Formation kinetics of hydroxymethylfurfural, lactulose and furosine in milk heated under isothermal and non-isothermal conditions

By WENDIE L. CLAEYS, LINDA R. LUDIKHUYZE and MARC E. HENDRICKX*

Department of Food and Microbial Technology, Faculty of Agricultural and Applied Biological Sciences, Katholieke Universiteit Leuven, Kardinaal Mercierlaan, 92, B-3001 Heverlee, Belgium

(*Received 28 June 2000 and accepted for publication 31 January 2001*)

Summary. A detailed kinetic study of hydroxymethylfurfural, lactulose and furosine formation was performed upon heating milk at temperatures between 90 °C and 140 °C. In case of prolonged heating, formation kinetics could be described by a fractional conversion model. Considering only the first phase of the model, kinetics could be simplified to a pseudo-zero order model. A first assessment of kinetic parameters was made by isothermal experiments. Data were analysed using both a 2-step linear and a 1-step non-linear regression method. Only for furosine, did the global 1-step regression approach seem to give better results than the individual 2-step regression approach. Next, the estimated parameters k_{ref} and E_a were re-evaluated under non-isothermal conditions by subjecting milk to a time variable temperature profile. Given the complexity of Maillard reaction, it seemed better to estimate kinetic parameters under non-isothermal conditions when using a simplified model. Formation of hydroxymethylfurfural, lactulose and furosine was characterized by an E_a value of 90·2 kJ/mol ($k_{110\,°C}$ = 1·2 μmol/l, min), 99·1 kJ/mol ($k_{110\,°C}$ = 51·5 mg/l, min) and 88·7 kJ/mol ($k_{110\,°C}$ = 16·3 mg/100 g protein, min) respectively. Additionally, 90 % joint confidence regions were constructed in order to obtain an accurate representation of the statistical confidence associated with the simultaneously estimated parameters.

Keywords: Milk, hydroxymethylfurfural, lactulose, furosine, kinetics, isothermal, non-isothermal, joint confidence region.

The microbiological contamination of fresh milk necessitates a heat treatment in order to guarantee a safe and shelf-stable product. To induce inactivation of thermoresistant spores and of all pathogenic and spoilage bacteria in milk, thereby making long-term preservation without refrigeration possible, sterilization temperatures between 90 and 140 °C are necessary. The main sterilization processes that can be distinguished are UHT processing (minimum 135 °C/1 s) with direct or indirect heat transfer, and the more classical in-bottle sterilisation (110–120 °C/10–20 min).

One of the methods used to classify UHT and in-bottle sterilized milk is the turbidity test or Aschaffenburg test, which depends on serum protein denaturation (International Dairy Federation, 1972). However, this test is reported to be incapable

* For correspondence: marc.hendrickx@agr.kuleuven.ac.be

of distinguishing between the two types of milk, for one because some UHT processes now used cause the same degree of whey protein denaturation as conventional 2-stage sterilization (sterilization before and after packaging) (Burton, 1984; de Koning, 1984). Besides, it is a semi-quantitative method, which gives no additional information about the impact of the process, whereas a quantitative measurement of the impact is of great importance in process design, evaluation, optimization and control. One way to distinguish between different heat treatments and, at the same time, to quantify the impact of the process is by time–temperature integrators (Taoukis & Labuza, 1989; De Cordt *et al.* 1992; Van Loey, 1996). To identify these integrators, extensive kinetic studies of components formed, denatured or inactivated under defined temperature–time conditions have to be performed.

The main events occurring upon sterilization are protein denaturation, Maillard reaction and lactose isomerization. Protein denaturation and sugar modification are responsible for the ''cooked'' taste, while the Maillard reaction induces a decrease of the protein nutritional value by irreversible alteration of the lysine residue. Evaluation of the extent of the early Maillard reaction in milk products can be achieved by determination of furosine formed during the hydrolysis of the Amadori product ϵ-N-deoxylactulosyl-L-lysine, whilst hydroxymethylfurfural (HMF) is an established indicator for the advanced stage. Isomerization of lactose is followed by measuring the amount of lactulose (4-O-β-D-galactopyranosyl-D-glucopyranose) (Corzo *et al.* 1994; Meissner & Erbersdobler, 1996). Although HMF, lactulose and furosine are well-known heat indicators, applications deal mainly with heat-load evaluation (Clawin-Rädecker *et al.* 1992; Morales *et al.* 1996) or identification of limiting values of thermal damage (Erbersdobler *et al.* 1987; Resmini & Pellegrino, 1994), and only a few elaborated quantitative kinetic studies, which take different experimental approaches and statistical data-analysis into account, have been published.

In the context of time–temperature integrators for controlling heat processing of milk, the objective of this paper was to perform a detailed kinetic analysis of HMF, lactulose and furosine formation upon heating milk. Formation rate and temperature sensitivity were estimated by isothermal experiments according to two different statistical approaches, namely an individual 2-step and a global 1-step approach. Since integrators represent the integrated impact of temperature and time of a thermal process and industrial processes usually never occur at isothermal conditions, next, kinetic parameters were re-evaluated under non-isothermal or variable temperature conditions. Precision and correlation of the parameters were examined by constructing 90% joint confidence regions.

Materials and methods

Milk

A lot of fresh raw bovine milk (with approximate concentrations of 13·0 g dry matter/100 g, 35·5 g total protein/l and 43 g fat/l) was purchased from a local dairy farm. The milk was divided into small portions of 50 ml and stored under frozen conditions (−18 °C).

Thermal treatment

Heat treatments took place in the temperature range between 90 °C and 140 °C. Samples of milk were heated in test tubes closed with screw caps (pyrex, 16 × 160 mm) and immersed in a thermostated oil bath. In the case of isothermal experiments,

samples were treated at a constant temperature, while in the case of non-isothermal experiments they were subjected to a time variable temperature profile. An example of such a profile is given in Fig. 1. Similar profiles were used in each assay, for which time–temperature data were measured at regular time intervals (2 or 15 s) using thermocouples connected to a datalogger (Ellab, TM 9616). At different pre-set times, samples were taken from the oil bath and immediately cooled in ice water to stop further formation of the chemical compound studied.

Analytical methods

Total HMF content was quantified spectrophotometrically at 443 nm (Pharmacia LBK-Biochrom) using thiobarbituric acid (TBA) as substrate following the method described by Keeney & Bassette (1959). Concentration of lactulose was determined using a D-glucose/D-fructose test combination of Boehringer Mannheim (1995). Formation of furosine was measured by reversed phase (RP)-HPLC (Amersham Pharmacia Biotech; RP-furosine-dedicated column, 250 × 4·6 mm, Metal-Free, Alltech) essentially according to the method of Resmini *et al.* (1990), with the exception that 100 μl of hydrolysate was injected instead of 10 μl in order to increase reproducibility and accuracy.

Data analysis: kinetic parameter estimation

Generally, the rate of a chemical reaction can be described by:

$$v = \frac{\mathrm{d}C}{\mathrm{d}t} = kC^n, \tag{1}$$

with v the rate of the reaction, C the concentration of the chemical compound formed, t the treatment time, k the reaction rate constant at the temperature studied, and n the order of the reaction. When studying kinetics under isothermal conditions and thus assuming constant extrinsic/intrinsic factors, k can be considered constant in time, and integration of expression (1) gives:

(a) for a first order reaction ($n=1$): $$\ln\left(\frac{C}{C_0}\right) = kt, \tag{2}$$

(b) for an nth order reaction: $$C^{(1-n)} = C_0^{1-n} + (1-n)\,kt, \tag{3}$$

When concentration C increase is linear as a function of treatment time t, kinetics can be modelled by a zero-order reaction ($n = 0$):

$$C = C_0 + kt. \tag{4}$$

The effect of temperature can often be expressed by the Arrhenius relation (Arrhenius, 1889), in which the temperature dependence of the rate constant k is quantified by the activation energy E_a (J/mol) according to:

$$k = k_{ref} \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right), \tag{5}$$

where R is the universal gas constant (8·314 J/mol, K), T the temperature concerned, and k_{ref} the reaction rate constant at reference temperature T_{ref}.

When the plot of concentration v. time exhibits an exponential increase approaching a plateau or a maximal value, formation can be described by fractional conversion, a concept that is widely used in chemical engineering (Levenspiel, 1972; Hill, 1977). In order to determine reaction kinetics, the extent of the reaction or the

https://doi.org/10.1017/S0022029901004745 Published online by Cambridge University Press

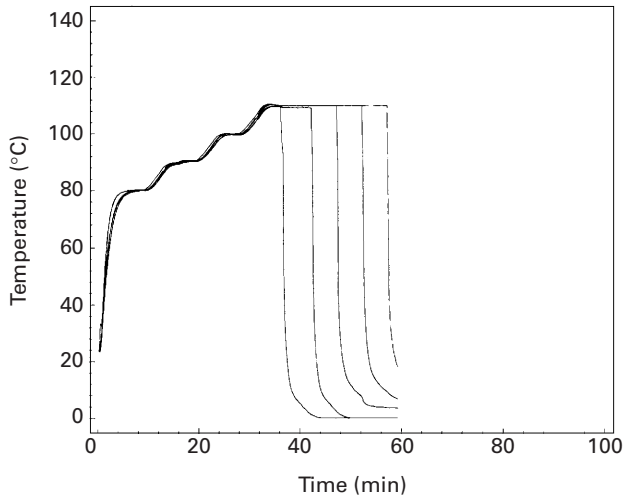

Fig. 1. Example of a time variable temperature profile applied during non-isothermal treatment of hydroxymethylfurfural, lactulose and furosine (resulting in five milk samples).

fraction that has been converted to product has to be known. The fractional conversion f of the reaction is defined as:

$$f = \frac{C_t - C_0}{C_\infty - C_0} = \frac{\text{what has been formed in a specified time } t}{\text{what can maximally be formed when reaction is completed'}}$$

with C_0 and C_∞ respectively the initial and maximal concentration. The plot of the logarithm of $(1-f)$ yields a straight line, resembling first order kinetics. Applying this on eqn (2) gives:

$$\ln(1-f) = kt = \left(\frac{C_\infty - C_t}{C_\infty - C_0} \right), \quad \text{or} \quad C = C_\infty - (C_\infty - C_0)\exp(kt). \tag{6}$$

Notice that when formation kinetics are studied under non-isothermal conditions, the integrated effect of temperature on k has to be taken into account, and e.g. eqn (4) becomes:

$$C = C_0 + \int_0^t \left[k_{ref} \exp\left(\frac{E_a}{R} \right) \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] \mathrm{d}t. \tag{7}$$

Statistical analysis

All regression procedures were performed in the statistical software package SAS (version 6.12). To verify the validity of the kinetic model and to measure linearity, regression coefficients (R^2) and asymptotic standard errors were calculated, and residual plots were checked for the absence of trends or correlations. If the model is appropriate for the data, residuals represent only the experimental error and the residual plot will have a random distribution of positive and negative residuals (Motulsky & Ransas, 1987; Straume & Johnson, 1992). Kinetic parameters were estimated from isothermal data using an individual linear regression approach or alternatively, using a global non-linear regression approach. In the case of non-isothermal experiments, kinetic parameters were calculated by non-linear regression using a numerical integration routine (e.g. Simpson) on the recorded time–temperature profile (Carnahan *et al.* 1969).

https://doi.org/10.1017/S0022029901004745 Published online by Cambridge University Press

For the evaluation of the precision and accuracy associated with the estimated parameters, besides 95% individual confidence intervals, 90% joint confidence regions were constructed, according to the expression (Draper & Smith, 1981):

$$\mathrm{SSQ} \leqslant \mathrm{SSQ}(\theta) \left\{ 1 + \frac{p}{n-p} F(p, n-p, 1-\varphi) \right\}, \tag{8}$$

where SSQ represents the error sum of squares at a specific parameter combination, SSQ(θ) the error sum of squares associated with the least squares estimate θ at optimal parameter values, p the number of parameters estimated simultaneously, n the number of observations and F the classical F-distribution with $(1-\varphi)$ the upper quantile ($\varphi = 0{\cdot}1$). This equation can be used in case of (i) independent observations and consequently $(n-p)$ degrees of freedom, and (ii) a linear fitting function.

RESULTS AND DISCUSSION

In order to facilitate kinetic analysis, HMF, lactulose and furosine formation was followed under isothermal conditions by heating milk at different constant temperatures between 90 °C and 140 °C. At each temperature studied, formation reached a plateau upon prolonged heating and could as such be described by a fractional conversion model according to eqn (6) (Fig. 2). However, taking only the first phase of the model into consideration, a linear relation between concentration and treatment time was observed (Fig. 3) and formation kinetics could be simplified to a pseudo-zero order model (eqn (4)). The fact that formation kinetics might be modelled using zero-order kinetics, implying that reaction rates are independent of concentration, is rather artificial (*cf.* the prefix 'pseudo') and a consequence of either experimental conditions, sampling procedure or the presence of competing or rate-limiting intermediate reactions (O'Brien, 1997).

Additional to the nature of the model, different regression methods can be distinguished. A common approach is to estimate kinetic parameters from experimental data using an individual 2-step approach (i.e. sequentially plotting C *v.* t to obtain k, and $\ln(k)$ *v.* $1/T$ to obtain E_a, according to eqn (4) and the logarithmic form of eqn (5)). Advantages of this procedure are that the validity of the model can easily be interpreted graphically and that calculation of the parameters follows directly from the regression. However, by performing successive linear least squares fits on the data, errors of the first regression can influence the exactness of the second regression, resulting in less accuracy and precision of the estimated parameters. Alternatively, a global 1-step approach can be used, in which the model parameters are determined in a single step performing non-linear regression analysis on response values (i.e. substituting k in eqn (4) with the Arrhenius equation). By this global fit the dataset is considered as a whole, which increases the number of degrees of freedom and should make confidence intervals for k_{ref} and E_a smaller (Haralampu *et al.* 1985; van Boekel, 1996). Kinetic parameters estimated on the basis of the pseudo-zero order model using the individual 2-step as well as the global 1-step regression method, together with their standard errors are summarized in Table 1.

From this table it can be seen that reaction rate constants k increased with increasing temperature, indicating a faster production of HMF, lactulose and furosine at higher temperatures. At 130–140 °C standard errors became rather high, which could be attributed to coagulation and caramelization of the milk at these temperatures, making it very difficult to pipette exactly the required amount of milk

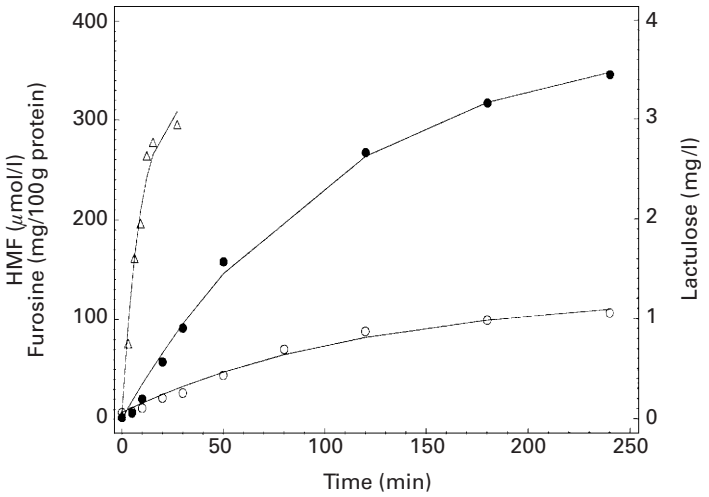

Fig. 2. Formation kinetics of hydroxymethylfurfural (HMF; ○), lactulose (●) and furosine (Δ) in raw milk heated at 110 °C according to the fractional conversion model.

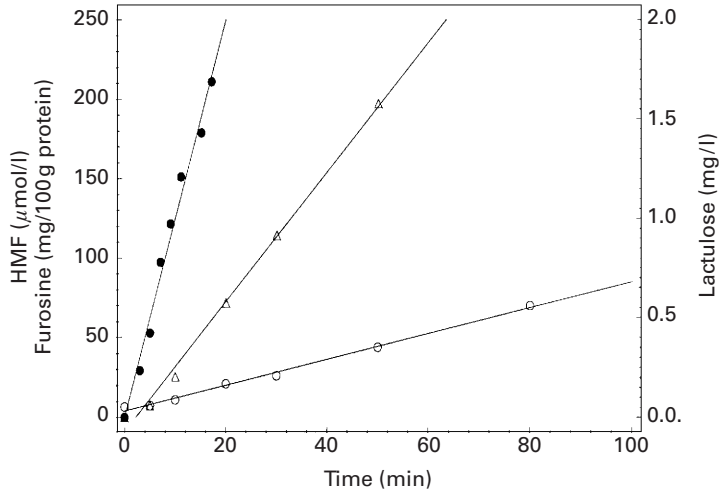

Fig. 3. Pseudo-zero order kinetics for hydroxymethylfurfural (HMF: ○), lactulose (●) and furosine (△) formation in raw milk heated at 110 °C.

for analysis from the heated test tubes. As depicted in Fig. 4, the Arrhenius equation could be used to fit temperature dependence of k. Corresponding activation energies, although in essence very close to each other, show a tendency of increasing from furosine over HMF to lactulose, indicating the distinction in temperature sensitivity with lactulose being the most sensitive to changes in temperature.

Comparing the individual 2-step and the global 1-step regression methods, based on 95% confidence level, both approaches seem to be comparable, except for furosine. Some authors indicate the global approach to be more efficient, primarily because in the individual analysis some unnecessary parameters are estimated (Haralampu *et al.* 1985), while others concluded that overall, none of the possible methods is convincingly superior to the other and that the performance of a specific regression approach depends on the dataset to which it is applied (De Cordt, 1994).

https://doi.org/10.1017/S0022029901004745 Published online by Cambridge University Press

Table 1. *Kinetic parameters describing hydroxymethylfurfural, lactulose and furosine formation during heat treatment of raw milk, calculated according to the pseudo-zero order model*

(Values ±SE for ten samples at each temperature in the case of isothermal experiments, for 30 samples divided between six temperature profiles in the case of non-isothermal experiments)

	Hydroxymethylfurfural			Lactulose			Furosine		
	Isothermal		Non-isothermal	Isothermal		Non-isothermal	Isothermal		Non-isothermal
T (°C)	k (μmol/l, min)	k_{ref} (μmol/l, min)	K_{ref} (μmol/l, min)	k (mg/l, min)	k_{ref} (mg/l, min)	k_{ref} (mg/l, min)	k (mg/100 g protein, min)	k_{ref} (mg/100 g protein, min)	k_{ref} (mg/100 g protein, min)
90	0·118±0·008			3·66±0·12			2·03±0·06		
100	0·273±0·009			11·17±0·25			5·69±0·31		
110	0·809±0·025	0·75±0·05	1·22±0·06	32·73±1·15	30·90±1·48	51·50±0·93	14·72±1·03	12·10±0·30	16·32±0·21
120	1·585±0·119			82·97±6·31			25·76±0·85		
130	4·363±0·653			199·85±13·94			43·44±2·51		
140	7·718±0·798			343·35±27·27			81·20±5·51		
E_a (kJ/mol)	106·7±3·2	105·6±3·2	90·2±5·6	114·2±3·0	105·6±2·3	99·1±3·6	89·8±5·1	83·5±1·4	88·7±1·5

k, reaction rate constant at temperature T (calculated by individual linear regression).
k_{ref}, reaction rate constant at reference temperature concerned (calculated by global non-linear regression).
E_a, activation energy.
For details, see Data Analysis section.

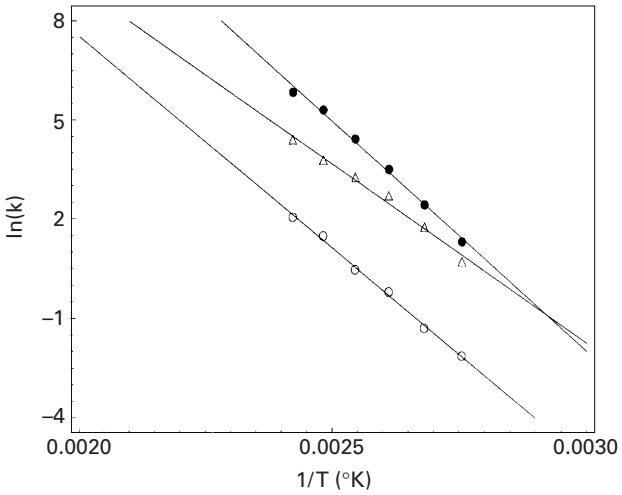

Fig. 4. Arrhenius plot for hydroxymethylfurfural (○), lactulose (●) and furosine (△) formation in raw milk.

Table 2. *Kinetic data on formation of hydroxymethylfurfural, lactulose and furosine in heated milk, as reported in the literature*

	Order	E_a (kJ/mol)	Treatment	Reference
Hydroxymethyl furfural	0	118·6	90–140 °C	Morales *et al.* 1995
	1	100·7	Shelf-life modelling (45–55 °C)	Patel *et al.* 1996
	0	135·1	75–103 °C	Peri *et al.* 1988
	0	104	khoa manufacturing	Sahai *et al.* 1992
Lactulose	0	102·2	100–120 °C/20–60 s	De Rafael *et al.* 1997
	0	74	Direct UHT milk processing	Geier, 1984
	0	118	Indirect UHT milk processing	Geier, 1984
	0	153·2	135–150 °C/10–40 s	Montilla *et al.* 1996
	0	114·4	120–150 °C	Nangpal *et al.* 1990
	1	125	100–125 °C/3–30 min	Olano & Calvo, 1989
	1	118·3	110–140 °C	Schlimme *et al.* 1996
Furosine	0	104·1	100–120 °C/20–60 s	De Rafael *et al.* 1997
	0	93·2	135–150 °C/10–40 s	Montilla *et al.* 1996
	0	100·2	120–150 °C	Nangpal *et al.* 1990
	0	86·2	110–140 °C	Schlimme *et al.* 1996

In spite of the great number of studies on HMF, lactulose and furosine formation, sometimes it is difficult to compare data because of: (i) different choice of analytical procedures, e.g. total HMF in milk is traditionally measured colorimetrically with TBA, but also HPLC techniques are available; (ii) effects of milk composition on concentration of compounds formed during heating. Some authors reported milk fat to protect against heat-induced changes through a decrease in heat transfer (Pellegrino, 1994; Morales & Jiménez-Pérez, 1999), but also contradictory results can be cited (Geier & Klostermeyer, 1983; de Koning *et al.* 1990); (iii) variations in experimental set-up (different temperature range, milk/reconstituted milk powder/model system, etc.) and (iv) variations in the applied kinetic model.

Taking this into account, our results for HMF, lactulose and furosine formation kinetics are generally of the same order of magnitude as those reported in literature (Table 2).

Because isothermal experiments represent an idealized situation and most

https://doi.org/10.1017/S0022029901004745 Published online by Cambridge University Press

industrial processes usually involve a heating and cooling phase, kinetics were re-evaluated under more realistic, variable temperature conditions. Kinetic parameters k_{ref} and E_a re-estimated on the basis of non-isothermal experiments and according to eqn (7) are presented in Table 1.

Based on 95% individual confidence intervals, significant differences were observed between reaction rate constants k_{ref} for isothermal and non-isothermal production of HMF, lactulose and furosine. The E_a-values on the other hand, were quite similar.

When the model is linear, individual confidence intervals are exactly defined and symmetric, and their determination is straightforward. A model is linear (in the parameters) if the first partial derivatives of the model with respect to the parameters are independent of the parameters as for the 2-step regression analysis of the isothermal data by eqn (4) and the logarithmic form of eqn (5). However, when applying the global 1-step non-linear regression on the isothermal data or when analysing the non-isothermal data by eqn (7), the model was of a non-linear nature. In that case, resulting individual confidence intervals are only approximate because they neglect the covariances of the simultaneously estimated parameters and assume normal distribution of the parameters. Alternative techniques taking into account the possible correlation between simultaneously estimated parameters, are the Monte Carlo technique and joint confidence regions (Bard, 1974; Motulsky & Ransas, 1987; Johnson, 1992). In the present study, 90% joint confidence regions were constructed in order to provide a realistic estimate of the confidence associated with the parameters calculated by non-linear regression (Fig. 5*a*, *b*, *c*). As the joint probability of two events at 95% probability approximates 90% (i.e. $(0{\cdot}95)^2 \approx 0{\cdot}90$), the limits of the 95% confidence intervals for the individual parameters coincide more or less with the extremes of the 90% joint contour plot (Haralampu *et al.* 1985; Van Loey, 1996). The rectangular region defined by the individual confidence intervals will approximate to the correct confidence region only if the correlation between k_{ref} and E_a is close to zero. No attempt should be made to interpret simultaneously the accuracy of the model parameters by constructing a rectangular joint confidence interval using the separate confidence intervals, because a parameter pair being within the separate 95% confidence intervals, can be situated far outside of the 90% joint confidence ellipse and consequently, will be very unlikely to occur. This is clearly discernible in Fig. 5*a*, *b* and *c*, which represent next to the 90% joint regions also the 95% individual confidence intervals. Individual confidence intervals are suitable only to describe the limits of one single parameter, despite the value of the other parameter. Comparison of the 90% contour plots for the kinetic parameters obtained from isothermal and non-isothermal data, confirmed the significant differences gathered from the individual 95% prediction intervals. Overlapping of the joint regions would indicate that based on a 90% significance level, parameters derived under static and variable temperature conditions do not differ from each other. The E_a values were more or less the same, but k_{ref} values differed. Consequently, it seems that the isothermally derived parameters are not simply applicable to describe HMF, lactulose and furosine formation under non-isothermal conditions. The joint regions for the isothermally derived parameters were smaller than those for the parameters determined from the non-isothermal experiments, possibly due to a larger dataset gathered in the isothermal experiments (for furosine the number of observations was more or less the same in both experiments).

In order to measure the reliability of the kinetic parameter estimates to predict HMF, lactulose or furosine concentration after heating milk, concentrations

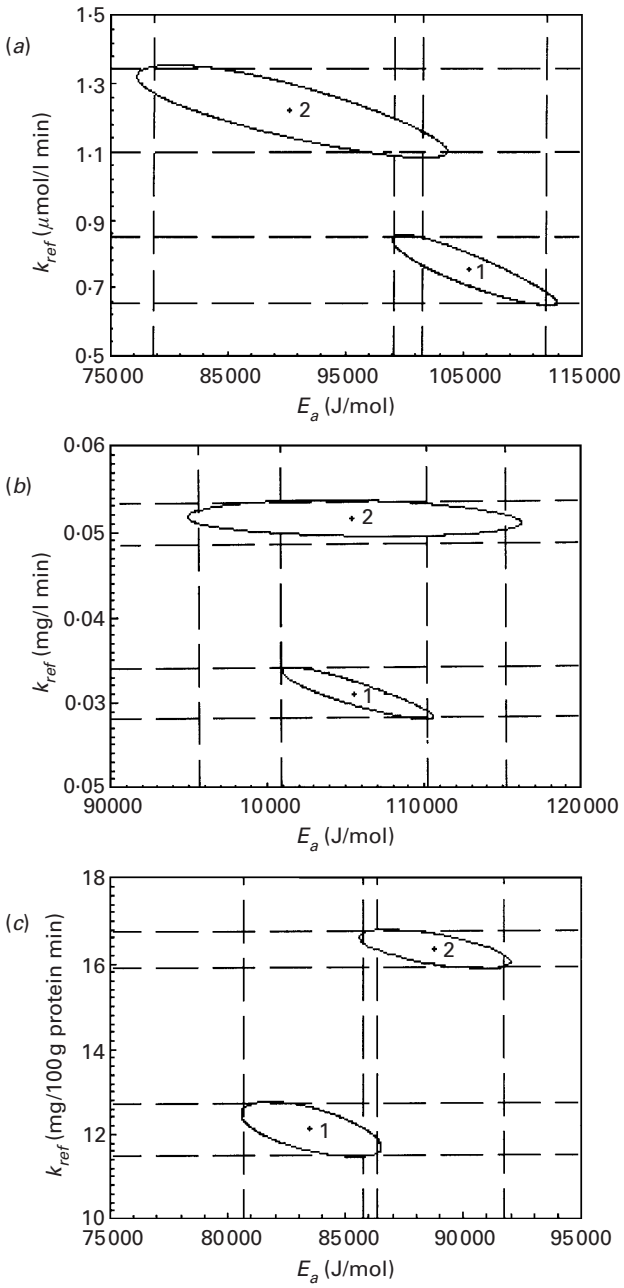

Fig. 5. 90 % joint confidence regions for simultaneously estimated kinetic parameters k_{ref} and E_a under isothermal (1) and non-isothermal (2) conditions at reference temperature $T_{ref} = 110$ °C according to the pseudo-zero order model for (*a*) hydroxymethylfurfural (correlation between k_{ref} and E_a: (1) 0·894; (2) 0·801), (*b*) lactulose (correlation between k_{ref} and E_a: (1) 0·899; (2) 0·224) and (*c*) furosine (correlation between k_{ref} and E_a: (1) 0·628; (2) 0·643). Broken lines represent individual 95 % confidence intervals.

predicted by integration of the recorded time–temperature profile using kinetic parameter estimates from both isothermal and non-isothermal data were compared with the experimentally observed ones (Fig. 6*a*, *b*, *c*). The correlation between the experimentally determined concentrations after non-isothermal processing and those

https://doi.org/10.1017/S0022029901004745 Published online by Cambridge University Press

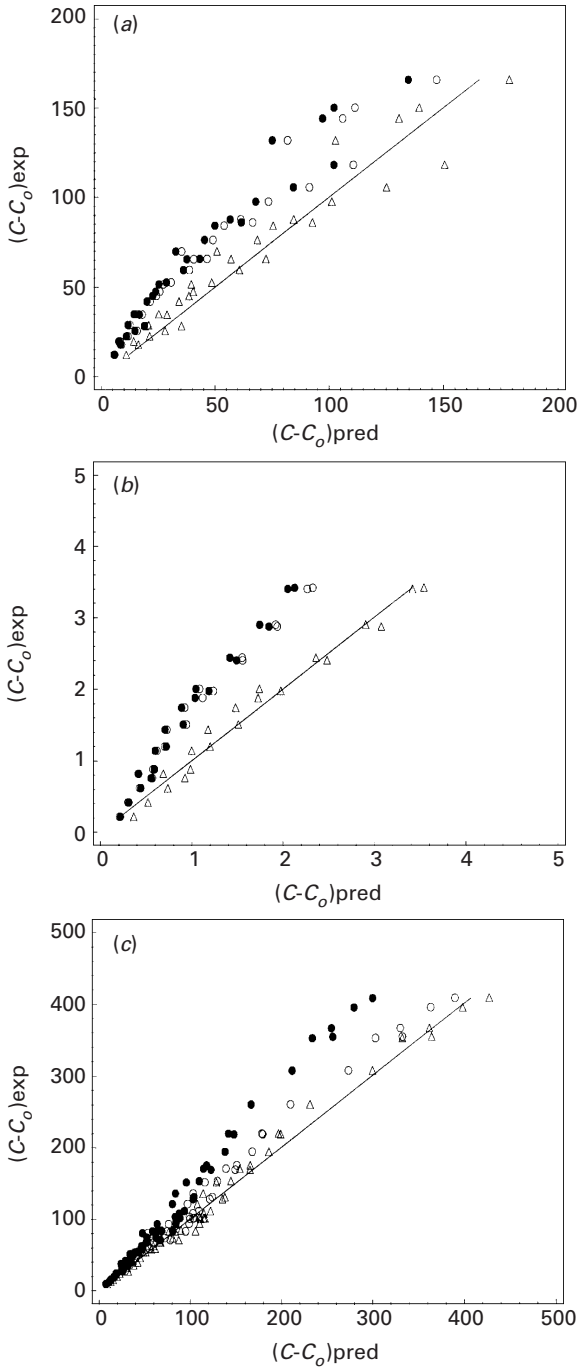

Fig. 6. Correlation between experimentally determined (exp) concentrations of (*a*) hydroxymethylfurfural, (*b*) lactulose and (*c*) furosine in milk formed after non-isothermal treatment and those calculated (pred) by means of kinetic parameter estimates from isothermal data using 2-step linear (●) and 1-step non-linear (○) regression and from non-isothermal data (△). C_o, initial concentration; C, final concentration.

https://doi.org/10.1017/S0022029901004745 Published online by Cambridge University Press

calculated by means of isothermal kinetic parameters was lower compared with the ones calculated with the non-isothermally derived kinetic parameters. However, for furosine the correlation between the experimental and predicted concentrations became somewhat higher when the isothermal kinetic parameters calculated by the 2-step linear regression were used instead of those obtained by the 1-step global non-linear regression. This could already be seen in Table 1 when for both regression methods the formation rate constant was compared with the non-isothermally derived k_{ref} value. Moreover, it seems that isothermal kinetic parameters underrated HMF, lactulose and furosine formation under variable temperature conditions because, when applying the isothermally derived model parameters on the non-isothermal data, predicted concentrations were consistently lower than experimentally determined ones.

Several potential explanations can be postulated for the observed discrepancy between kinetics estimated under isothermal and non-isothermal conditions. From an experimental point of view, small deviations in temperature and/or time registration can partly explain the difference in k values. With regard to the design, attention should be paid to the length of the temperature profile in the non-isothermal experiments. While the actual aim was to evaluate kinetic parameters of the pseudo-zero order model, it was observed that after a certain treatment time maximal concentration is reached and formation could as such be described by a fractional conversion model. So, when measuring concentrations in the curvature resulting in the plateau phase, non-isothermally derived reaction rate constants will be lower than expected. However, this could not account for the observed differences since the non-isothermally derived k_{ref} values were higher than the ones derived from isothermal experiments (Table 1).

Another possible explanation is connected with the complexity of the Maillard reaction, rendering modelling and interpretation of kinetics difficult. Published data describe lactose isomerization into lactulose as an irreversible zero-order or first-order reaction:

$$\text{lactose} \xrightarrow{k_0/k_1} \text{lactulose},$$

while in fact, as expected for an isomerization reaction, the reaction is reversible, and lactulose degrades further to galactose and other constituents:

$$\text{lactose} \xleftrightarrow{k_1/k_{-1}} \text{lactulose} \xrightarrow{k_2} \text{galactose} + \text{C5/C6-compounds}.$$

The mechanism is even more complicated because lactose not only isomerizes into lactulose, but also into very small amounts of epilactose, and both lactose and lactulose are involved in the Maillard reaction (Olano & Calvo, 1989; O'Brien, 1997; van Boekel, 1998). Likewise, the pathway of HMF formation is a complex one. The total HMF amount in milk can be formed by Maillard reaction as well as through the acid-catalysed degradation of lactose via 3-deoxyosulose (van Boekel, 1998; Morales & Jiménez-Pérez, 1999). Furthermore, during the course of the Maillard reaction, HMF formation is dependent upon the availability of lysine residues in both casein and serum proteins. This availability is related to the accessibility of such residues to lactose, which in turn is connected with intermolecular protein interactions (Morales *et al.* 1995).

Hence, chemical changes produced during heat treatment of milk are the result of many separate reactions, each with its own kinetics and a different dependence on reaction conditions. Compared with the isothermal experiments, the non-isothermal

experiments were each time conducted over a broader temperature range resulting in a different history of the sample. Consequently, different reaction equilibria might have dominated, which could explain the deviation of reaction rate constants under isothermal and variable temperature conditions. Besides, if the rate constant describes multi-step reactions with several rate-controlling steps, deviation from the Arrhenius model may be expected (van Boekel, 1996). Because our results were taken over a limited temperature range (Fig. 4), it is rather difficult to say whether the Arrhenius relationship is linear or slightly curved.

Despite the complexity, use of a simple reaction order for complex formation pathways is useful for describing chemical changes during processing or for modelling shelf-life, when knowledge of pure chemistry or mechanism of the reaction is of no importance. From our results, it seems that when using a simple reaction order, the best method to determine overall kinetic parameters appears to be the non-isothermal one.

In conclusion, HMF, lactulose and furosine formation could be described by a pseudo-zero order model, *i.e.* concentration increased linearly as a function of time. When calculating kinetic parameters k and E_a using both a 2-step linear and a 1-step non-linear regression approach, results were comparable and superiority of one of the two methods was not clear.

When isothermally estimated parameters were re-evaluated under time variable temperature conditions, significant differences were observed between reaction rate constants, while activation energies were more or less the same. Results were discussed based on individual confidence intervals as well as on joint confidence regions. As a measure of the reliability of the kinetic parameter estimates to predict HMF, lactulose and furosine formation in milk, concentrations predicted by parameters resulting from isothermal and the non-isothermal experiments were compared with experimentally observed concentrations. It was concluded that estimates from regression on the non-isothermal data were preferred to those from regression on the isothermal data, because the former bring about the best correlation between the predicted and the observed responses under variable temperature conditions, while the latter seem to underestimate consistently the actual formation.

This research has been supported by the Office of the Federal Office for Scientific, Technical and Cultural Affaires as a part of project NP/01/034.

REFERENCES

Arrhenius, S. 1889 [About the reaction rate of the inversion of non-refined sugar at souring.] *Zeitschrift für Physikalische Chemie* **4** 226–248

Bard, Y. 1974 *Nonlinear Parameter Estimation*. Academic Press, New York

Boehringer Mannheim (1995) Lactulose in milk. Enzymatic Bioanalysis Food Analysis. Cat. No 139 106

Burton, H. 1984 Reviews of the progress of dairy science: The bacteriological, chemical, biochemical and physical changes that occur in milk at temperatures of 100–150 °C. *Journal of Dairy Research* **51** 341–363

Carnahan, B., Luther, H. & Wilkes, J. 1969 Numerical integration. In: *Applied Numerical Methods*. John Wiley & Sons, New York, 69–140

Clawin-Rädecker, I., Kuhlmann B., Weiss G., Klostermeyer H. & Schlimme E. 1992 Lactulose content in commercial UHT-milks. *Kieler Milchwirtschaft Forschungsberichte* **44** 129–141

Corzo, N., Delgado, T., Troyano, E. & Olano, A. 1994 Ratio of lactulose to furosine as indicator of quality of commercial milks. *Journal of Food Protection* **57** 737–739

De Cordt, S., Vanhoof, K., Maesmans, J., Hendrickx, M. & Tobback, P. 1992 Thermostability of soluble and immobilized alpha-amylase from Bacillus licheniformis. *Biotechnology and Bioengineering* **40** 396–402

De Cordt, S. 1994 Feasibility of development of protein-based time-temperature-integrators for heat-process evaluation. Ph.D.Thesis, K. U. Leuven, Laboratory of Food Technology, Leuven, Belgium

De Koning, P. 1984 The definition of UHT milk: product-based, process-based, or both? *Voedingsmiddelentechnologie* **17** 25–27

De Koning, P., Badings, H., van der Pol, J., Kaper, J. & Vos-Klompmaker, E. 1990 Effect of heat treatment and fat content on UHT-milk. *Voedingsmiddelentechnologie* **23** 11–14

De Rafael, D., Villamiel, M. & Olano, A. 1997 Formation of lactulose and furosine during heat treatment of milk at temperatures of 100–120 °C. *Milchwissenschaft* **52** 76–78

Draper, N. & Smith, H. 1981 *Applied Regression Analysis*. John Wiley & Sons, New York

Erbersdobler, H., Dehn, B., Nangpal, A. & Reuter, H. 1987 Determination of furosine in heated milk as a measure of heat intensity during processing. *Journal of Dairy Research* **54** 147–151

Geier, H. & Klostermeyer, H. 1983 Formation of lactulose during heat treatment of milk. *Milchwissenschaft* **38** 475–477

Geier, H. 1984 [Study of the analytical control of the impact of a heat treatment on consumption milk.] Ph.D.Thesis Technische Universität, Munich

Haralampu, S., Saguy, I. & Karel, M. 1985 Estimation of Arrhenius model parameters using three least squares methods. *Journal of Food Processing and Preservation* **9** 129–143

Hill, X. 1977 *An Introduction to Chemical Engineering Kinetics and Reactor Design*. John Wiley & Sons, New York

International Dairy Federation 1972 Monograph on UHT milk. *Federation Internationale de Laiterie-International Dairy Federation*, Brussels, Annual Bulletin **5** 158–164

Johnson, M. 1992 Review: why, when and how biochemists should use least squares. *Analytical Biochemistry* **206** 215–225

Keeney, M. & Bassette, R. 1959 Detection of intermediate compounds in the early stages of browning reaction in milk products. *Journal of Dairy Science* **42** 945–960

Levenspiel, O. 1972 *Chemical Reaction Engineering*. 2nd edn. John Wiley & Sons, New York

Meissner, K. & Erbersdobler, F. 1996 Maillard reaction in microwave cooking: comparison of early Maillard products in conventionally and microwave-heated milk. *Journal of the Science of Food and Agriculture* **70** 307–310

Montilla, A., Calvo, M., Santa-Maria, G., Corzo, N. & Olano, A. 1996 Correlation between lactulose and furosine in UHT-heated milk. *Journal of Food Protection* **59** 1061–1064

Morales, F., Romero, C. & Jiménez-Pérez, S. 1995 New methodologies for kinetic study of 5-(hydroxymethyl)-furfural formation and reactive lysine blockage in heat-treated milk and model systems. *Journal of Food Protection* **58** 310–315

Morales, F.-J., Romero, C. & Jimenez-Perez, S. 1996 Evaluation of heat-induced changes in Spanish commercial milk: hydroxymethylfurfural and available lysine content. *International Journal of Food Science and Technology* **31** 411–418

Morales, F. & Jiménez-Pérez, S. 1999 HMF formation during heat-treatment of milk-type products as related to milkfat content. *Journal of Food Science* **64** 855–859

Motulsky, H. & Ransas, L. 1987 Fitting curves to data using nonlinear regression: a practical and nonmathematical review. *FASEB* **1** 365–374

Nangpal, A., Reuter, H., Dhen-Müller, B. & Erbersdobler, H. 1990 Formation of furosine during UHT treatment of milk – comparison between direct and indirect heating. *Kieler Milchwirtschaftliche Forschungsberichte* **42** 43–51

O'Brien, J. 1997 Reaction chemistry of lactose: non-enzymatic degradation pathways and their significance in dairy products. In: *Advanced Dairy Chemistry*, Volume 3: *Lactose, Water, Salts and Vitamins*. Ed. P. Fox, Chapman & Hall, London 155–231

Olano, A. & Calvo, M. 1989 Kinetics of lactulose galactose and epilactose formation during heat-treatment of milk. *Food Chemistry* **34** 239–248

Patel, A., Gandhi, H., Singh, S. & Patil, G. 1996 Shelf-life modeling of sweetened condensed milk based on kinetics of Maillard browning. *Journal of Food Processing and Preservation* **20** 431–451

Pellegrino, L. 1994 Influence of fat content on some heat-induced changes in milk and cream. *Netherlands Milk and Dairy Journal* **48** 71–80

Peri, C., Pagliarini, E. & Pierucci, S. 1988 A study on optimizing heat treatment of milk. I. Pasteurization. *Milchwissenschaft* **43** 636–639

Resmini, P. & Pellegrino, L 1994 HPLC of furosine for evaluating Maillard reaction damage in skimmilk powders during processing and storage. *International Dairy Federation Bulletin no.* 298 31–36

Resmini, P., Pellegrino, L. & Battelli, X. 1990 Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Italian Journal of Food Science* **3** 173–183

Sahai, D., Adhikari, A. & Mathur, O. 1992 Formation kinetics of total hydroxy methylfurfural during manufacture of khoa. *Lebensmittel Wissenschaft und Technologie* **25** 146–149

Schlimme, E., Clawin-Rädecker, I., Einhoff, K., Kiesner, C., Lorenzen, P., Martin, D., Meisel, H., Molkentin, J. & Precht, D. 1996 Studies on distinguishing features for evaluating heat treatment of milk. *Kieler Milchwirtschaftliche Forschungsberichte* **48** 5–36

Straume, M. & Johnson, M. 1992 Analysis of residuals: criteria for determining goodness-of-fit. *Methods in Enzymology* **210** 87–105

Taoukis, P. & Labuza, T. 1989 Applicability of time temperature integrators as shelf life monitors of food products. *Journal of Food Science* **54** 783–788

van Boekel M. 1996 Statistical aspects of kinetic modeling for food science problems. *Journal of Food Science* **61** 477–485, 489

van Boekel, M. 1998 Effect of heating on Maillard reactions in milk. *Food Chemistry* **62** 403–414

Van Loey A. 1996 Enzymic time temperature integrators for the quantification of thermal processes in terms of food safety. Ph.D.Thesis, K. U. Leuven, Laboratory of Food Technology, Leuven, Belgium