlu}{dt} = k_1 la - k_2 lu - k_3 lu - k_{10} lu.lys \qquad (5.22)$$

$$\frac{dgal}{dt} = k_3 lu + k_7 laly - k_8 gal.lys - k_9 gal \qquad (5.23)$$

$$\frac{dX}{dt} = k_3 lu + k_7 laly - k_4 X + k_9 gal \qquad (5.24)$$

$$\frac{dform}{dt} = k_4 X \qquad (5.25)$$

$$\frac{d[lys]}{dt} = -k_5[la].[lys] + k_6[laly] + k_7[laly] - k_8[gal].[lys] - k_{10}[lu].[lys] \quad (5.26)$$

$$\frac{d[laly]}{dt} = k_5[la].[lys] - k_7[laly] - k_6[laly] - k_{11}[AMP] \quad (5.27)$$

la = lactose concentration
lu = lactulose concentration
lys = lysine concentration
gal = galactose concentration
laly = lactulosyllysine concentration
X = unknown intermediate C6 compound
form = formic acid
AMP = advanced Maillard products

Several simplifications had to be introduced in this model.

We have not determined the formation of advanced Maillard products. One way to take these into account is to simply assign the moles lost from the mass balance calculations (Tables 3.8, and 4.4) as advanced Maillard products. This would, however, imply that not all equations are independent any more, which may cause problems in the statistical analysis (Box et al., 1973, McLean et al., 1979), due to the fact that this response is not measured but assumed to be true from the model. Validation of the model from a statistical point of view is then not well possible. Another problem is that not the whole loss is caused by advanced Maillard products, but also by such products as epilactose and tagatose and possible further degradation of galactose, especially at the higher temperatures.

A problem that remains to be solved is the activity coefficient of lactose. No independent measure can be introduced in the ODE's, for example by introducing the mole fraction of lactose in the open chain f_x (which is believed to be the active form of lactose, as discussed in Chapter 1), into the equations (i.e. $-k_1.f_x[la]$ instead of $-k_1[la]$ in Eq. (5.21)). This would make it impossible to estimate the reaction rate constants independently. As discussed in Ch. 3, the activity coefficient for lactose

is estimated to be 0.1 or less, for the other sugars and lysine it is just not known. The reaction rate constant k_1 obtained from curve fitting is, of course, exactly correlated with f_x, hence the value obtained by simulation is the product $f_x k_1$. Although it would in principle be better to include activity coefficients in the model, it makes no difference for the calculations if we do not, if we only realize that the value obtained by simulations comprises both the reaction rate constant and the activity coefficient, and it should be realized that f_x is also likely to be temperature dependent.

Reaction step 5 is not a single step reaction but comprises a sequence of steps, starting with the condensation of lactose with lysine residues and ending with the Amadori rearrangement into the Amadori product laly. The final Amadori rearrangement is believed to be irreversible, but the preceding steps are reversible; hence the equilibrium depicted in step 5. We found indeed lactose after heating the Amadori product (Table 3.19). Since we have no experimental access to the intermediates in these steps, we can only treat it as a single step. It should be realized, therefore, that reaction rate constants k_5 and k_6 neither are true elementary rate constants.

As explained in the previous chapters, we have only indirectly obtained data on lactulosyllysine, namely via the HMF content which was recalculated to lactulosyllysine through the relationship obtained from Dehn-Müller (1989) in the case of UHT treated milks, via the relation HMF-lysine in the case of model solutions and via lysine determinations in the case of sterilized milks. It is assumed that the decrease in lysine content equals the lactulosyllysine concentration in both cases. Therefore, a considerable error may be involved in the experimental establishment of lactulosyllysine.

All these considerations must be taken into account when evaluating the reaction model. Nevertheless, we considered it worthwhile to fit the proposed model to the experimental data; the validity of the above assumptions will be discussed afterwards.

5.3 Numerical and statistical procedures

The task is now to find out how well the proposed model is able to fit the experimental data. First of all, a procedure is needed to simulate the reactions which are mathematically described by the ODE's, derived in section 5.2. It is clear

that these equations cannot be solved analytically, so a numerical procedure was needed. The well-known and much used Runge-Kutta numerical integration routines with adaptive step size were not well suited for this job because the ODE's were stiff. Therefore, we used the so-called Gear-routine specifically designed for stiff ODE's, as described by Chesick (1988) and Stabler and Chesick (1978); the routine, written in Turbo Pascal, was kindly provided by dr. Chesick. The question how well the proposed model describes the experimental data must be addressed from a statistical point of view. Our problem is typically a case of analyzing multiresponse data, an approach that is used to some extent in chemical engineering. To our knowledge, such an approach is not yet used in food science, maybe because reaction networks in foods are even more complicated than in chemical engineering.

The most simple, (but mostly incorrect) approach to fit the model to the data is to minimize the combined sum of squares (*SS*) for all the responses (Hunter, 1967):

$$\sum_{i=1}^{r}\sum_{u=1}^{n}[y_{iu} - \hat{y}_{iu}]^2 \qquad (5.28)$$

in which y_{iu} is the *i*th observed response of *r* responses obtained after various reaction times *u* ($u = 1..n$), and $\hat{y}_{iu}$ the response according to the model. $\hat{y}_{iu} = f_i(\underline{x}_u,\underline{k})$, describing the expectation model for response *i* ($i = 1,2,...r$) at the experimental design point x_u (reaction time, in our case) depending on a set of parameters $\underline{k}$ (in our case the reaction rate constants). The task is to find values of the constants *k* which minimize the above mentioned combined *SS*, followed by an analysis of the goodness of fit. However, Hunter (1967) showed that this criterion is only valid under the following restrictions:

a. each of the responses has a normally distributed uncertainty
b. the data on each response have the same variance
c. there is no correlation between the deviations of the individual measurements of the responses.

These restrictions are mostly not met. Condition b, however, can be taken into account by weighting the data with their own variance and minimize:

$$\sum_{i=1}^{r} w_i \sum_{u=1}^{n} (y_{iu} - \hat{y}_{iu})^2 \qquad (5.29)$$

in which w_i is the inverse of the variance of y_i: $(\frac{1}{\sigma^2_{yi}})$. Effectively, this comes down to the χ^2 statistic. If the above restrictions a and c are met, the goodness-of-fit can be judged by testing χ^2 with v degrees of freedom ($v = n\text{-}p$, n is the number of observations and p the number of rate constants). If, however, restrictions a, b and c are not met, the proper criterion would be to minimize the determinant of the matrix $\underline{\mathbf{V}}$ of the sums of squares and cross products of the differences between the observed and predicted values of the responses (according to the model) (Box and Draper, 1965; Hunter, 1967).

$\underline{\mathbf{V}}$ =

$$\begin{bmatrix} \sum_{u=1}^{n} (y_{1u} - \hat{y}_{1u})^2 & \sum_{u=1}^{n} (y_{1u} - \hat{y}_{1u})(y_{2u} - \hat{y}_{2u}) & \cdots & \sum_{u=1}^{n} (y_{1u} - \hat{y}_{1u})(y_{ku} - \hat{y}_{ku}) \\ \sum_{u=1}^{n} (y_{2u} - \hat{y}_{2u})(y_{1u} - \hat{y}_{1u}) & \sum_{u=1}^{n} (y_{2u} - \hat{y}_{2u})^2 & \cdots & \sum_{u=1}^{n} (y_{2u} - \hat{y}_{2u})(y_{ku} - \hat{y}_{ku}) \\ \sum_{u=1}^{n} (y_{ku} - \hat{y}_{ku})(y_{1u} - \hat{y}_{1u}) & \sum_{u=1}^{n} (y_{ku} - \hat{y}_{ku})(y_{2u} - \hat{y}_{2u}) & \cdots & \sum_{u=1}^{n} (y_{ku} - \hat{y}_{ku})^2 \end{bmatrix}$$

However, this appeared to be rather difficult in our case because of the very low value of the determinant causing numerical instabilities; probably, the number of data points was too small. We decided therefore to fit the model to the data by means of minimizing the combined sum of squares, despite the above mentioned objections. Though we have some idea about the variation in the determinations (Chapter 3), we only derived standard deviations from replicate heating experiments for sterilized milks. In all experiments we determined duplicates from one heat treated sample, not from duplicate heating experiments. With the results, we will present the sum of squares (SS) and the error variance of the reaction rate data, which equals the residual variance (s^2) if the reaction model is correct (which is, of course, not necessarily true). This may be compared to the variances in the

experiments as described in Chapter 3. Although this is not an independent measure of the goodness of fit, it gives at least some impression.

To optimize the k values a method which minimize the combined sum of squares described by Lobo and Lobo (1991) was used. This is a direct search method, and implies performing an optimalization without having to differentiate with respect to the reaction constants, which would be quite complicated in our case.

5.4 Results

The model depicted in Fig. 5.5, which is referred to as model 1, was fitted to the data of all the experiments of heated model solutions and milks. After fitting the data of the model solutions and the sterilized milk it appeared that the formic acid formation did not fit quite well. In order to improve the fit, the model was adjusted as indicated in Figure 5.6, referred to as model 2. The change was that in model 2 formic acid is assumed to be formed simultaneously with galactose and not via the intermediate X. Since, however, usually more galactose was found than formic acid, we had to introduce two reaction steps for the formation of galactose out of lactulose (k_3 and k_4). First, a description of the results of the model solutions and heated milk is given, after that the values of the reactions constants found are compared.

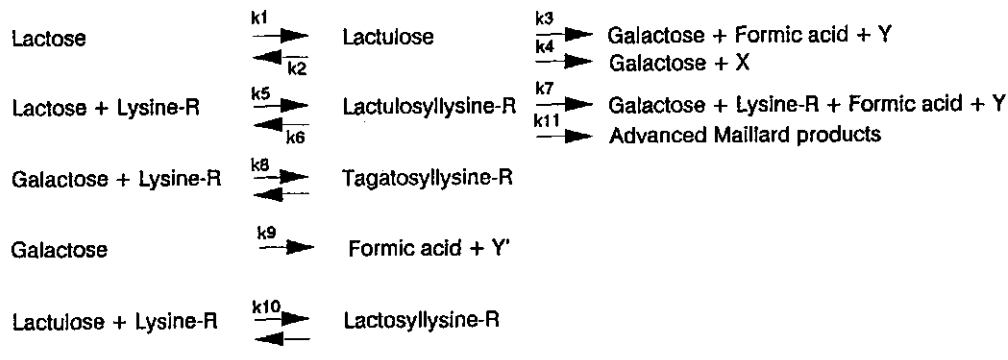


Figure 5.6 Steps in the proposed reaction network that describes the degradation of lactose during heating of milk, referred to as model 2

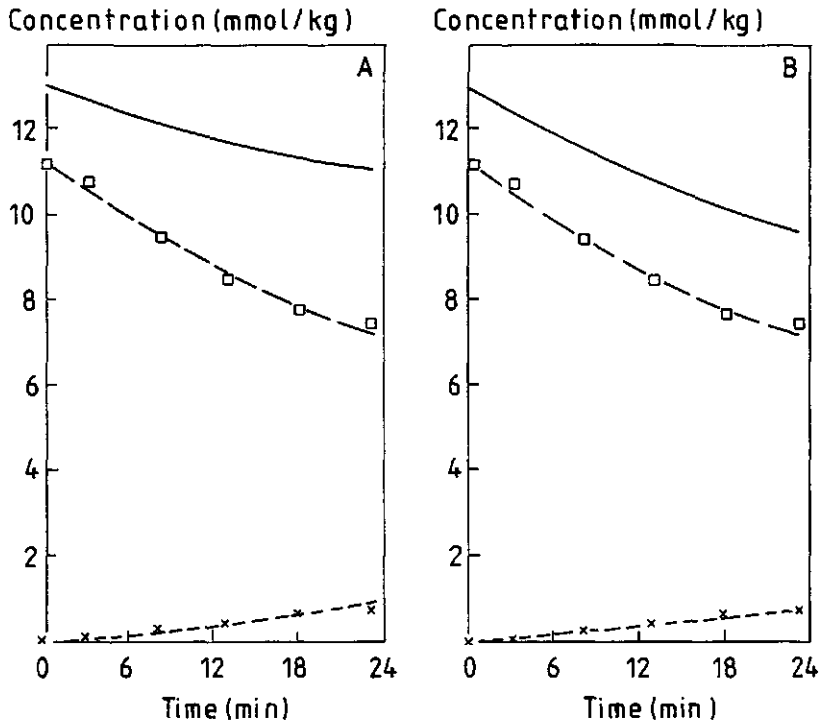


Figure 5.7 Fit for the galactose-casein(2.6%) model solution after heating at 413 K calculated by model 1 (A) and 2 (B). □ = galactose, × = formic acid; ——— = lysine fit, -------- = formic acid fit, — — = galactose fit

5.4.1 Results for the model solutions

Galactose-casein model solution

Henle (1991) found that galactose is more reactive to lysine than lactose is. However, the lysine degradation in the galactose-casein model solution was not determined, and it could only be estimated from the HMF concentration (Eqs. 3.3 and 3.4). As the free HMF formation in this model solution was low, the calculated lysine degradation is also very small and probably not correct, as the Eqs. are derived for lactulosyllysine in milk and are probably not valid in this case in which tagatosyllysine has formed. Only k_4, k_8 and k_9 of model 1 are applicable and only k_8 and k_9 of model 2 (Figure 5.7). Both model 1 and 2 fit very well. However, only qualitative conclusions can be drawn from the results of the galactose-casein model solution because the lysine degradation was not determined. One important aspect is the relatively large amounts of formic acid formed.

The k values found are given in Table 5.1. The residual variance was lower than the variance due to experimental uncertainty, derived in Ch. 3. This may indicate a reasonable fit.

k	model 1 413 K	model 2 413 K
1	0.0	0.0
2	0.0	0.0
3	0.0	0.0
4	8.3e-4	0.0
5	0.0	0.0
6	0.0	0.0
7	0.0	0.0
8	1.3e-5	2.3e-5
9	1.7e-4	5.8e-5
10	0.0	0.0
11	0.0	0.0
SS	0.26	0.18
s^2	0.04	0.02

Table 5.1 k-values (s^{-1}, except for k_8: $l.mmol^{-1}.s^{-1}$) for galactose-casein model solution calculated by model 1 and 2. SS = sum of squares, s^2 = residual variance

Maillard model solution

The model solution containing the Amadori product is very important for giving more information about reaction constants concerning the Maillard reaction. As initially no lactose was present and only small amounts of lactose were formed during heating and no lactulose could be determined at all, some reaction constants can be left out of consideration. In model 1 only k_4, k_6, k_7, k_8, k_9 and k_{11} are important and in model 2 k_4 can also be left out of consideration (Figure 5.8). Unfortunately, the lysine degradation during heating of this model solution was not determined, so lactulosyllysine concentration could only be determined by means of the HMF concentration and Eqs. (3.3) and (3.4), which means that no independent estimation of the lactulosyllysine concentration can be made. So, only

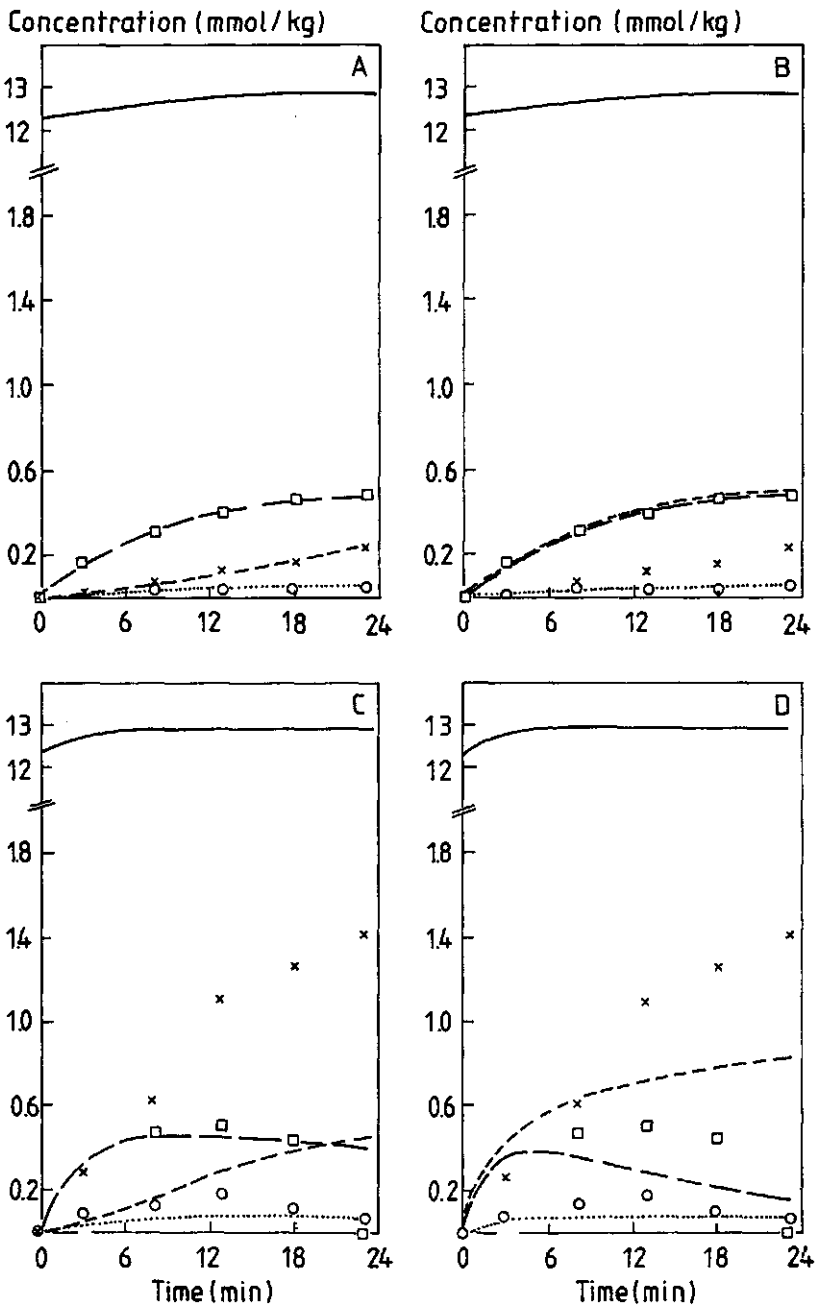


Figure 5.8 Fits for the Maillard model solutions. A = at 393 K calculated by model 1, B = at 393 K calculated by model 2, C = at 413 K calculated by model 1, D = at 413 K calculated by model 2. ○ = lactose, □ = galactose, △ = lysine, × = formic acid; ——— = lysine fit, ... = lactose fit, ------ = formic acid fit, — — = galactose fit

the initial concentrations of lysine and lactulosyllysine in the unheated model solution were used to calculate the fits.

The k values found for both models are given in Table 5.2. The residual variance is lower than the variance due to experimental uncertainty; this may indicate that the fit is reasonable.

k	model 1		model 2	
	393 K	413 K	393 K	413 K
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0
4	6.7e-4	1.3e-3	0.0	0.0
5	0.0	0.0	0.0	0.0
6	1.7e-4	6.7e-4	1.7e-4	1.3e-3
7	1.7e-3	5.0e-3	1.7e-3	8.3e-3
8	1.3e-6	1.7e-6	1.3e-6	1.7e-6
9	1.7e-5	1.7e-4	1.7e-5	8.3e-4
10	0.0	0.0	0.0	0.0
11	8.3e-6	1.7e-5	1.7e-5	1.7e-4
SS	0.0016	2.5	0.34	0.86
s^2	0.0002	0.28	0.03	0.09

Table 5.2 k-values (s^{-1}, except for k_8: $l.mmol^{-1}.s^{-1}$) for Maillard model solution calculated by model 1 and 2. SS = sum of squares, s^2 = residual variance

The results of this model solution appear to fit well at 120°C with model 1. With model 2, at 120°C, the results can never be fitted exactly; because if model 2 starts with only lactulosyllysine the fit can never result in the formation of more galactose than formic acid (see Fig. 5.6). At 140°C both fits are not very nice despite the low s^2, probably because in the end more formic acid is formed than sugar is present in the initial solution. However, in the case of model 2, the fit results in a continuously increasing formic acid concentration and an initially increasing and later on decreasing galactose concentration. There are at least two possible explanations for these effects. First, it is possible that model 1 is valid at

lower temperatures and model 2 at higher temperatures. Second, there may also be two reaction paths for the formation of galactose out of lactulosyllysine just the same as with degradation of lactulose; one resulting in galactose and X (unknown C-6 compound) and the other in galactose, formic acid and Y (unknown C-5 compound). As formic acid is preferably formed at higher temperatures this is in agreement with the first explanation. However, this refinement is only of relevance to this model solution with the Amadori compound, but not to skim milk, because then this reaction route is not very important.

Lactulose-casein model solutions

The data of the lactulose-casein model solutions were also fitted with models 1 and 2. As lactose was only formed in very small amounts the values of k_1, k_5, k_6, k_7, k_8 and k_{11} were kept at 0 (the reactions with lactose are effectively zero). Especially at 140°C, it appeared that the fit of the formation of formic acid was better in the case of model 2 (Figure 5.9). From these results it was concluded that model 2 was the best. The k values resulting from model 1 and 2 are given in Table 5.3. The calculated residual variance is lower than the experimental variance (Ch. 3), indicating that the fit is reasonable.

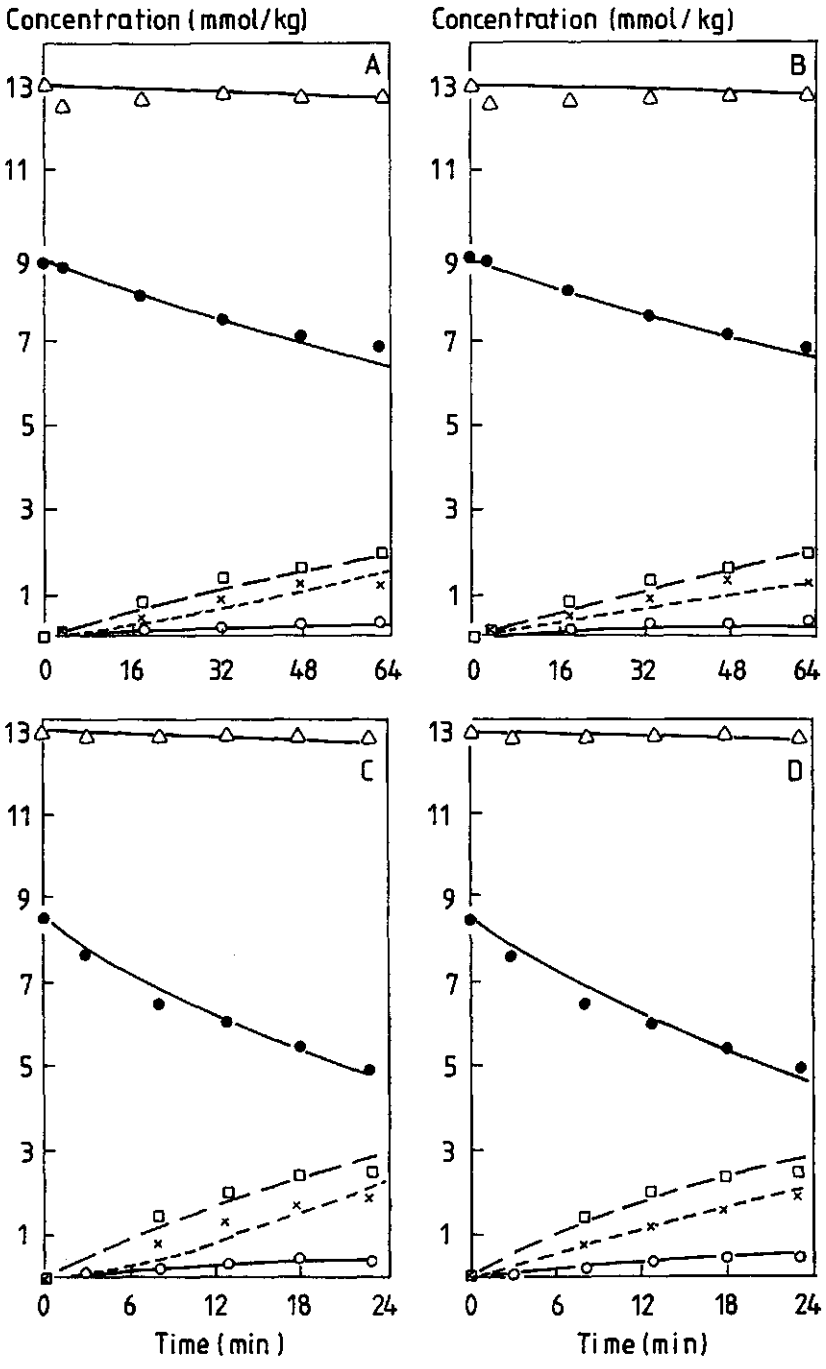


Figure 5.9 Fits for the lactulose-casein model solutions. A = at 393 K calculated by model 1, B = at 393 K calculated by model 2, C = at 413 K calculated by model 1, D = at 413 K calculated by model 2 ○ = lactose, ● = lactulose, □ = galactose, △ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, -------- = formic acid fit, — — = galactose fit

k	model 1		model 2	
	393 K	413 K	393 K	413 K
1	0.0	0.0	0.0	0.0
2	8.3e-6	5.7e-5	6.7e-6	6.0e-5
3	6.7e-5	3.3e-4	4.2e-5	2.0e-4
4	1.0e-3	2.2e-3	2.5e-5	1.6e-4
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0
9	6.7e-6	6.3e-5	6.7e-6	1.6e-4
10	8.3e-7	1.7e-6	6.2e-7	1.6e-6
11	0.0	0.0	0.0	0.0
SS	1.0	1.1	0.91	0.76
s^2	0.05	0.06	0.05	0.04

Table 5.3 k-values (in s^{-1}, except for k_{10}: $l.mmol^{-1}.s^{-1}$) for lactulose-casein model solution calculated by model 1 and 2. *SS* = sum of squares, s^2 = residual variance

Lactose-casein model solutions

The data of the lactose-casein solutions were fitted with models 1 and 2. Model 2 gave the best fit, especially at 120 and 140°C (Figures 5.10 and 5.11). Also with higher casein contents (see section 5.6), model 2 gave the best fit; however, in this case the galactose fit appeared to be somewhat better with model 1. The k values are shown in Table 5.4. The residual variance is lower, or in the same order of magnitude as the experimental variance, indicating that the fit may be reasonable.

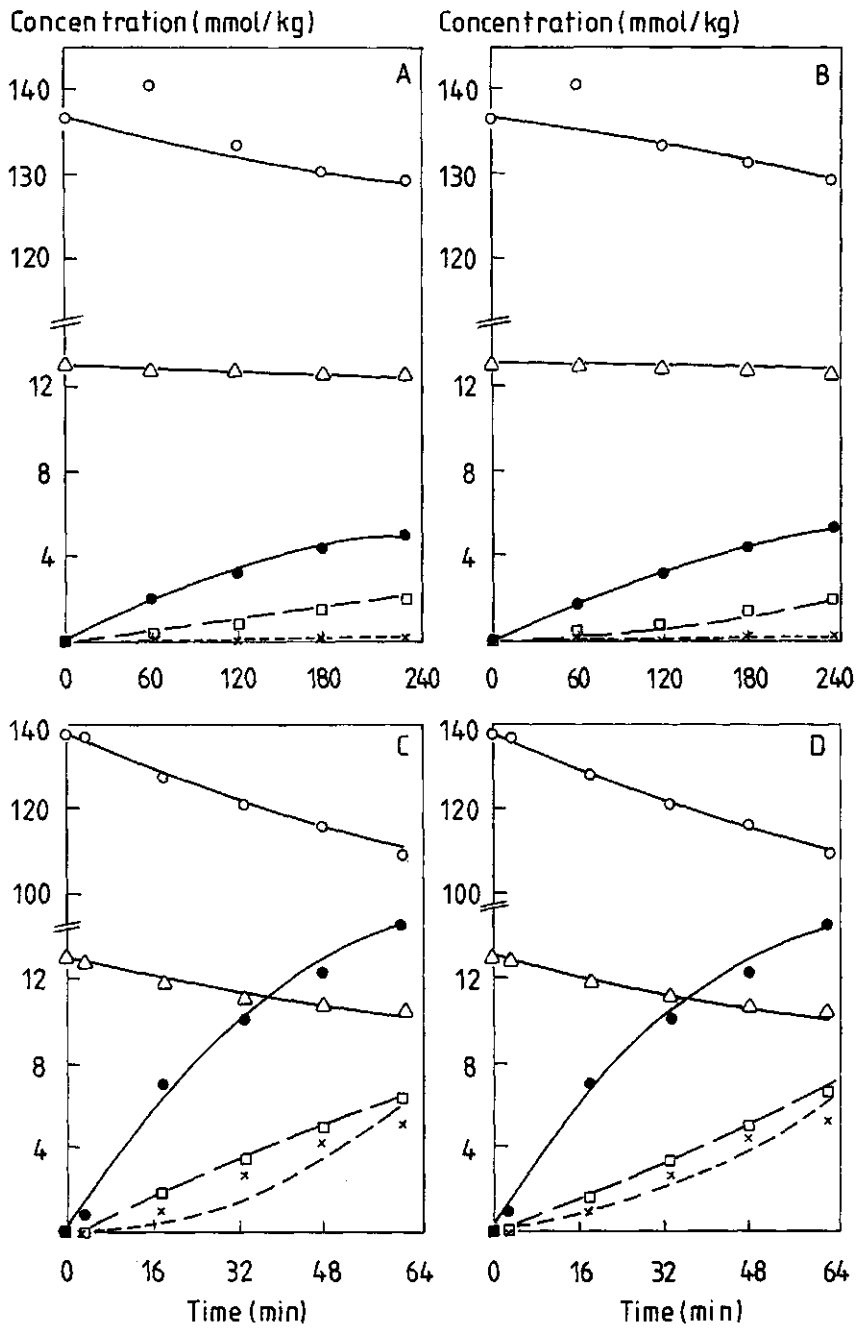


Figure 5.10 Fits for the lactose-casein model solutions. A = at 368 K calculated by model 1, B = at 368 K calculated by model 2, C = at 393 K calculated by model 1 and D = at 393 K calculated by model 2.○ = lactose, ● = lactulose, □ = galactose, △ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, -------- = formic acid fit, — — = galactose fit

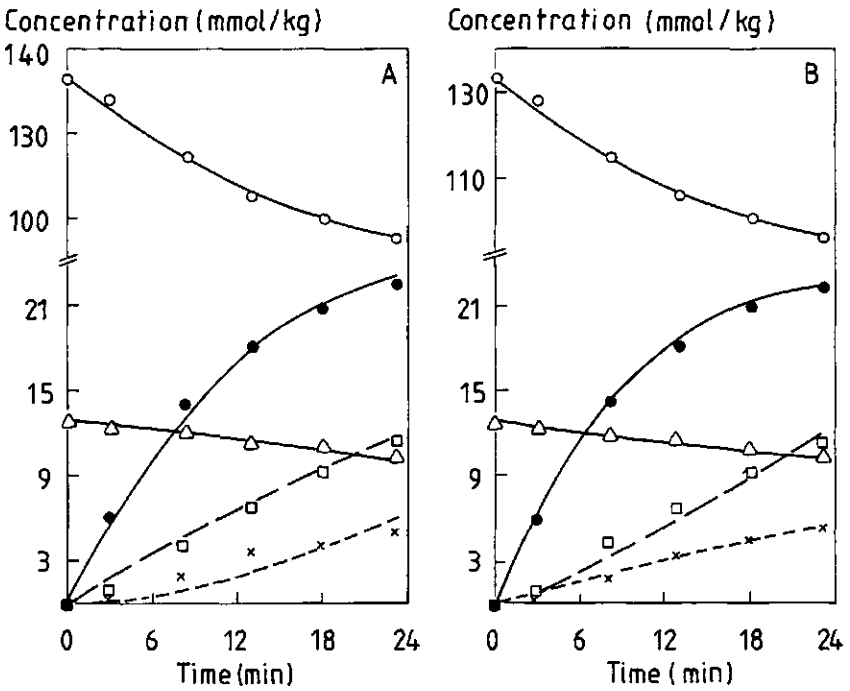


Figure 5.11 Fits for the lactose-casein model solutions. A = at 413 K calculated by model 1, B = at 413 K calculated by model 2. ○ = lactose, ● = lactulose, □ = galactose, △ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, -------- = formic acid fit, — — = galactose fit

k	model 1			model 2		
	368 K	393 K	413 K	368 K	393 K	413 K
1	5.0e-6	5.3e-5	2.7e-4	3.8e-6	5.7e-5	3.3e-4
2	8.7e-5	1.1e-4	7.0e-4	0.0	9.8e-5	9.2e-4
3	2.5e-6	1.4e-4	6.8e-5	2.3e-6	1.7e-6	1.8e-5
4	1.7e-5	4.0e-4	1.1e-3	4.5e-5	2.3e-4	3.2e-4
5	9.3e-8	1.4e-6	7.2e-6	2.8e-8	1.2e-6	4.8e-6
6	8.3e-5	2.2e-4	4.8e-3	5.7e-3	3.2e-3	9.3e-3
7	8.3e-4	3.0e-3	2.5e-2	1.0e-3	6.3e-3	2.2e-2
8	2.5e-8	2.0e-7	5.2e-7	2.3e-6	1.7e-7	0.0
9	0.0	3.3e-4	4.0e-5	0.0	3.2e-4	0.0
10	9.5e-7	4.5e-6	9.3e-6	0.0	1.7e-8	5.7e-6
11	0.0	5.0e-4	6.5e-4	8.3e-4	8.3e-3	4.7e-3
SS	5.2	9.5	1.1	1.4	7.2	17
s^2	0.37	0.68	0.08	0.10	0.51	1.21

Table 5.4 k-values (in s^{-1}, except for k_5, k_8 and k_{10}: $l.mmol^{-1}.s^{-1}$) for lactose-casein model solution calculated by model 1 and 2. *SS* = sum of squares, s^2 = residual variance

5.4.2 Results for sterilized milks

The data of the sterilized milks were fitted with both model 1 and 2. At lower temperatures (110 and 120°C) both models appeared to fit quite well. At 130 and 140°C model 2 appeared to result in the best fit. At 150°C both models did not result in a nice fit of the experimentally determined data, probably because advanced Maillard reactions become important at this high temperature (Figures 5.12 and 5.13). The k values are given in Table 5.5. The residual variance is, except for 423 K, lower or in the same order of magnitude as compared to the experimental variance, meaning that the fit may be reasonable.

k	383 K	393 K	403 K	413 K	423 K
1	1.2e-5	4.7e-5	1.1e-4	3.3e-4	7.3e-4
2	3.3e-5	2.0e-4	2.5e-4	1.1e-3	2.3e-3
3	1.3e-6	1.7e-6	1.3e-5	1.8e-4	2.5e-4
4	2.0e-4	5.2e-4	6.3e-4	6.8e-4	1.7e-3
5	7.3e-7	1.0e-6	2.7e-6	8.2e-6	2.0e-5
6	8.3e-5	1.2e-3	1.7e-3	7.3e-3	1.1e-2
7	1.3e-4	9.8e-4	1.7e-3	3.2e-3	6.7e-3
8	1.5e-7	1.7e-7	1.3e-6	2.3e-6	1.2e-5
9	1.7e-5	7.8e-4	8.0e-4	1.1e-3	5.3e-3
10	1.7e-7	6.7e-7	1.7e-6	7.5e-6	8.3e-6
11	1.7e-4	2.5e-3	1.8e-3	1.8e-3	2.2e-3
SS	0.63	1.9	24	19	63
s^2	0.05	0.14	1.71	1.36	4.50

Table 5.5 *k*-values (in s^{-1}, except for k_5, k_9 and k_{10}: $l.mmol^{-1}.s^{-1}$) for skim milk, calculated with model 2. *SS* = sum of squares, s^2 = residual variance

The values for k_1 and k_5 appeared to be rather critical; if these were changed, the fit changed greatly. Some other *k* values could easily be changed without much changing the fit. The reason may be that the other reaction constants are not very important in the degradation described by the model. Some of these non-critical *k*-values were adjusted while keeping the *SS* constant. After that, the fits for 110°C to 150°C gave a reasonable result. In Table 5.6 limits are given for the *k*-values at 383 and 423 K; changing the *k*-value within these limits has no effect on the *SS* value. This, then, gives some idea of the uncertainty in the *k*-values found. Admittedly, this is only a crude confidence interval, which, moreover, does not take into account possible correlations between parameters, which are undoubtedly present. A more detailed statistical analysis would be needed to reveal such things, but, then, more experimental data are needed to do so.

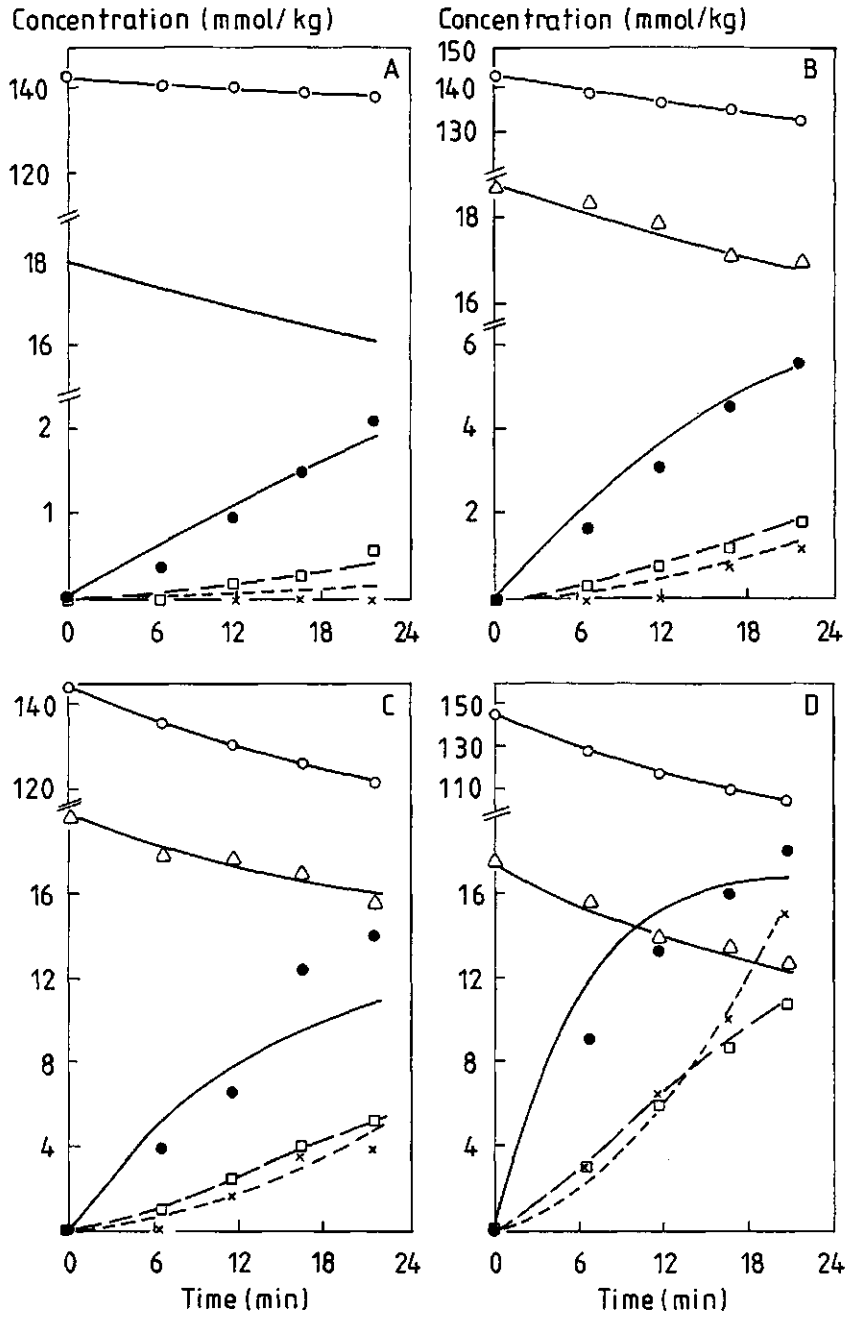


Figure 5.12 Fits for sterilized milks calculated by model 2. A = 383 K, B = 393 K, C = 403 K, D = 413 K. ○ = lactose, ● = lactulose, □ = galactose, △ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, -------- = formic acid fit, — — = galactose fit

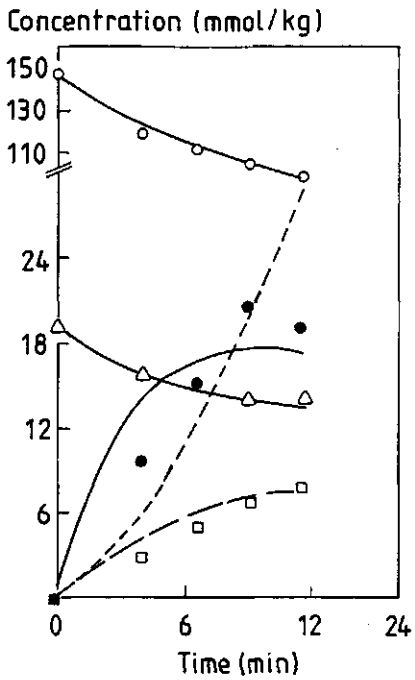


Figure 5.13 Fit for milk sterilized at 423 K calculated by model 2. ○ = lactose, ● = lactulose, □ = galactose, △ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, ------- = formic acid fit, — — = galactose fit

In the model solutions some reaction pathways can be more important than in skim milk. For example, in the lactulose-casein model solutions k_2 and k_{10} are more important than in skim milk, as almost no lactose is formed in the solution and only lactulose can be degraded. However, if we use the k_2 value found in the lactulose-casein solution for skim milk, the fit becomes poor. Using the k_{10} of the lactulose-casein solution for skim milk had hardly any effect on the fit of skim milk. Likewise k_8 and k_9 are the only k-values that play a role in the galactose-casein solution, whereas they seem to be less important in skim milk. If k_8 and k_9 found in the galactose-casein solution are used for skim milk, the fit became worse. From the experiment with the Amadori compound solution, k_6, k_7 and k_{11} can be derived.

k	383 K		423 K	
	Minimum (%)	Maximum (%)	Minimum (%)	Maximum (%)
1	-1.4	+0.1	-0.5	+0.2
2	-10*	+2.5	-0.4	+0.7
3	-10*	+10*	-4	+10*
4	-3.3	+10*	-0.6	+2
5	-5.7	+0.2	-1.7	+0.7
6	-10*	+5	-1.5	+4.3
7	-1.3	+10*	-7.5	+10*
8	-10*	+10*	-10*	+10*
9	-10*	+10*	-4.1	+1.6
10	-10*	+10*	-10*	+10*
11	-10*	+10*	-10*	+10*

Table 5.6 Limits for the k-values at 383 and 423 K. Within these limits changing of the k-value has no effect on Q. *: 10% is the maximum change tried, so 10% means: 10% change has no effect on Q, but probably a higher change neither has effect

However, these k-values did not fit in the model for skim milk: the fits became worse. The values of k_3 and k_4 from the lactulose-casein solution can also be compared with those of skim milk. The use of the k_3 values of the lactulose-casein solution for skim milk only results in a somewhat higher formic acid formation. The values of k_4 of the lactulose-casein solution can not be used for skim milk. Finally, k_1 and k_5 have to be compared with the results of the lactose-casein solution. At 120°C k_1 of the lactose-casein solution did not fit well in the skim milk model; at 140°C the k_1-values of the lactose-casein solution and skim milk are the same. The k_5-values of lactose-casein solution and skim milk are almost the same.

Consequently, most of the results on the model solutions cannot be used in exactly the same way for the skim milk. This means that the model solutions are not always representative for skim milk, but, of course, results of model solutions have given insight into the reaction paths, which are the same as for milk. Probably, the change of pH during heating is different, or other components of the milk have also a catalytic effect on the reactions that take place during heating.

5.4.3 Results for UHT heat-treated milks

The results of the fit of sterilized milks were compared with the results of UHT treated milks. The k-values found for sterilized milks were used for UHT-treated milk. These results appeared to fit quite well, especially if k_1 and k_5 were increased a bit because the milk was UHT heated at 135, 145 and 155°C. The results of the best fits are given in Table 5.7 and Figure 5.14. The residual variance is only at 408 K in the same order of magnitude as the experimental variance, at 418 and 428 K it is much higher, indicating that the fit is not very accurate. After that we studied the influence of every k-value on the fit and set those to zero that did not influence the fit. It is seen that at higher temperatures more k-values become important. Despite the high s^2, the fits look reasonable except for lactose. The high experimental error in the lactose determinations in these experiments is responsible for the high s^2. If we use the variance of sugar determination in the calculation of reaction constants by means of model 2 for UHT-treated milk, we see that SS (sum of squares) is lower, and thus s^2, as especially the variance in lactose determination played an important role in UHT-treated milk.

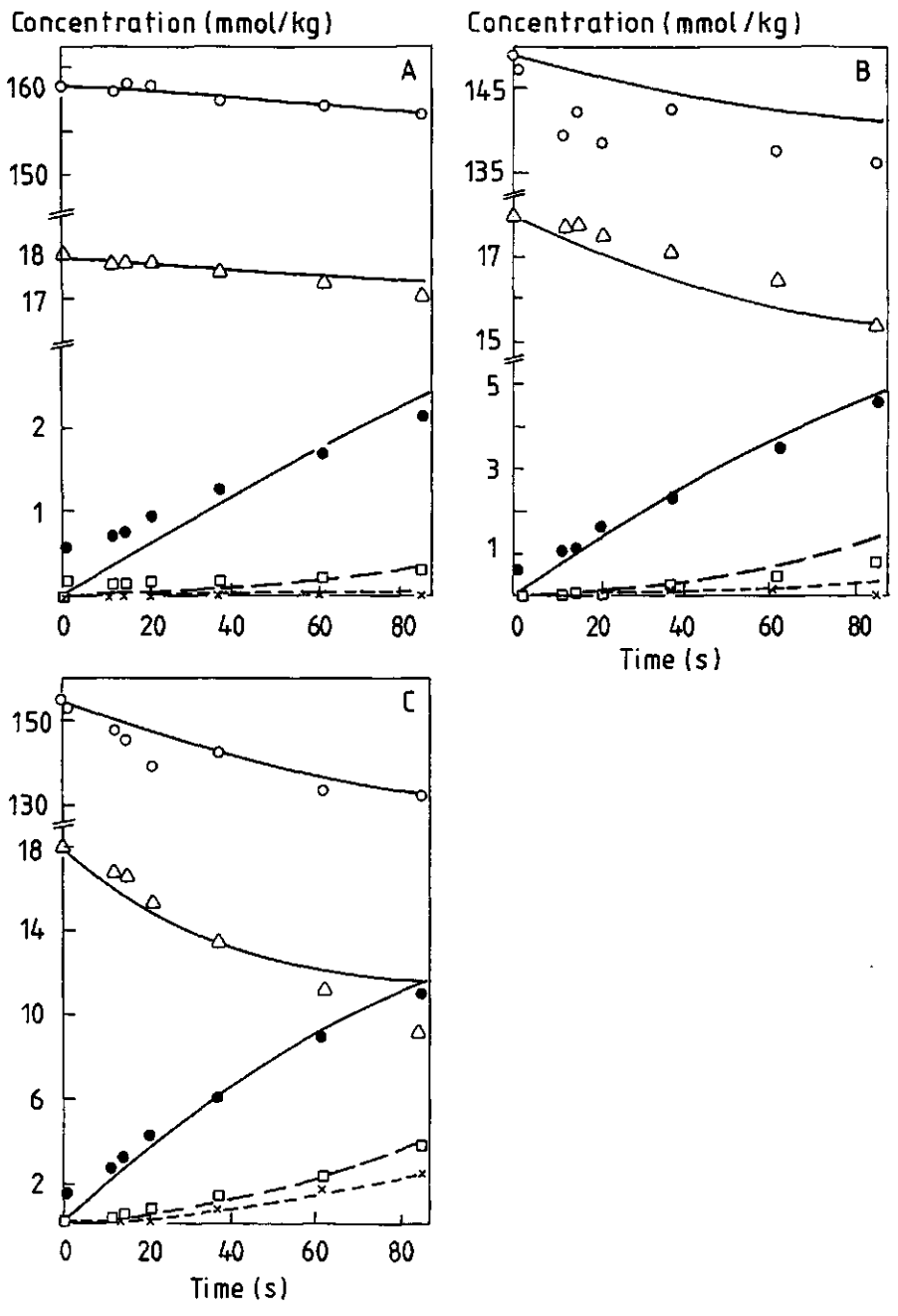


Figure 5.14 Fits for UHT heat treated milks. A = 408 K, B = 418 K and C = 428 K. ○ = lactose, ● = lactulose, □ = galactose, △ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, ------- = formic acid fit, — — = galactose fit

k	408 K		418 K		428 K	
	a	b	a	b	a	b
1	2.0e-4	2.0e-4	5.0e-4	5.0e-4	1.3e-3	1.3e-3
2	2.5e-4	0.0	1.1e-3	0.0	2.3e-3	2.3e-3
3	1.3e-5	0.0	6.0e-5	0.0	2.5e-4	0.0
4	3.0e-3	3.0e-3	5.0e-3	5.0e-3	5.0e-3	5.0e-3
5	2.7e-6	2.7e-6	1.8e-5	1.8e-5	7.0e-5	7.0e-5
6	1.7e-3	0.0	7.3e-3	7.3e-3	1.1e-2	1.1e-2
7	1.7e-3	0.0	2.2e-3	0.0	5.0e-3	5.0e-3
8	1.3e-6	0.0	2.3e-6	0.0	1.2e-5	0.0
9	8.0e-4	0.0	1.1e-3	0.0	5.3e-3	5.3e-3
10	1.7e-6	0.0	7.5e-6	0.0	8.3e-6	0.0
11	1.8e-3	0.0	1.8e-3	0.0	2.2e-3	0.0
SS	3.7	3.7	190	190	120	120
s^2	0.41	0.22	21.1	11.9	8.57	6.67

Table 5.7 k-values (in s^{-1}, except for k_6, k_8 and k_{10}: $l.mmol^{-1}.s^{-1}$) for UHT-treated milks calculated by model 2. a: with starting k-values estimated from results of sterilized milks; b: k-values set to zero for unimportant reactions in UHT-treated milk. SS = sum of squares, s^2 = residual variance

The fact that the k-values found in sterilized milks fitted quite well (taking into account that only the fits for lactose are bad) in the model of UHT-treated milks is surprising. During heating of sterilized milks the pH dropped rather fast, but, in UHT-treated milks the pH hardly dropped. This suggests that the reactions involved in our model are not greatly influenced by pH, as the reaction kinetics are almost the same in both sterilized and UHT-treated milks. This would confirm our analysis, given in section 5.2, that the pH should not have an effect on the reactions. The discrepancy with the experimentally observed effect of pH on lactulose formation (Martinez-Castro and Olano, 1980) and Maillard reaction (Nursten, 1986) remains.

5.5 Effect of temperature on reaction rates

As indicated in section 1.3, activation enthalpy ($\Delta H^{\ddagger}$) and activation entropy ($\Delta S^{\ddagger}$) can be calculated by means of the theory of Eyring. The two parameters in the Eyring equation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, were estimated from non-linear regression, after reparameterization as described by Himmelblau (1970); reparameterization is necessary because the parameters are highly correlated, and it was done by centering the independent parameter T about an intermediate temperature:

$$T^{*} = \frac{T - \bar{T}}{T} \qquad (5.30)$$

where $\bar{T}$ is the average of the five temperatures employed. An approximate confidence region was obtained as described by Himmelblau (1970), and the values reported are approximately 95% confidence intervals, calculated as t_{n-2} * standard deviation of the parameter (Students t-parameter; t_{n-2} is 3.18 for $n = 5$). The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values calculated for the sterilized milk are given in Table 5.8, derived from the effect of temperature on rate constants of model 2.

k	$\Delta H^{\ddagger}$ kJ.mol^{-1}	$\Delta S^{\ddagger}$ J.mol^{-1}.K^{-1}
1	121 ± 17	-22 ± 5
2	121 ± 41	-12 ± 6
3	109 ± 119	-59 ± 91
4	67 ± 55	-142 ± 93
5	130 ± 15	-29 ± 6
6	96 ± 63	-58 ± 45
7	96 ± 19	-61 ± 15
8	215 ± 81	167 ± 187
9	179 ± 145	131 ± 266
10	81 ± 93	-151 ± 173
11	21 ± 76	-248 ± 199

Table 5.8 Activation enthalpy and entropy of the degradation reactions of lactose in sterilized milks with the calculated 95% confidence interval

For the UHT-treated milk the same calculations were made. The activation enthalpies and entropies for UHT-treated milk are given in Table 5.9. From Table 5.7 it can be concluded that k_2, k_3, k_6, k_7, k_8, k_9, k_{10} and k_{11} are negligible for UHT-treated milk, so they are left aside.

k	$\Delta H^{\ddagger}$ kJ.mol^{-1}	$\Delta S^{\ddagger}$ J.mol^{-1}.K^{-1}
1	126 ± 10	3 ± 1
4	26 ± 152	-227 ± 209
5	188 ± 94	129 ± 86

Table 5.9 Activation enthalpy and entropy of the degradation reactions of lactose in UHT-heat treated milks

The activation enthalpies and entropies of the reactions ought to be the same for sterilized and UHT-treated milk. However, they are not always exactly the same. For the UHT experiment we only have three values for each k from which the activation enthalpy and entropy are calculated, so these are less accurate than those for sterilized milks; however, they are of the same order of magnitude. The activation enthalpies found are quite normal for chemical reactions. Geier (1984) found an activation energy (ΔE_a) of 114 kJ.mol^{-1} for lactulose formation in sterilized milks (60-120°C), 118 kJ.mol^{-1} for lactulose formation in indirect UHT heated milks and 74 kJ.mol^{-1} for lactulose formation in direct UHT heated milks (130-150°C). Andrews (1985) found a ΔE_a of 152 kJ.mol^{-1} for lactulose formation in sterilized and UHT treated milk samples and Andrews and Prasad (1987) reported a ΔE_a of 127.8 kJ.mol^{-1} for lactulose formation in sterilized milk. Olano and Calvo (1989) determined ΔE_a of the formation of lactulose, galactose and epilactose during heat treatment of milk over a wide temperature/time range (100-150°, 1-30 min): they found 125.7, 139.4 and 131.3, respectively. Troyano et al. (1992a) determined ΔE_a of galactose and tagatose formation during heating of milk (5-105 min, 115-135°C); they found 113 kJ.mol^{-1} for galactose formation and 115 kJ.mol^{-1} for tagatose formation. Horak (1980) found an activation energy of 108 kJ.mol^{-1} for lysine degradation. These results are of the same order of magnitude as our results for $\Delta H^{\ddagger}$; however, these ΔE_a values are only determined for one step, without

taking into account further degradation steps. The activation entropy for chemical reactions is usually negative for bimolecular reactions, and near zero for unimolecular reactions (Maskill, 1985). Usually, our $\Delta S^{\ddagger}$ values are slightly negative, although for some k's the confidence intervals are too wide to draw conclusions.

Considering the confidence intervals given in Tables 5.8 and 5.9, it may be concluded that the activation enthalpies of the reactions in both sterilized and UHT-treated milks are comparable. It should be added that the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for k_3, k_4, k_9 and k_{11} are actually not very reliable because the fits were poor. This may indicate that these reaction steps are not very well defined in our model (the actual reaction scheme may be somewhat different). In the case of k_{11} it may be that the formation of advanced Maillard products becomes significant only at temperatures $\geq$ 140°C, which explains its unusual behaviour with temperature. The same may be valid in the case of k_3, because formic acid formation seems to be promoted especially at higher temperatures.

5.6 General conclusions

The model used to describe the degradation reactions of lactose during heat treatment of milk appeared to fit the experimentally obtained results for heated milk fairly well. However, the k-values obtained from the model solutions could not be used directly for the skim milk: mostly they were of the same order, but not exactly the same. From this it can be concluded that the model solutions do not represent skim milk exactly; probably, pH changes and/or salt changes are so much different that the reaction rate constants are significantly affected.

The k-values found in sterilized milks could also be used for UHT-treated milks; consequently, the k-values found are exchangeable for the same system. This means that the influence of pH in the range between 6.6 and 5.5 on the reaction rate constants is very slight, as the pH dropped hardly during UHT-heating, whereas during sterilization the pH dropped remarkably. This confirms the conclusion from section 5.2 that pH does not have a large effect on the degradation reactions.

To determine whether the model can predict the reactions that take place if the composition of the system is changed, milk with different lactose and casein concentrations and model solutions with different lactose concentrations were

heated. The experimentally obtained results of sugar and formic acid formation in heated milk with varying lactose concentrations were compared to the model using the *k*-values of normal skim milk (Figure 5.15). Figure 5.15A gives the results of sugar formation in diafiltered milk heated at 130°C after addition of 183.5 mmol lactose per kg milk. The formation of lactulose is much less than predicted by the model and the galactose formation is somewhat higher, suggesting that k_3 and k_4 as obtained for skim milk are too low in the case of diafiltered milk with added lactose. Unfortunately, lysine degradation was not determined, so we cannot see whether this compound "fits" the results or not. Figures 5.15B to 5.15D give the results of formic acid formation in dialysed milks heated at 140°C after addition of 13.73, 68.6 or 137.3 mmol lactose per kg dialysed milk. The predicted formic acid formation in Figures 5.15C and 5.15D is somewhat higher than the experimentally observed formic acid concentration, though the trend is the same.

The lactose-casein model solutions with varying lactose concentrations were heated at 130°C. However, the lactose-casein model solutions with normal lactose concentration were heated at 95, 120 and 140°C, so the *k*-values for 130°C had to be determined by means of Q_{10} values (Table 5.10).

k	*k* at 403 K	Q_{10}
1	1.4e-4	2.4
2	3.0e-4	3.1
3	5.5e-6	3.3
4	2.7e-4	1.2
5	2.4e-6	2.0
6	5.4e-3	1.7
7	1.2e-2	1.9
8	0.0	-
9	0.0	-
10	3.1e-7	-*
11	6.2e-3	0.8

Table 5.10 Q_{10} and *k*-value (s^{-1}, except for k_6 and k_{10}: $l.mmol^{-1}.s^{-1}$) for lactose-casein model solution at 403 K. *: cannot be determined as k_{10} was almost 0 at 393 K

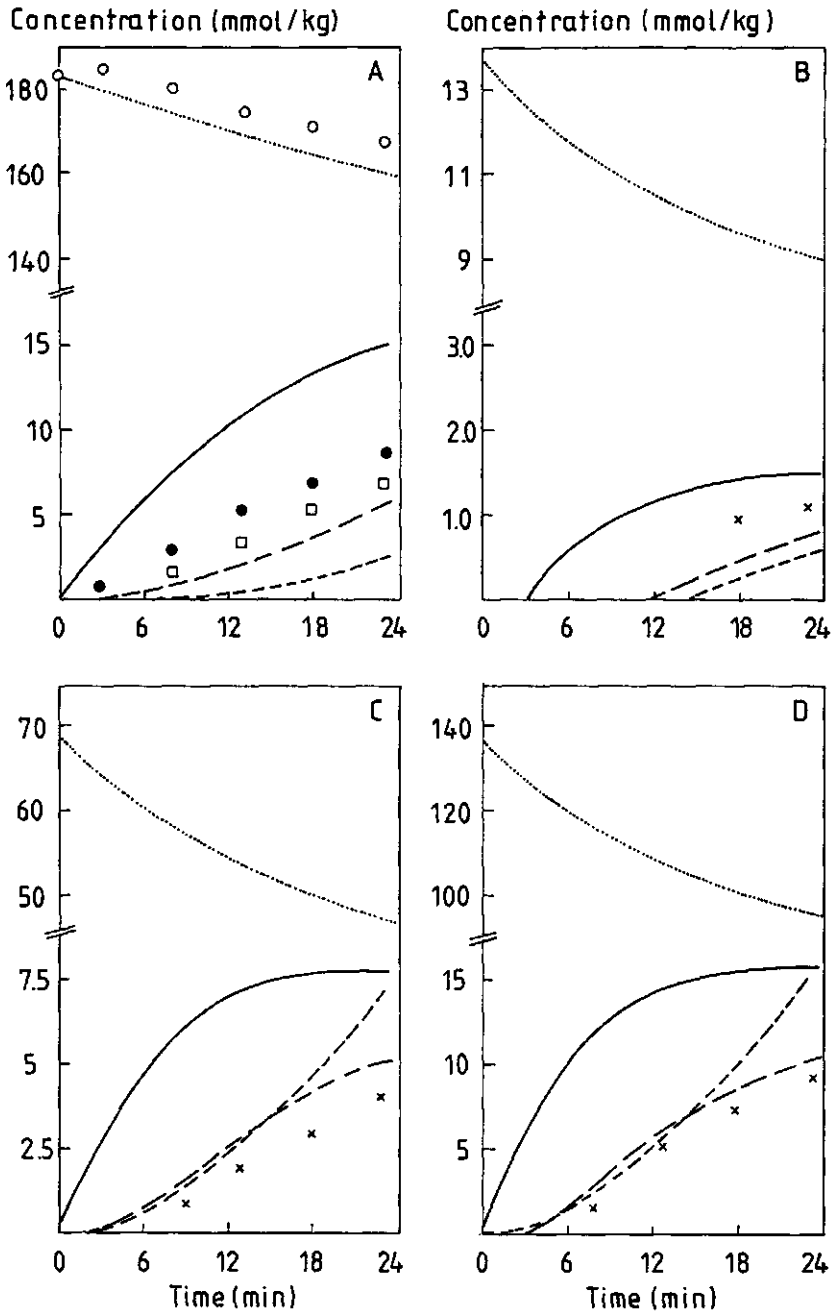


Figure 5.15 Fits for diafiltered and dialysed skim milk using the *k*-values found for sterilized milks calculated by model 2. A = diafiltered skim milk heated at 130°C after addition of 183.5 mmol lactose/l, B, C and D = dialysed skim milk heated at 140°C after addition of 13.73 mmol lactose/l, 68.6 mmol lactose/l, and 137.3 mmol lactose/l, respectively. ○ = lactose, ● = lactulose, □ = galactose, × = formic acid; ..... = lactose fit, ——— = lactulose fit, ------- = formic acid fit, — — = galactose fit

The results of the model solutions with different lactose concentration are given in Figures 5.16A to D and 5.17A and B. It is seen that for lower lactose concentrations (Figures 5.16 A and B) the predicted lactose degradation and lactulose formation are lower than the results experimentally found. The prediction for the model solution with 3/4 times the normal lactose concentration and of the model solution with the normal lactose concentration fit quite well with the experimentally determined results. In the case of a higher lactose concentration the predicted lactose degradation is somewhat higher than experimentally observed, the galactose formation is also higher than experimentally found (Fig. 5.17A). It suggests that the rate constants found are only valid for a particular solution composition from which they were derived. This may be due to the difference in pH of these heated model solutions, although we cannot explain the pH effect. These results are different from the results of diafiltered milk with a higher lactose concentration than in normal skim milk.

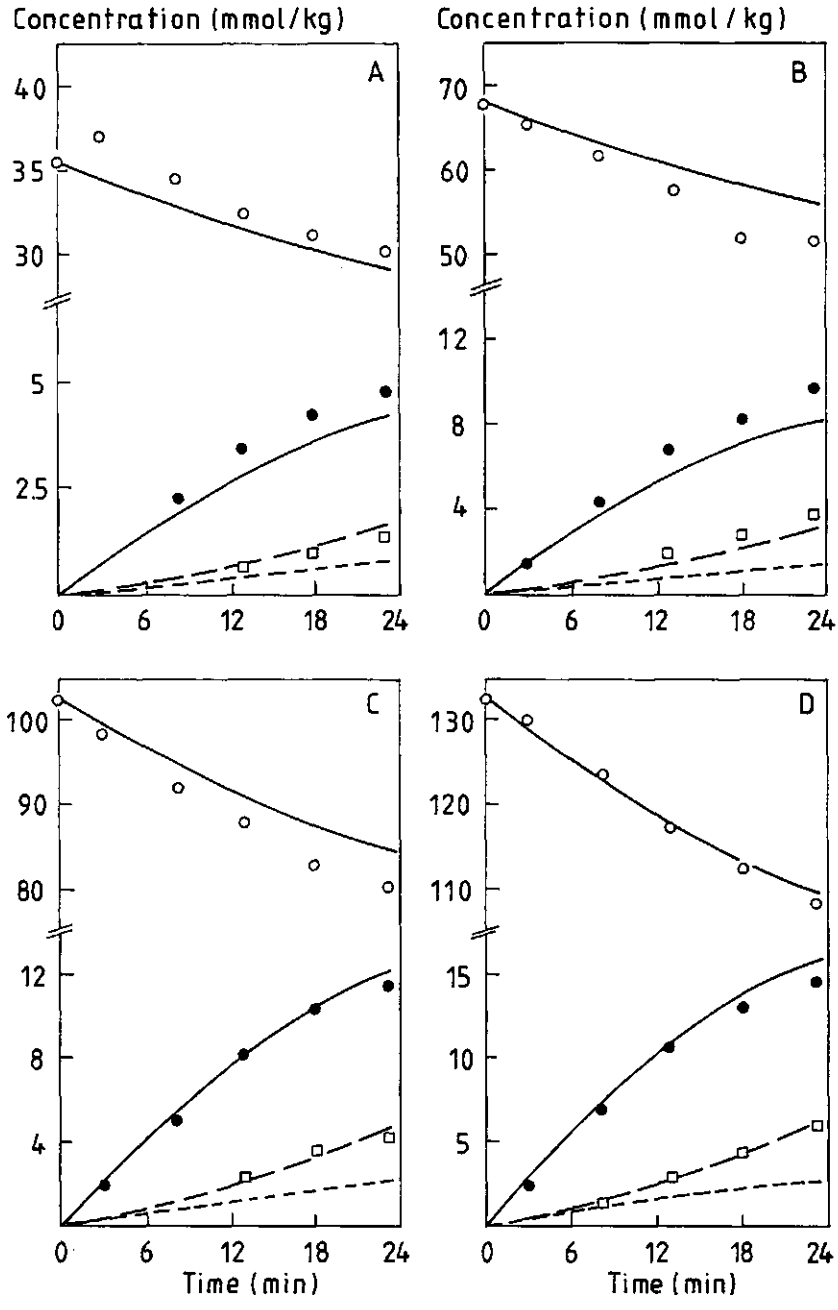


Figure 5.16 Fits of lactose-casein model solutions in water with varying concentrations of lactose after heating at 130°C using the *k*-values found for lactose-casein model solutions. A = 35 mM, B = 70 mM, C = 105 mM, D = 134 mM lactose added. ○ = lactose, ● = lactulose, □ = galactose; ——— = lactose or lactulose fit, ------- = formic acid fit, — — = galactose fit

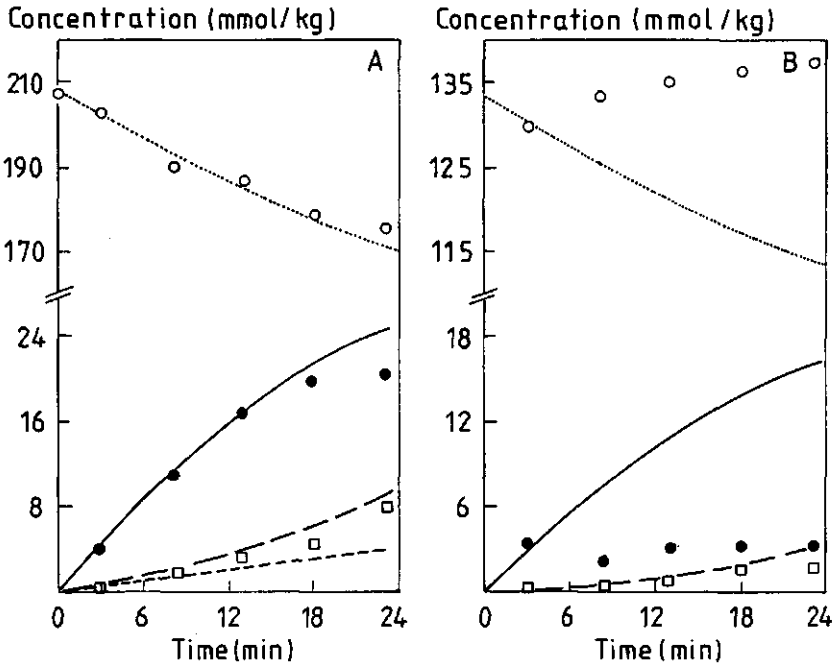


Figure 5.17 Fits of lactose-casein model solutions in water with varying concentrations of lactose after heating at 130°C using the *k*-values found for lactose-casein model solutions. A = 210 mM, B = 134 mM without casein. ○ = lactose, ● = lactulose, □ = galactose; ——— = lactulose fit, ... = lactose fit, ------- = formic acid fit, — — = galactose fit

For the lactose-casein model solutions with higher casein concentration (5.2%) the fit using the *k*-values of the model solutions with 2.6% casein was not very good. At 120°C the predicted lactose degradation was less than the experimentally found degradation; too much lysine was degraded compared to the observed degradation; the predicted lactulose and galactose formation were less; and the predicted formic acid formation was more than the experimentally found values (Figure 5.18). At 140°C, the predicted formation and degradation comes closer to the experimentally found results; the predicted lactose degradation is less; the predicted lysine degradation is more; and the lactulose formation is somewhat too small compared to the experimentally found results. This finding suggests that upon changing the composition of model solutions, or milk, the mechanism for degradation changes somewhat. The effect of protein concentration is not so much found on the Maillard reaction (as our model suggests) but more on the sugar

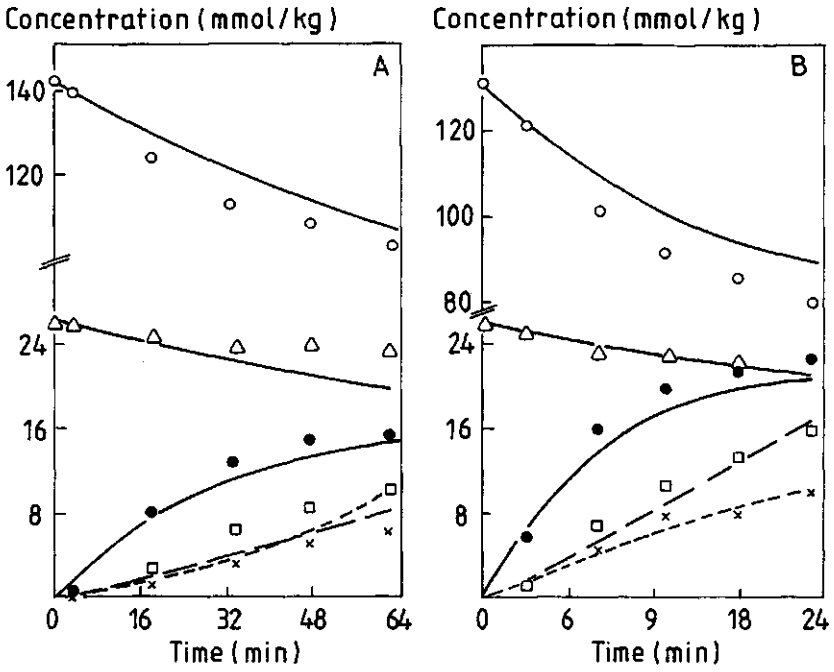


Figure 5.18 Fits of lactose-casein model solutions with 5.2% casein using the *k*-values found for lactose-casein(2.6%) model solutions. A = 393 K, B = 413 K. ○ = lactose, ● = lactulose, □ = galactose, ▵ = lysine, × = formic acid; ——— = lactose, lactulose or lysine fit, ------- = formic acid fit, — — = galactose fit

isomerization and subsequent degradation. Probably, amino acid residues in casein promote these latter reactions, either directly or via pH buffering. The effect of pH, however, is questionable. We suggest that, maybe, the effect of pH is not so much due to the pH itself, but to a pH-induced change in chemical properties of amino acid residues, which in turn affect isomerization and degradation.

The major conclusion from these results is that the model can not yet predict accurately the degradation reactions of lactose, only trends; the model describes the degradation of lactose in heated milk rather good if the milk is of normal composition. Probably other parameters, that are not taken into account in the model, play also an important role during heating which come into play when milk composition is altered. Nevertheless, the model has allowed us to test the hypothesized mechanism for degradation of lactose, and it appears that the proposed mechanism is able to explain the experimental observations, be it not always quantitatively. Consequently, such mathematical modelling allows rigorous

checking of a reaction scheme: several other reaction schemes, that were (almost) as likely as the scheme adopted, could not be fitted at all in a corresponding simulation model.

The *k*-values were determined by means of minimizing the combined sum of squares. However, the criteria mentioned in section 5.3 were not always met. The data were not weighted with their variance, as the variation of the determination was only determined for sterilized milks. This is one reason why the *k*-values found are not optimal; as discussed in section 5.3, a better criterion would be minimization of the determinant, but this was not well possible with our present data set.

The criterion that there should be no correlation between the measurements of the responses is not always met either. In the UHT-treated milks and the model solutions the lysine concentration was not determined, but calculated from the HMF-concentration; however, the HMF-concentration is not used in the model. The formation of lactulosyllysine itself was mostly calculated from the change in lysine concentration, it was not used in the model to avoid statistical dependencies. Only lactose, lactulose, galactose, formic acid and lysine concentration were used in the model describing the lactose degradation.

The temperature dependency of the reactions does not differ greatly. The activation enthalpy is of the same order of magnitude for most rate constants, only for the rate constant describing the formation of galactose (k_4) the activation enthalpy may be somewhat lower. However, due to the uncertainty in the parameters, we cannot make exact statements.

The reaction constants that describe the formation of lactulose, the Amadori compound lactulosyllysine and galactose (k_1, k_5 and k_4) are the most important ones; as k_5 is rather low, (for example, after heating skim milk for 20 min at 140°C, 18.2 mmol lactulose is formed and only 4.9 mmol lactulosyllysine) the conclusion can be drawn that the isomerization reaction is the most important reaction from a quantitative point of view in the degradation of lactose during heat treatment of milk.

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SUMMARY

The purpose of this study was to determine the kinetics of the chemical reactions associated with lactose degradation, as it occurs during heat treatment of milk. The literature available at the beginning of this study clearly suggested that the Maillard reaction plays an overriding part in the lactose degradation. The Maillard reaction starts with a reaction between a reducing sugar (in milk: lactose) and an amino group (in milk: mostly lysine residues of milk proteins). In Chapter 1 a literature review is given on reactions that appear to play a role in the degradation of lactose. Based on this literature overview, we derived what compounds might be involved in the degradation of lactose and were of interest to determine in heated milk. Initially, we focused on the Maillard reaction, including any toxicological effects. However, from the experimental results it appeared that isomerization and degradation are far more important in a quantitative sense than the Maillard reaction. Neither was any mutagenic activity found in heated milk. Hence, during this study the emphasis was shifted towards isomerization reactions. Results from literature suggested that the reactions of lactose in heated milk resemble those of lactose in alkaline solutions, reason why alkaline degradation reactions of lactose are described in some detail in Chapter 1.

Skim milk was "sterilized" - i.e. heat treated as in conventional sterilization processes - or UHT heated (Ultra-High-Temperature). Sterilization was performed in a glycerol-bath for various times (1.5-60 min) at temperatures varying from 110 to 150°C. UHT treatment was performed using a pilot-plant UHT-apparatus suitable for direct and indirect heating for 1.5-85 s at temperatures varying from 120 to 155°C.

To simplify the milk system, model solutions containing lactose, lactulose, galactose, formic acid, hydroxymethylfurfural (HMF) and furfural, furfuryl alcohol, deoxyribose or lactulosyllysine (bound in casein) in Jenness-Koops-buffer (a salt solution which simulates that of milk serum), both in the presence and absence of casein, were used in addition to skim milk.

In Chapter 2 the methods used for heating milk and model solutions and the analytical methods are described. The sugars lactose, lactulose, galactose, tagatose, glucose and deoxyribose were determined using High Performance Liquid Chromatography (HPLC). Organic acids, HMF, furfural and furfuryl alcohol were also determined by HPLC. Lysine was determined by means of a dye-binding

procedure. To study the influence of varying concentrations of lactose in milk, milk was dialysed or diafiltered.

In Chapter 3 results of heating milk and the model solutions in a glycerol-bath are described. Reaction products were determined and the influence of varying lactose and casein concentration on the formation of these products was studied. It was observed that lactose, after isomerization into lactulose, degraded into galactose, formic acid and a C-5 compound, part of which appeared to be deoxyribose, a very unstable compound that was immediately degraded into other components. The heat-induced acidity was considerable and could almost completely be ascribed to formic acid. Another reaction pathway was the condensation of lactose and lysine residues into the Amadori compound lactulosyllysine (bound to protein). After heating lactulosyllysine residues in the absence of lactose, galactose, lactose, HMF and formic acid were formed, but no lactulose. From these results, a model describing the steps in the reaction network of the degradation reactions of lactose during heating of milk is proposed.

Chapter 4 describes the results of the degradation reactions of lactose during UHT heat treatment of milk. These results are compared with those of "sterilized" milks to see whether they fitted in the same model for lactose degradation. The same products were formed as in sterilized milks and model solutions, only in much lower concentrations because of the less intense heat treatment. The formic acid concentration was so low that pH decrease was very limited. Hence, the same degradation pathways appear to be followed in the case of sterilized and UHT-treated milks, despite the difference in pH decrease. In Chapter 4, the influence of fat and protein concentration on the formation of lactulose and HMF is also given. No significant influence of the fat content on lactulose and HMF formation could be detected in this study, at least not in the range of 0-4.5% fat. At 140 and 145°C a slight effect of protein concentration on lactulose formation was found, it being higher at lower protein concentration. No correlation was found between galactose and HMF formation and protein concentration. Increasing formation of formic acid with increasing protein concentration was found at 155°C, probably because degradation reactions increase with protein content.

In Chapter 5 an attempt is made to establish kinetic parameters for the model proposed in Chapter 3. It was tried to model the degradation of lactose by computer simulation in order to predict the quantities of the various degradation products in the course of time. The experimental results described in Chapters 3

and 4 were compared to the results of the simulation. The model appeared to fit the experimentally obtained results for sterilized and UHT-treated skim milk reasonably well. However, the reaction constants found for the model solutions were not quite the same as those found for skim milk, though of the same order of magnitude. The temperature dependencies of the various reaction steps were not significantly different and were quite normal for chemical reactions. The results for the milk with varying lactose concentration and the model solutions with varying lactose and casein concentrations could not well be predicted by the model. This suggests that the rate constants found are only valid for the particular composition of the system from which they were derived. Probably, other parameters than those taken into account in the model, play a role during degradation of lactose. One of them may be the effect of pH; our model predicts no effect of pH whereas in literature a large (though quite variable) effect is described. Altogether the model has allowed us to test the hypothesized mechanism for degradation of lactose and it appears that the proposed mechanism is able to explain the observations for milk and model solutions resembling milk. Several other reaction schemes, that were (almost) as likely as the scheme adopted, could not be fitted at all in a corresponding simulation model. Mathematical modelling thus allows rigorous checking of a proposed reaction scheme.

Two main conclusions can be drawn from this work. First, mathematical modelling is a very powerful method to check complicated reaction networks in foods. It clearly shows that wrong conclusions can be drawn if one only studies one or two reaction products instead of the whole reaction scheme. Second, in the case of milk it has been found that, from a quantitative point of view, the isomerization reaction is much more important than the Maillard reaction in the degradation of lactose during heat treatment of milk.

SAMENVATTING

Het doel van het onderzoek beschreven in dit proefschrift was het verkrijgen van meer informatie over de reakties waarbij lactose betrokken is die optreden tijdens het verhitten van melk. Hoofdstuk 1 geeft een overzicht van de relevante literatuur. In de literatuur zijn veel reakties beschreven waarbij lactose een rol speelt; op het moment dat dit onderzoek begon, was de aandacht vooral gericht op de Maillard-reaktie. Deze begint met een reaktie van een reducerende suiker (in melk: lactose, melksuiker) en een reaktieve aminogroep (in melk: de vrije aminogroepen van lysine uit melkeiwit); als vervolg op deze reaktie kunnen bruinkleuring, smaakverandering en verlies aan voedingswaarde optreden. De literatuur suggereerde dus dat de Maillard-reaktie een zeer belangrijke rol speelt bij de afbraak van lactose tijdens het verhitten van melk. In eerste instantie vestigden we daarom in dit onderzoek alle aandacht op de Maillard-reaktie, ook op de toxicologische aspekten ervan. Uit de literatuur bleek namelijk dat intermediairen uit de Maillard-reaktie mutageniteit zouden kunnen initiëren. Uit onze resultaten bleek echter dat de Maillard-reaktie helemaal niet zo'n grote rol speelt, maar dat isomerisatie en degradatie van lactose in kwantitatieve zin veel belangrijker zijn. Ook kon geen mutageniteit worden aangetoond in verhitte melk. Als gevolg hiervan werd de nadruk van het onderzoek verschoven naar de isomerisatie- en degradatiereakties. Uit het literatuuronderzoek kwam naar voren dat de reakties van lactose in verhitte melk overeenkomst vertoonden met reakties van lactose in alkalische omstandigheden. Deze zijn daarom uitgebreid beschreven in hoofdstuk 1.

Ondermelk werd "gesteriliseerd" (verhit zoals dat globaal gebeurt bij klassieke sterilisatie van melk) of ultrahoog verhit (UHT, korte tijd op een hoge temperatuur). De sterilisatie werd uitgevoerd in een glycerolbad, waarin gedurende 1,5-60 minuten bij 110 tot 150°C verhit werd; de UHT verhitting werd in een kleinschalig UHT-apparaat in een continue stroom uitgevoerd, waarbij gedurende 1,5-85 s bij 120 tot 155°C verhit werd.

Aangezien melk zeer veel bestanddelen bevat, werden model-oplossingen gebruikt die een vereenvoudigd melk-systeem voorstellen. Deze model-oplossingen werden gemaakt door lactose, lactulose, galactose, mierezuur, hydroxymethylfurfural (HMF) en furfural, furfurylalcohol, desoxyribose of lactulosyllysine (gebonden aan caseïne) met of zonder caseïne op te lossen in Jenness-Koops-buffer (dit is een zoutoplossing die de samenstelling van melkserum

zo goed mogelijk benadert).

In hoofdstuk 2 worden de materialen en methoden van het verhitten van ondermelk en model-oplossingen en de analytische methoden beschreven. De suikers lactose, lactulose, galactose, glucose, tagatose en desoxyribose werden met behulp van hoge-druk vloeistofchromatografie (HPLC) bepaald. Organische zuren, HMF, furfural en furfurylalcohol werden ook met behulp van HPLC bepaald. Lysine werd bepaald met behulp van een kleurstofbindingsmethode. Om de invloed van variatie van de lactoseconcentratie te bestuderen, werd melk ook gediafiltreerd en gedialyseerd; tevens werden model-oplossingen gemaakt om de invloed van lactoseconcentratie en caseïne-concentratie te kunnen bestuderen.

In hoofdstuk 3 worden de resultaten van de gesteriliseerde ondermelk en model-oplossingen beschreven. De verschillende reaktieprodukten werden bepaald en de invloed van lactoseconcentratie en eiwitconcentratie werd bestudeerd. Lactose isomeriseert tot lactulose en lactulose kan weer afgebroken worden, waarbij galactose, mierezuur en een C-5-verbinding gevormd worden. Een deel van de gevormde hoeveelheid C-5-verbinding werd verklaard door het aantonen van afbraakprodukten van desoxyribose in de verhitte melk en model-oplossingen. Dit desoxyribose bleek erg reaktief te zijn en al snel door te reageren tot verdere afbraakprodukten. De zuurvorming tijdens het verhitten van melk bleek nagenoeg helemaal veroorzaakt te worden door de vorming van mierezuur. Verder reageert lactose met lysine-residuen waarbij de Amadori-verbinding lactulosyllysine (gebonden aan eiwit) gevormd wordt. Tijdens het verhitten van een model-oplossing met lactulosyllysine (gebonden aan eiwit) werden lactose, galactose, mierezuur en HMF gevormd, maar geen lactulose. Dit geeft aan dat de Amadori-verbinding tijdens verhitten niet hydrolyseert in lactulose en lysine-residuen. Naar aanleiding van de resultaten van deze experimenten werd een model opgesteld voor het complex van reakties die optreden bij de afbraak van lactose tijdens het verhitten van melk.

In hoofdstuk 4 worden de resultaten van de UHT verhittingen beschreven. Deze resultaten werden vergeleken met de resultaten van gesteriliseerde melk, om na te gaan of ze in hetzelfde model voor de afbraak van lactose passen. Dezelfde reaktieprodukten werden gevormd, alleen in veel kleinere concentraties, als gevolg van de minder intensieve hittebehandeling. Ook werd veel minder zuur gevormd, zodat de pH nauwelijks daalde. Alleen bij 155°C werden waarneembare hoeveelheden mierezuur gevormd. Hieruit kan geconcludeerd worden dat de

afbraak van lactose in gesteriliseerde melk en UHT melk op dezelfde wijze verloopt, ondanks het door verhitting geïnduceerde pH-verschil. Tevens werd in hoofdstuk 4 de invloed van het vet- en het eiwitgehalte op de vorming van lactulose en HMF bestudeerd. De hoeveelheid vet, variërend van 0 tot 4,5% vet, bleek geen signifikante invloed op lactulose- en HMF-vorming te hebben. De eiwitconcentratie bleek bij 140 en 145°C een lichte invloed op de lactulosevorming te hebben: er werd meer lactulose gevormd bij een lagere eiwitconcentratie. Galactose- en HMF-vorming werden niet beïnvloed door de eiwitconcentratie. Bij 155°C bleek dat bij toenemende eiwitconcentratie ook de vorming van mierezuur toeneemt; dit is mogelijk een gevolg van het feit dat afbraakreakties gestimuleerd worden door eiwit.

In hoofdstuk 5 werd getracht om kinetische parameters vast te stellen voor het in hoofdstuk 3 voorgestelde model. Er werd een computerprogramma geschreven om de afbraak van lactose tijdens het verhitten te simuleren. De resultaten van hoofdstuk 3 en 4 werden vergeleken met de resultaten van de simulatie. De intentie was om met behulp van deze simulatie de reakties van lactose in verhitte melk te kunnen voorspellen. De simulatie bleek de resultaten van gesteriliseerde ondermelk en UHT-verhitte ondermelk goed te kunnen beschrijven. Alleen bleken de reaktiesnelheidsconstanten van de model-oplossingen niet volledig overeen te komen met die van de model-oplossingen, al waren ze wel van dezelfde orde van grootte. De temperatuurafhankelijkheid van de diverse reakties verschilde niet veel en kwam in het algemeen overeen met wat meestal voor chemische reakties wordt gevonden. De resultaten van melk en model-oplossingen met gevarieerde lactose- en eiwitconcentraties konden echter niet goed voorspeld worden door de simulatie. Dit suggereert dat de gevonden reaktiesnelheidsconstanten alleen gelden voor het specifieke systeem waar ze van afgeleid zijn. Het is goed mogelijk dat andere factoren, die niet bij het opstellen van het simulatiemodel betrokken zijn, ook een rol spelen bij de afbraakreakties. Een voorbeeld hiervan is de pH; volgens het simulatiemodel heeft pH geen invloed op het verloop van de reakties, maar in de literatuur wordt aangegeven dat pH wel degelijk een invloed heeft (alhoewel het resultaat van die beïnvloeding in de literatuur nogal kan verschillen). Het belang van het simulatiemodel is dat het ons in staat heeft gesteld om het voorgestelde reaktiemechanisme voor het verloop van de lactose afbraak kwantitatief te toetsen en wel met redelijk succes. Allerlei andere reaktieschema's, die kwalitatief eveneens in overeenstemming leken met de resultaten van hoofdstuk 3, bleken de

toets van een simulatiemodel niet te kunnen doorstaan. Hieruit blijkt dat het opstellen van een mathematisch model een rigoreuze methode is om de juistheid van een voorgesteld reaktiemechanisme te controleren.

Twee belangrijke conclusies kunnen naar aanleiding van dit onderzoek worden getrokken. Ten eerste, een mathematisch model is een krachtig middel om gecompliceerde reaktienetwerken in levensmiddelen te achterhalen. Duidelijk is dat verkeerde conclusies getrokken worden als maar één of twee reaktieprodukten worden bestudeerd in plaats van het gehele reaktiemechanisme. Ten tweede, in het geval van melk, kan geconcludeerd worden dat de Maillard-reaktie in kwantitatieve zin veel minder belangrijk is dan de isomerisatie- en degradatiereakties van lactose die optreden tijdens het verhitten van melk.

CURRICULUM VITAE

Iekje Berg werd op 3 november 1962 in Rijswijk (N.Br.) geboren. In 1981 behaalde zij haar VWO-diploma aan het Develsteincollege te Zwijndrecht. In hetzelfde jaar begon zij haar studie Levensmiddelentechnologie aan de toenmalige Landbouwhogeschool te Wageningen. Zij koos voor de doctoraalvakken levensmiddelenchemie, levensmiddelenmicrobiologie, organische chemie en industriële bedrijfskunde. In november 1987 slaagde zij voor het doctoraalexamen. Van 1 september 1987 tot 13 oktober 1991 was zij aangesteld als assistent in opleiding bij de sectie Zuivel en Levensmiddelennatuurkunde van de Landbouwuniversiteit. In deze periode werd het in dit proefschrift beschreven onderzoek uitgevoerd. Met ingang van 19 oktober 1992 is zij in dienst bij Buro Gort, adviseurs in bakkerij- en zoetwarentechnologie, te Zwijndrecht.