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Kinetics of Lactulose, Galactose and Epilactose Formation during Heat-treatment of Milk

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ABSTRACT

The kinetics of formation of lactulose, galactose and epilactose during heating of milk were examined over a wide temperature/time range (100–150°C/1–30 min). Formation of the studied carbohydrates was best described with apparent first order reactions. The Arrhenius plot of the logarithm of the rate constant as a function of the reciprocal of the temperature (°K) enabled calculation of the activation energies (125 kJ/mol for lactulose, 131 kJ/mol for epilactose and 139 kJ/mol for galactose). By using the kinetics parameters for calculating lines of equal degrees of formation in a plot of log-time versus reciprocal of absolute temperature it was possible to predict the effect of different heat-treatments on the formation of individual carbohydrates.

INTRODUCTION

Although the occurrence of free lactulose in heated milk was reported in 1958 (Adachi, 1958), data on the differences in lactulose content between pasteurized, sterilized and UHT milks were published for the first time twenty years later (Martinez-Castro & Olano, 1978). For the last ten years, considerable research has been carried out on the presence of lactulose in milk (Andrews, 1984; Geier & Klostermeyer, 1983; Fink & Kessler, 1988) and it has been proposed that the formation of lactulose in heated milk proceeds by the Lobry de Bruyn-Alberda Van Ekenstein transformation catalyzed by the milk salt system (Martinez-Castro *et al.*, 1986; Andrews & Prasad, 1987).

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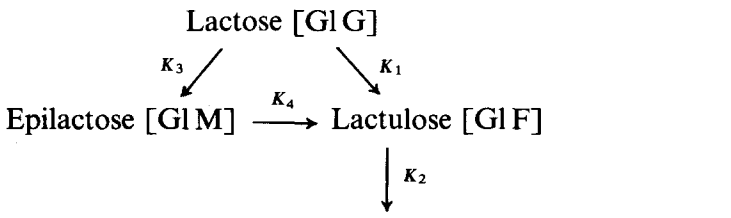

Scheme 1

The aldose–ketose isomerization has been intensively studied and the formation of the epimeric aldose as well as the degradation of the ketose into isosaccharinic acids have been reported (Speck, 1958; Rendleman & Hodge, 1979). In the case of lactose–lactulose isomerization during heating of milk, the formation of the epimeric aldose (epilactose) and degradation of lactulose have been proved (Martinez-Castro & Olano, 1980; Olano *et al.*, 1988) but no kinetics of galactose and epilactose formation have been reported.

The main reaction pathway of lactose in heated milk is outlined in Scheme 1.

Since the determination of lactulose in heated milks has been shown to be useful in distinguishing milks submitted to different thermal treatments, it is of interest to discover whether the heat-treatment conditions undergone by milk during processing can be determined from its galactose and epilactose content. In this paper we report a study of the formation of lactulose, epilactose and galactose with an interpretation by reaction kinetics.

MATERIALS AND METHODS

Heat treatments

Milk was heated by two different methods.

a-Portions

Milk (10 ml) was heated at 100, 105, 110, 120 or 125°C for 3, 10, 15, 20, 25 or 30 minutes in a silicone oil bath, in tightly stoppered pyrex glass tubes (16 × 162 mm).

b-Portions

Milk (1 ml) was heated at 140 or 150°C for 1, 2, 4, 8 or 10 minutes in a silicone oil bath in sealed glass-capillary thin-walled tubes (1 m × 1·3 mm i.d. × 1·8 mm o.d.).

In order to take into account the errors introduced by the heating and cooling periods, the effect of the shortest heating period was subtracted from the effect of longer heat-treatments.

Preparation of derivatives

Heated milk (0·5 ml) was mixed with 0·5 ml of 0·5% phenyl-β-D-glucoside in 60% methanol. The mixture was diluted to 5 ml with methanol, kept for 1 h at room temperature and filtered. One millilitre of the filtrate was evaporated under vacuum at room temperature and converted to trimethylsilyl (TMS) derivatives using trimethylsilylimidazole as reported (Martinez-Castro & Olano, 1980).

Gas chromatography

The gas chromatographic analyses were performed on a Sigma 3B gas chromatograph (Perkin Elmer) equipped with a 3 m × 1·0 mm i.d. stainless steel column (Chrompack) packed with 2% OV-17 on non-silanized 120/140 Volaspher A-2 (Merck). The temperature of the injector and detector was 300°C; the analysis was performed using temperature programming from 200°C to 270°C at a heating rate of 15°C/min with an initial holding at 200°C for 2 min.

RESULTS AND DISCUSSION

Lactulose

Previous studies have shown that the transformation of reducing sugars on basic media proceed according to first order reaction kinetics (Rendleman & Hodge, 1979). Thus, taking into account that, at the beginning of the reaction, the concentration of lactulose is zero, the rate at which this disaccharide appears may be expressed by eqn (1):

$$\ln \frac{[\mathrm{Gl\,G}]_0}{[\mathrm{Gl\,G}]_0 - [\mathrm{Gl\,F}]_t} = K_1 t \qquad (1)$$

K values were calculated for the formation of lactulose with eqn (1) in which $[\mathrm{Gl\,G}]_0$ is the lactose concentration at time zero and $[\mathrm{Gl\,F}]_t$ is the lactulose concentration at time t. The results are shown in Fig. 1; the rate constants, K, for lactulose formation were from $5{\cdot}56 \times 10^{-6}$ to $7{\cdot}17 \times 10^{-4}$/s in the range of 100–150°C studied.

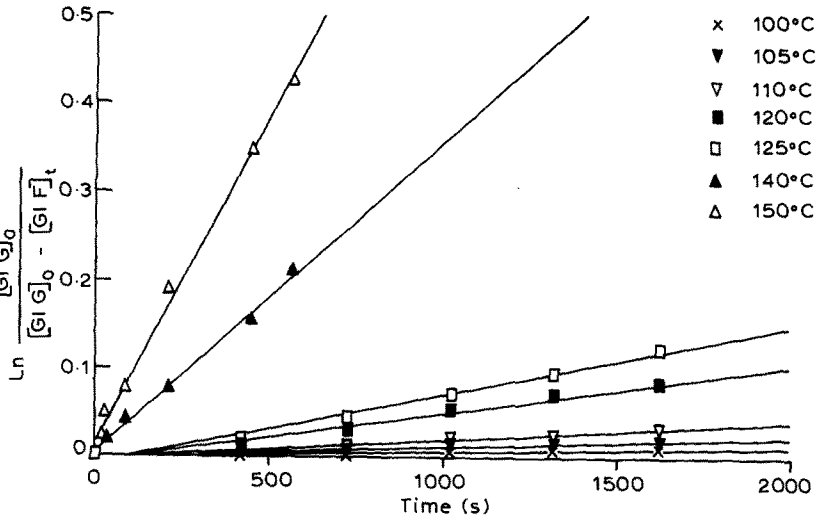

Fig. 1. Formation of lactulose in milk, plotted according to a first order reaction.

The energy of activation (E_a) was obtained by the Arrhenius equation

$$K = K_0 \exp(-E_a/RT) \tag{2}$$

by plotting $\ln K$ against the reciprocal of the absolute temperature (see Fig. 2). For the conversion of lactose to lactulose the E_a calculated was $125{\cdot}7 \pm 2{\cdot}35$ kJ/mol which compares reasonably well with the values 111–120 kJ/mol and 128 kJ/mol obtained by Nangpal and Reuter (1987) and by Andrews and Prasad (1987) respectively. Greig and Payne (1985) obtained a value of $E_a = 125$ kJ/mol for the conversion in model systems which suggest that the same mechanism is involved in both media.

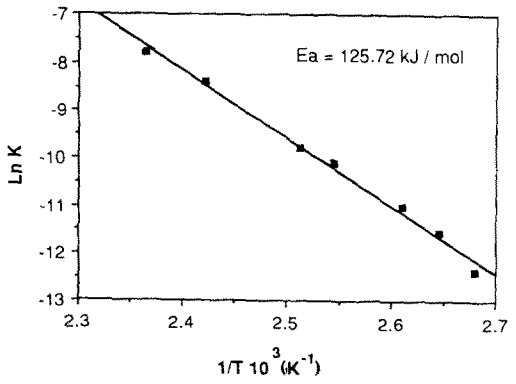

Fig. 2. Arrhenius plot for the formation of lactulose in milk.

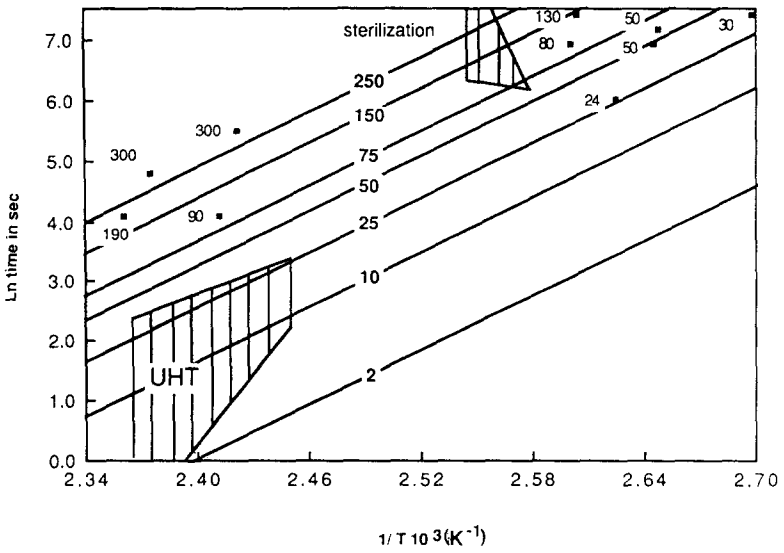

Fig. 3. Formation of lactulose in milk, calculated and measured (values at measuring points stated in mg/100 ml).

The temperature/time dependent formation of lactulose [Gl F] is given by the following equation:

$$[\text{Gl F}] = 1 - (1 + K_0 \exp(-E_a/\text{RT}))^{-1} \quad (3)$$

This equation was used to obtain the data shown in Fig. 3, which is a plot of the natural logarithm of time against the reciprocal of the absolute temperature and shows seven lines of equal lactulose formation. These data can be compared with experimentally obtained data shown as squares in the graph. It was therefore easily possible to predict the effect of the heat treatment conditions on the changes in lactulose concentration. In the sterilized region there were lactulose concentrations higher than 75 mg/100 ml and in the UHT region concentrations of 2–25 mg/100 ml. These values are slightly lower than those found in commercial milks, probably due to the formation of lactulose during the preheating in the processing plant before reaching the final processing temperature.

Epilactose

The K values in the epilactose formation were calculated supposing that the epilactose formation was a first order reaction. The rate constants, K, calculated are shown in Fig. 4; the rate constants, K found for epilactose formation were from $4{\cdot}96 \times 10^{-7}$ to $7{\cdot}30 \times 10^{-5}$/s in the range studied.

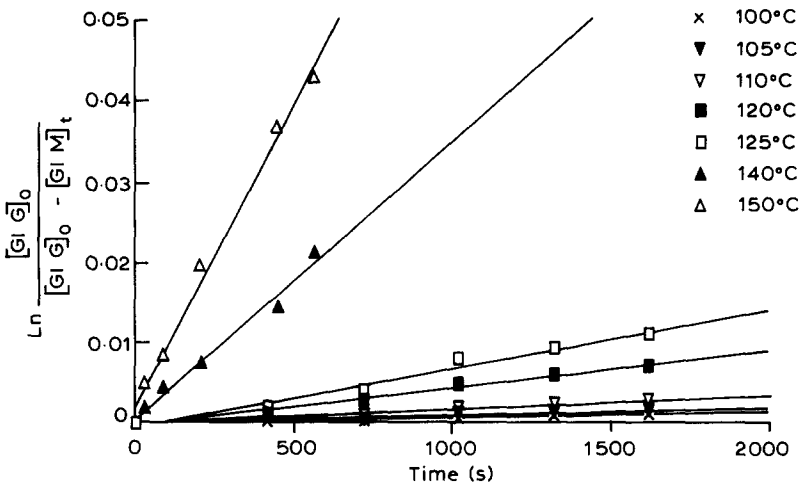

Fig. 4. Formation of epilactose in milk, plotted according to a first order reaction.

The Arrhenius plot for the epilactose formation is shown in Fig. 5; the E_a calculated was 131·3 ± 1·54 kJ/mol.

The temperature/time dependence for epilactose formation is shown in Fig. 6. The epilactose formation lines showed 1–35 mg/100 ml of epilactose. Epilactose concentrations higher than 5 mg/100 ml were found in the sterilized region, and in the UHT region there were 1–3 mg/100 ml of epilactose. These concentrations were similar to that found by Martinez-Castro and Olano (1980) and Olano *et al.* (1988) in milks submitted to UHT or sterilization processes.

Galactose

Considering that the rate of galactose formation is very much faster than that of epilactose, the concentration of galactose formed at time t is:

$$[\mathrm{Gl}]_t = [\mathrm{Gl\,G}]_0 - [\mathrm{Gl\,G}]_t - [\mathrm{Gl\,F}]_t \tag{4}$$

where [Gl], [Gl G] and [Gl F] respectively are the concentrations of galactose, lactose and lactulose.

By analogy with eqn (4) the reaction for the galactose formation may be described by eqn (5)

$$[\mathrm{Gl}]_t = [\mathrm{Gl\,G}]_0 - [\mathrm{Gl\,G}]_0 \exp(-(K_1 t)) - [\mathrm{Gl\,G}]_0 \frac{K_1}{K_2 - K_1} \exp(-(K_1 t)) - \exp(-(K_2 t)) \tag{5}$$

In order to simplify this equation, and taking into account that the

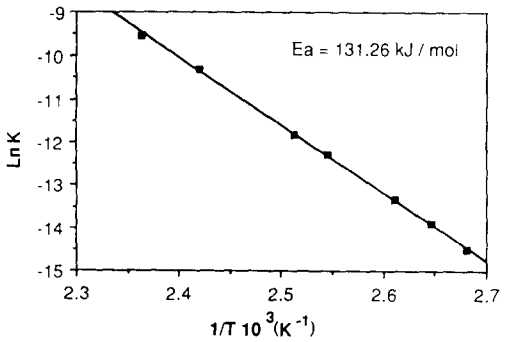

Fig. 5. Arrhenius plot for the formation of epilactose in milk.

formation of lactulose is much faster than that of galactose, it can be considered that $K_1 \gg K_2$ and eqn (5) becomes

$$[\mathrm{Gl}]_t = [\mathrm{Gl\,G}]_0(1 - \exp(-K_2 t)) \tag{6}$$

For this one gets

$$\frac{[\mathrm{Gl}]}{[\mathrm{Gl\,G}]_0} - 1 = -\exp(-K_2 t) \tag{7}$$

and K_2 was calculated from

$$K_2 t = \ln\left(\frac{[\mathrm{Gl\,G}]_0}{[\mathrm{Gl\,G}]_0 - [\mathrm{Gl}]_t}\right) \tag{8}$$

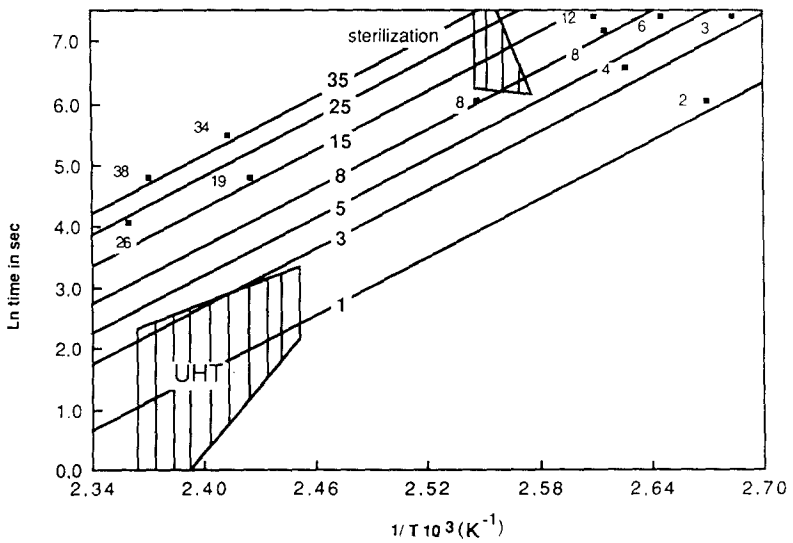

Fig. 6. Formation of epilactose in milk, calculated and measured (values at measuring points stated in mg/100 ml).

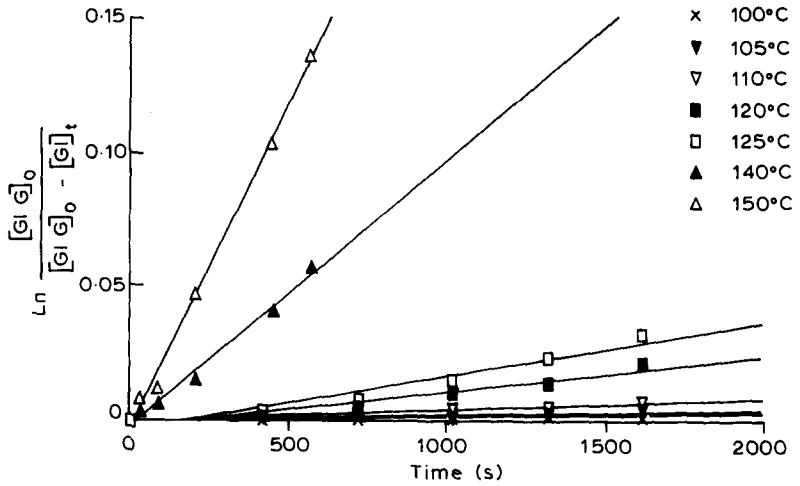

Fig. 7. Formation of galactose in milk, plotted according to a first order reaction.

The results are shown in Fig. 7. It can be seen that the amount of galactose formed increases with increasing temperatures and times in the range of 100–150°C.

The Arrhenius plot is shown in Fig. 8 and the E_a calculated was 139·4 ± 3·92 kJ/mol.

The lines of equal galactose formation in the range of concentrations found in milks submitted to different thermal treatments (Olano *et al.*, 1988) are shown in Fig. 9. Under sterilization conditions, concentrations of galactose were higher than 16 mg/100 ml, whereas for UHT treatments, concentrations of galactose were in the range of 9–12 mg/100 ml.

Even if the orders of reactions determined experimentally were not necessarily identical with the reaction molecularity actually occurring, these

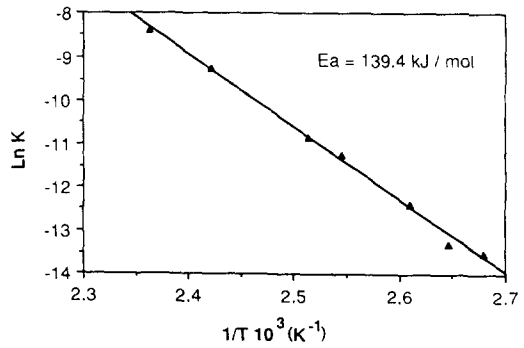

Fig. 8. Arrhenius plot for the formation of galactose in milk.

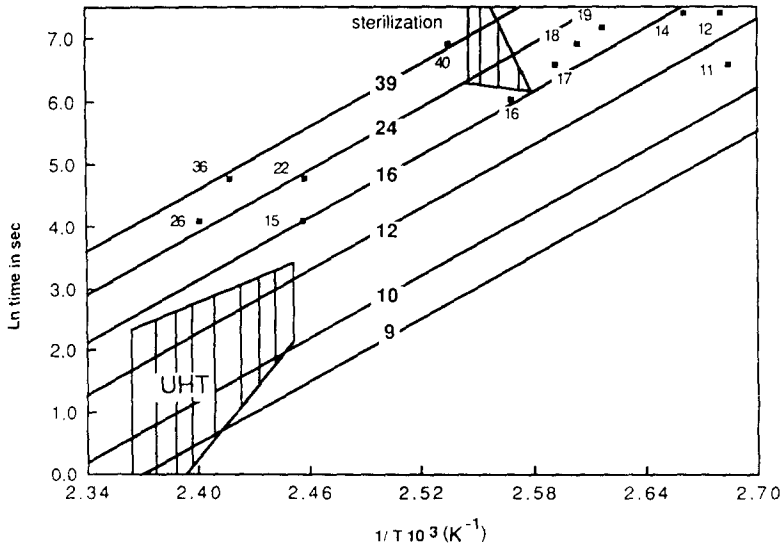

Fig. 9. Formation of galactose in milk, calculated and measured (values at measuring points stated in mg/100 ml).

determinations can be used to evaluate changes in carbohydrate composition. These results allow the conclusion that not only lactulose, but also galactose and epilactose formation, can be considered as heat damage indicators.

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