Food Chemistry **21** (1986) 211–221

Modifications and Interactions of Lactose with Mineral Components of Milk During Heating Processes

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(Received: 22 January, 1986)

ABSTRACT

The isomerization and degradation of lactose during the heating of 5% lactose solutions in different buffer systems were studied. The extent of formation of the isomeric disaccharides, lactulose and epilactose, and of galactose increased as the pH of the solution was increased. Precipitation of calcium phosphate during heating resulted in a decrease in isomerization and degradation of lactose. Formation of lactulose during the heating of lactose, as a 5% solution in synthetic milk ultrafiltrate, was lower than that found in milk submitted to similar heat treatments.

INTRODUCTION

During heat treatment of milk, a number of interactions among components take place which cause changes in their physical, chemical and biological properties. Knowledge of the mechanisms of chemical conversions in this complex system is very limited. Most of the changes in lactose during the heating of milk are attributed to the Maillard reaction which occurs between lactose and proteins. Nevertheless,

Food Chemistry 0308-8146/86/$03·50

reactions with other milk components may also occur. Early work has shown that lactulose can be formed during heat-treatment of aqueous, alkaline solutions of lactose (Corbett & Kenner, 1953; Speck, 1958), as well as in heated milks (Adachi, 1959). Olano & Martinez-Castro (1981) observed that sterilization of solutions of lactose in the presence of different cations, amines or amino acids gave lactulose, epilactose and monosaccharides, as has been observed during sterilization of milk (Martinez-Castro & Olano, 1980; Geier & Klostermeyer, 1983). However, it is not yet clear whether the isomeric disaccharides of lactose, present in heated milks, are formed during the earlier stages of the Maillard reaction or during basic catalysis caused by amino groups or inorganic constituents of milk (Adachi & Patton, 1961).

Although there are some notable differences between model systems and milk, model systems are of great value in elucidating the mechanisms of the reactions and also in predicting changes taking place during heating, which are particularly important for the optimization of heating processes.

The present study aims to evaluate the interactions of milk salts with lactose. The rôle of milk salts was investigated, first with different phosphate and citrate buffers, then with a mixed-salts solution, which simulates milk ultrafiltrate. Although caseins play an important rôle in the salt equilibrium of milk, only inorganic ions and citrate were used here, to avoid the presence of an amino group, and hence the Maillard reaction.

MATERIALS AND METHODS

All chemicals used were reagent grade. Buffer solutions A (Na_2HPO_4/NaH_2PO_4) (Gomori, 1955) and B (Na_2HPO_4/sodium citrate) (McIlvaine, 1921) were prepared at different concentrations. A synthetic milk ultrafiltrate solution, simulating milk serum and containing no N (SMUF/C), was prepared according to the method of Jenness & Koops (1962). Another salt solution (SMUF/D) was prepared as for SMUF/C but omitting the $CaCl_2$ and $MgCl_2$; the pHs of SMUF/C and D were 6·6 for all experiments.

Crystalline lactose (pure by gas–liquid chromatography) was dissolved at 5% w/v in the different buffers and then adjusted to the desired pH with 1 N NaOH.

Heat treatments

Portions (10 ml) of lactose solution were heated in a silicone oil bath for a stated period in tightly stoppered pyrex glass tubes (16 × 162 mm). For treatments at high temperature, sealed glass-capillary, thin-walled tubes (1 m long, 1·31 mm id and 1·80 mm od) were used. They were coiled as chromatographic columns and could be introduced in a silicone bath at 145°C, attaining the desired temperature in a few seconds.

Gas chromatography

After cooling to room temperature, phenyl-β-D-glucoside was added as an internal standard and the mixture evaporated under vacuum. Blanks containing lactose and salts without heating were included to observe the effect of concentration during evaporation. Samples were dissolved in pyridine and kept for 1 h at 70°C in order to achieve a constant equilibrium composition. *N*-Trimethylsilylimidazole at 50% in pyridine was added to sylilate the samples; the reaction was complete in 30 min at 70°C (Martínez-Castro & Olano, 1980). The trimethylsilyl ethers of the carbohydrates were injected into a Perkin–Elmer model Sigma 3 B gas chromatograph (Perkin–Elmer Co., Norwalk, Conn.) equipped with a fused silica capillary column, 25 m × 0·22 mm, coated with OV-17 (Chrompack, PO 3, 4330 AA Middelburg, The Netherlands). The injector, at 300°C, was operated in the split mode with a ratio 1:100. The oven was at 190°C for 14 min to elute monosaccharides and then raised to 240°C at a heating rate of 39°C/min until elution of disaccharides was completed. Response factors were calculated with a series of pure standards, at different concentrations versus the peak height for phenyl-β-D-glucoside.

Identification of peaks was undertaken by retention times and chromatographic patterns, according to reported results of previous work carried out by gas–liquid chromatography/mass spectrometry (Martinez-Castro & Olano, 1980).

RESULTS

Carbohydrate formation

Heating lactose in buffer solutions of pH near 7, under similar conditions to the classical sterilization of milk, caused isomerization and degradation of lactose, so that lactulose, epilactose and galactose were formed.

Only traces of glucose were found, since the conversion of lactose to lactulose is followed by the rapid degradation of the lactulose into galactose and isosaccharinic acids (Corbett & Kenner, 1953). Simultaneously, a loss of total carbohydrate and a change of the colourless solution to a pale yellow colour were observed. Only traces of lactulose were detected when solutions of lactose in pure water were heated at 120°C for 30 min.

A chromatogram of the carbohydrate fraction of a heated solution of lactose in buffer is shown in Fig. 1. Peaks corresponding to galactose, epilactose, lactulose and lactose are clearly visible. The high performance of the column used afforded the separation of the four galactose anomers; this pattern was clearly distinguishable from that of a mixture of glucose and galactose. The disaccharides were also well resolved except β-epilactose and lactulose. The separation was better than that previously reported using packed columns (Martinez-Castro & Olano, 1980).

Effect of simple buffer systems

The effects of concentration, pH and type of buffer were studied. Molarity varied from a value similar to that found in milk to higher values. Of all the buffer systems able to interact with sugars, only phosphate and citrate, which are present in milk, were investigated. A factorial experiment with three factors, buffer concentration (0·01–0·2 M), pH (6·2 to 6·8) and type of buffer (A and B), was designed.

Multiple regression analysis of lactulose formed in twenty-four samples heated at 120°C for 20 min showed that the pH was the most important source of variation, whereas concentration and type of buffer were not significant. Figure 2 shows the formation of galactose, epilactose and lactulose and that the levels of these carbohydrates increased with the pH of the buffer.

Solutions simulating the milk salts system

When SMUF/C was heated above 100°C a precipitate containing calcium phosphate was formed which partly redissolved on cooling, as was previously observed by Jenness & Koops (1962). The reaction which takes place is roughly:

$$3Ca^{2+} + 2HPO_4^{2-} \rightarrow Ca_3(PO_4)_2\downarrow + 2H^+$$

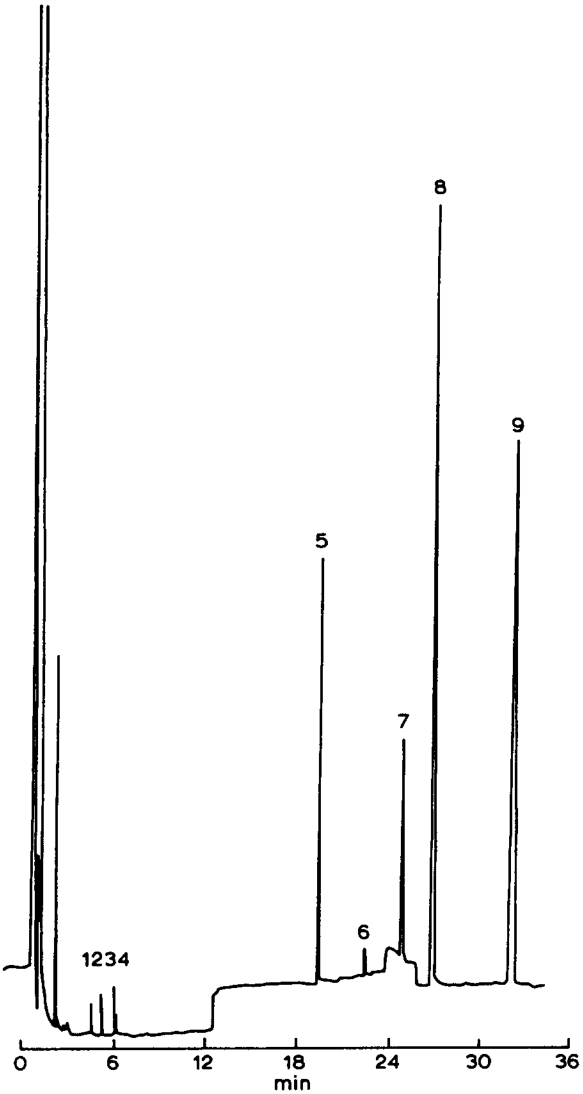

Fig. 1. Chromatogram of trimethylsilyl derivatives of mono- and disaccharides in 5% lactose buffer solution heated at 120°C for 30 min. 1–4, Galactose. 5, Phenyl-β-D-glucoside (internal standard). 6,. α-Epilactose. 7, Lactulose + β-epilactose. 8, α-Lactose. 9, β-Lactose.

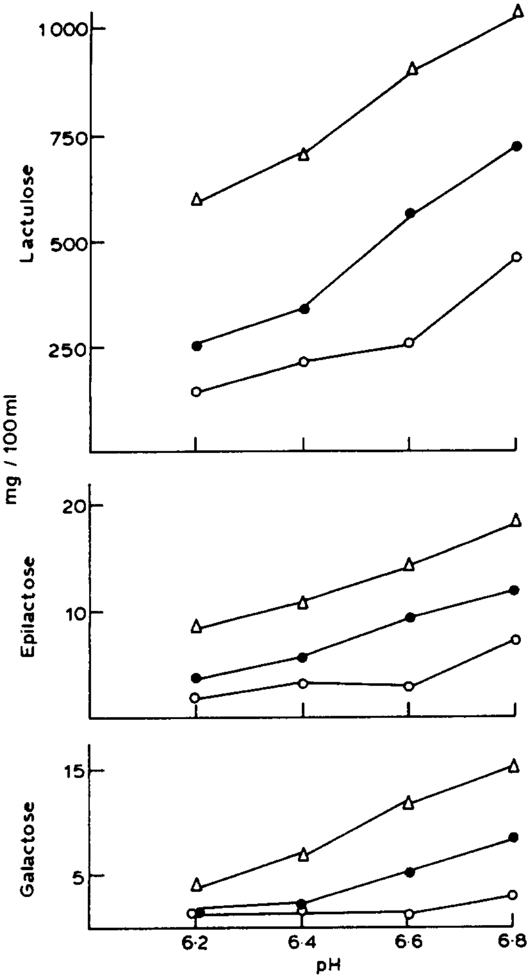

Fig. 2. Average values of the amounts of lactulose, epilactose and galactose formed when heating 5% lactose in buffer solutions (A and B) at 120°C at different pH values. ○, 10 min. ●, 20 min. △, 30 min.

This implies a drop in pH (Walstra & Jenness, 1984). Transformation of lactose under these conditions was less than with buffers A and B (Fig. 3). Up to 116 mg/100 ml lactulose was formed at 120°C, whereas, at 100°C and 65°C, the amounts of lactulose formed were less than 35 mg/100 ml. Proportional quantities of galactose were always observed. Epilactose could not be detected after heating at temperatures below 120°C.

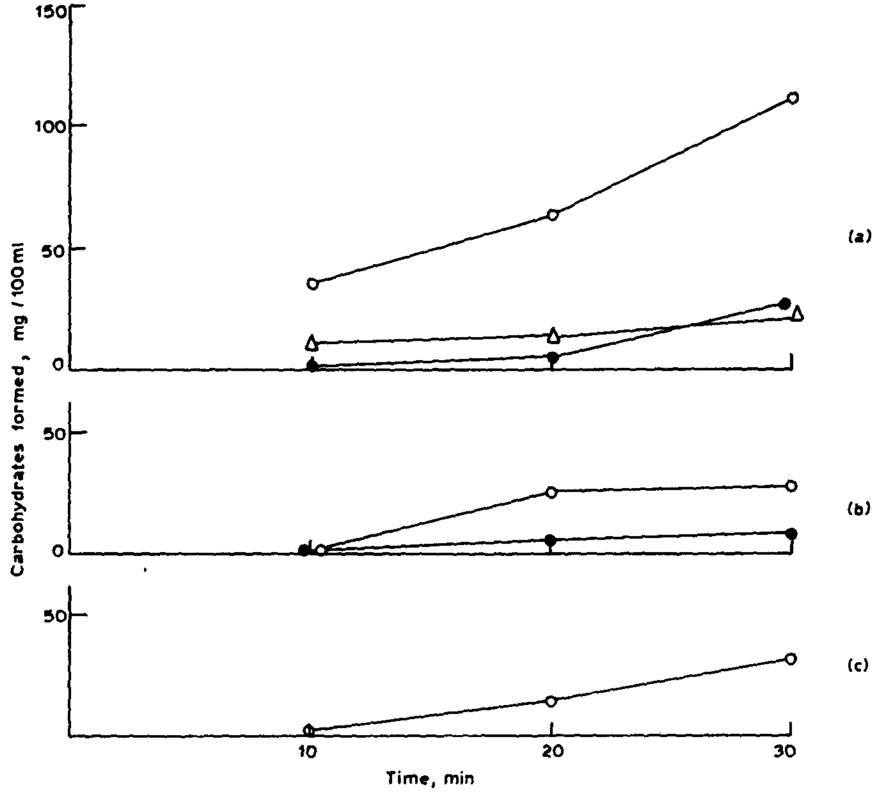

Fig. 3. Formation of lactulose, epilactose and galactose during heat treatment of synthetic milk ultrafiltrate (SMUF/C, pH 6·6) at several temperatures. (a) 120°C. (b) 100°C. (c) 65°C. ○, Lactulose. ●, Galactose. △, Epilactose.

In order to explain the effect of calcium phosphate on the isomerization of lactose, parallel experiments were carried out in the absence of Ca^{++} (SMUF/D). Under these conditions no precipitation was observed during heating (Fig. 4). Transformation of lactose was higher than that attained under the same heating conditions in SMUF/C and similar to, but slightly lower than, that observed with buffers A and B. The amount of lactulose formed was 600 mg/100 ml after 20 min at 120°C, whereas only 116 mg/100 ml were formed when SMUF/C was heated under the same conditions. These differences can be attributed to the decrease in pH of SMUF/C from 6·6 to 6·0 due to precipitation of insoluble calcium phosphate. It is necessary to take into account the fact that part of the

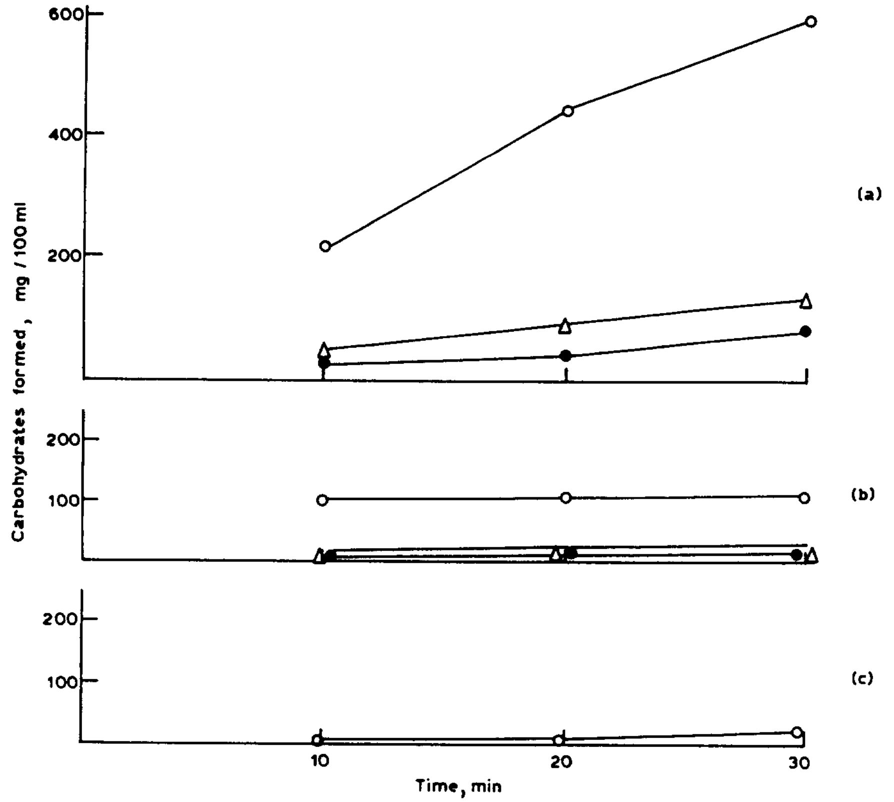

Fig. 4. Formation of lactulose, epilactose and galactose during heat treatment of a 5% lactose solution containing synthetic milk ultrafiltrate in the absence of calcium and magnesium (SMUF/D at pH 6·6) at different temperatures. (a) 120°C. (b) 100°C. (c) 65°C. ○, Lactulose. ●, Galactose. △, Epilactose.

TABLE 1

Mono- and Disaccharides Formed (Per cent of Total Carbohydrates) when Lactose is Dissolved at 5% in SMUF/C[a] and SMUF/D[b] (pH, 6·6) and Heated at 145°C for 10 and 20 s.

Time (s)	*SMUF/C*			*SMUF/D*		
	Galactose	*Epilactose*	*Lactulose*	*Galactose*	*Epilactose*	*Lactulose*
10	1·9	—	6·5	1·1	4·0	20·2
20	5·1	—	11·3	4·4	11·3	61·3

[a] Synthetic milk ultrafiltrate (Jennes & Koops, 1962).

[b] Modified synthetic milk ultrafiltrate.

precipitate redissolves after cooling, so that the final pH measured in SMUF/C was higher than the actual pH during heating.

Some experiments, at high temperature during short periods of time, were carried out using SMUF/C and SMUF/D. SMUF/C promoted lactulose formation in amounts similar to those found in ultra-high temperature (UHT)-treated milks (Martinez-Castro & Olano, 1978; Geier & Klostermeyer, 1983; Andrews, 1984). The concentration of lactulose formed during UHT treatment of SMUF/D was considerably higher (Table 1).

DISCUSSION

It is clear that the formation of lactulose, under the conditions described above, proceeds by the Lobry de Bruyn–Alberda van Ekenstein reaction, which takes place via enolization, and is subject to general acid–base catalysis (Speck, 1958). Of the salt components of milk, phosphates and citrates are mainly responsible for its buffer capacity and consequently for the formation of lactulose. When primary phosphates were added to milk as stabilizers, the formation of lactulose during heat-treatment increased significantly (Martinez-Castro & Olano, 1980).

Calcium phosphate precipitates during the heating of milk, probably as a hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (Fox, 1981) which is able to exert a catalytic effect on lactose isomerization. Klostermeyer (1984) collected the precipitate formed during the thermal treatment of milks and observed that addition of the precipitate, to milk or lactose solutions, accelerated the formation of lactulose during heating. In SMUF/C there are two competitive effects; one is the drop in pH during heating, which decreases the isomerization of lactose, and the other is the catalytic effect of the formed precipitate which increases isomerization. Since the formation of lactulose was higher when precipitation did not occur, as in the case of SMUF/D, it is clear that the catalytic effect of formed precipitate in SMUF/C is minimized by the corresponding decrease in pH.

Our results show that the salt system of milk can interact with lactose through the Lobry de Bruyn–Alberda van Ekenstein transformation to form lactulose. The amounts of lactulose formed during heating at 120°C for 20 and 30 min in SMUF/C (63·5 and 116 mg/100 ml, respectively) are close to the range reported for sterilized milks:

90–190 mg/100 ml (Martinez-Castro & Olano, 1978); 99–247 mg/100 ml (Geier & Klostermeyer, 1983); 69–120 mg/100 ml (Andrews, 1984). This suggests that most of the lactulose in heated milk is formed in alkaline rearrangement of lactose due to the buffering of milk. Since the phosphates and citrates mainly determine the pH and the buffer capacity of milk, it can be concluded that the salt system of milk is also responsible for most of the lactulose formed during the thermal processing of milk.

Differences between the lactulose formed in milk and in SMUF could be attributed to the presence in milk of other constituents such as urea and ionizable groups of the proteins, which have a buffering action and can isomerize lactose during heating of aqueous solutions (Cerbulis *et al.*, 1978; Olano & Martinez-Castro, 1981).

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the Comisión Asesora de Investigación Científica y Técnica (CAICYT) for this research. They also wish to thank María José Rodriguez for her technical assistance.

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