ELSEVIER

0308-8146(95)00245-6

Food Chemistry, Vol. 57, No. 3, pp. 423–428, 1996

0308-8146/96 $15.00 + 0.00

Fluorescence associated with Maillard reaction in milk and milk-resembling systems

Francisco J. Morales, Carmen Romero & Salvio Jiménez-Pérez*

Unidad de Productos Lácteos, Instituto del Frío (CSIC), Ciudad Universitaria s/n, E-28040, Madrid, Spain

(Received 4 April 1995; revised version received 8 August 1995; accepted 27 October 1995)

The progress of the Maillard reaction in milk and milk-resembling systems during heating was followed by monitoring free fluorescent intermediary compounds. Fluorescence intensity was examined over a wide temperature/time range (90–140°C/0.5–30 min). Different kinetic behaviours were found in the presence or absence of amino groups in the system. Traces of fluorescence were detected when the lactose system without proteins was heated; the isomerization and degradation reactions of lactose could also generate fluorescent compounds. Fluorescence accumulation in the lactose system (without proteins) proceeded according to first-order reaction kinetics, whereas systems with lactose and casein, lactose and whey protein and milk fitted zero-order reaction kinetics. Apparent activation energies calculated were 61.3, 78.6, 59.6 and 83.9 kJ mol^{-1} for lactose system, lactose/casein system, lactose/whey protein system and milk, respectively. Moreover, levels of intermediary fluorescent compounds in Spanish commercial milk (pasteurized, UHT-treated and in-bottle sterilized milk) have been studied. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Food components react with each other during processing, storage and cooking of food. Heat treatment is the most widely used processing operation in the dairy industry. The main reason for heat treatment of milk is to improve the keeping quality and safety of the product. In this way, one of the major chemical reactions taking place in milk and milk products is non-enzymatic browning, known as the Maillard reaction (MR). In an early step, the free amino groups of milk protein (mainly from κ-casein) react non-enzymatically with reducing sugars (lactose) to form a Schiff base, which is stabilized through Amadori rearrangement (lactylosyllysine). In the advanced step of the reaction, proteins are modified into coloured, fluorescent and cross-linked molecules. In the process, many reactive compounds are formed as intermediates, such as keto and aldoseamine compounds (Hodge, 1953), 3-deoxyosones (Troyano *et al.*, 1992; Madson & Feather, 1981), furans (Patton, 1950; Shaw & Berry, 1977), maltol (Potter & Patton, 1956), pyrrole derivatives (Klein *et al.*, 1992), pyrazines (Milic & Piletic, 1984), pyranones (Ledl *et al.*, 1983), lactones (Shigematsu *et al.*, 1971), substituted imidazoles (Davidek *et al.*, 1992), etc. The Maillard reaction plays an important role in the production of undesirable flavour compounds (Ferretti & Flanagan, 1972), decrease of nutritional quality (Baltes, 1982), as well as in the development of fluorescence and the brown colour associated with proteins (Choi *et al.*, 1949; Adrian, 1975).

Many chemical heat-induced indices based on products of the MR have been proposed for assessment of heat treatment in milk, milk products and model systems. Some of these parameters are related to an early stage of the MR evaluated by analysis of Amadori products using an amino acid analyzer (Henle *et al.*, 1991) and loss of essential amino acids as lysine (Fink & Kessler, 1988; Resmini *et al.*, 1990) and carboxymethyllysine analysed by reverse-phase high-performance liquid chromatography (RP-HPLC; Lüdemann & Erbersdobler, 1990). Heat-induced parameters related to the advanced MR are: total 5-hydroxymethylfurfural (HMF), analysed colorimetrically (Fink & Kessler, 1988) or by RP-HPLC (Morales *et al.*, 1992, 1995); the browning index (Pagliarini *et al.*, 1990); ε-lysylpyrrole, analysed by amino acid analyser (Henle & Klostermeyer, 1993); galactosylisomaltol analysed by HPLC (Kramholler *et al.*, 1992).

In stages prior to the formation of brown pigments, fluorescent compounds are formed. This has been studied in detail by Baisier & Labuza (1992) and Burton *et al.* (1963) in model systems of amines/sugars. Baisier & Labuza (1992) stated that fluorescence accumulation in a model system of glucose/lysine is due to interaction between reactive reducing compounds and amines by an irreversible reaction. The formation of major fluorescent

*To whom correspondence should be addressed.

compounds is linked to the presence of casein and occurs simultaneously with browning (Tarassuk & Simonson, 1950; Jenness & Coulter, 1948).

The aim of this paper is study the accumulation of fluorescent compounds in milk and milk-resembling systems over a wide range of temperature/time as well as determining the kinetics of formation of fluorescent intermediary compounds (FIC) not bound to proteins in each system. In addition, FIC indices have been tested as a non-specific heat-induced parameter for processing milk and a statistical study on the presence of fluorescent compounds in commercial Spanish milk has been carried out.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical grade. Whole raw milk was supplied by Clesa (Madrid, Spain). Synthetic milk ultrafiltrate solutions (SMUF) were made according to Jenness & Koops (1962) and in all cases pH was adjusted to 6.62 with KOH (1 N) before use. Three different systems were studied; lactose (5.0%, w/v), sodium caseinate (2.5%, w/v) and whey proteins (0.6%, w/v). Nine pasteurized, 36 UHT-treated and six in-bottle sterilized milks from representative commercial companies were purchased at local markets in Spain, and were stored in their containers at 6°C until analysis.

Heat treatments

Samples of lactose (L), lactose/casein (LC), lactose/whey protein (LW) in SMUF solutions and whole raw milk (M) were heated in stoppered test tubes (100 ×10 mm) in an oil-bath (Precisterm S-387; P-Selecta, Spain) under controlled conditions at 90–140°C for heating times of 0–1500 s (average estimated heating-up time 45 s). Samples were cooled in ice–water immediately after a predetermined heating time and were stored at −80°C until analysed.

Sample preparation

Five millilitres of well-mixed sample were slowly deproteinized with 5 ml of trichloroacetic acid solution (40%, w/v). The sample was kept for 30–50 min at ambient temperature and was then filtered through Whatman 42 paper filter.

Table 1. Average blank values of fluorescence intensity calculated for unheated systems

Sample	Mean ± SD	Range	*n*
L	1.5 ± 0.1	1.4–1.6	8
LC	9.4 ± 0.4	8.6–10.1	8
LW	6.3 ± 0.3	6.0–6.9	8
M	19.0 ± 1.1	17.5–21.3	8

L, lactose; LC, lactose/casein; LW, lactose/whey proteins; M, whole milk. SD, standard deviation.

Fluorescence spectroscopy

Intermediate-stage products were analysed by measuring fluorescent Maillard compounds in the deproteinized sample. Spectra were recorded on an Kontron Instruments (Milan, Italy) fluorescence spectrophotometer in 500 µl of sample diluted with 2.5 ml of phosphate buffered saliane (20 mM, pH 7.0, and 15 mM NaCl). Samples were measured at 347 nm excitation, 415 nm emission. A slit width of 5 nm was used. A quinine sulphate solution of 10 µg ml^{-1} in 0.1 N H_2SO_4 was prepared daily as a standard for calibration of the instrument at 100% relative fluorescence, but a concentration of 0.1 µg ml^{-1} was used with system L because of the lower fluorescence intensity (FI) of samples. All samples were analysed in triplicate.

Statistical analysis of data

Statistical analysis of data was performed by analysis of variance following a simple, balanced one-way model. The *t*-test was used to compare means and the level of significance was set at 95%.

RESULTS AND DISCUSSION

This study was designed using model lactose/protein systems to determine various kinetic parameters concerning the formation of fluorescent compounds and temperature-dependent reaction rates. The average blank values of fluorescence intensity (FI_b) calculated for L, LC, LW and M are summarized in Table 1. Blank values were subtracted in each analysis. Repeatability of the method for different heated-treated milk samples is shown in Table 2.

Average values of fluorescent compounds obtained on the studied systems (L, LC, LW and M) are shown in Fig. 1 for different temperatures and heating times. Accumulation of free fluorescent intermediary compounds (FIC) measured as FI (%) was proportional to both temperature and heating time in each model system and in milk. It should be taken into consideration that the value of free FIC (FI %) is not related to a single chemical compound: it is a non-specific measurement of fluorescence content in the sample.

Table 2. Repeatability study in samples of heated milk

Sample[a]	FI (%)	*n*	CV (%)
a	30.2 ± 1.2	10	4.00
b	50.6 ± 2.9	10	5.83
c	53.8 ± 2.4	10	4.35
d	55.3 ± 2.7	10	4.99

[a]Samples: direct-heated UHT commercial (a); whole untreated milk sample heated at 140°C/400 s (b), at 120°C/1000 s (c), at 130°C/600 s (d).
FI, fluorescence intensity with standard deviation; *n*, number of trials; CV, coefficient of variation.

Few literature data were available about fluorescent compounds in food or model systems. Most of the workers used a Maillard model system (reducing sugar and amino acid or protein) for studying fluorescence accumulation. Narayan & Andreotti (1989) did not find a linear formation of fluorescence in model systems of glucose/lysine and Matsuda *et al.* (1991) even detected a reduction of fluorescence in a system lactulose/β-lactoglobulin with prolonged storage. On the other hand, Cerrutti *et al.* (1985) found a linear increase of fluorescence with heating time in lysine/glucose systems with high water activity. The data are difficult to compare, however, because reactants and heating conditions were different.

We measured traces of fluorescence in system L at more severe heating conditions. As mentioned in 'Materials and methods', a calibration 100 times more accurate was required to measure free FIC in system L (Fig. 1(a)). These results suggest that isomerization and degradation reactions of lactose can contribute somewhat to the development of fluorescence. Different maximum peaks of emission and excitation were detected between system L and systems with proteins (LC, LW and M). Probably, the formation route of these fluorescent compounds was different, depending on the presence or absence of amino groups in the reaction medium. Our results agree with results of Cerrutti *et al.* (1985), who found trace levels of fluorescence intensity in a buffered model system with glucose without amino groups, but it is not possible to determine whether fluorescence occurred in the same order of magnitude.

To compare the development of fluorescence in the various systems, a kinetic study was carried out. We had to take into account that fluorescent compounds are intermediates in a very complex reaction and it is impossible to measure all the highly reactive compounds

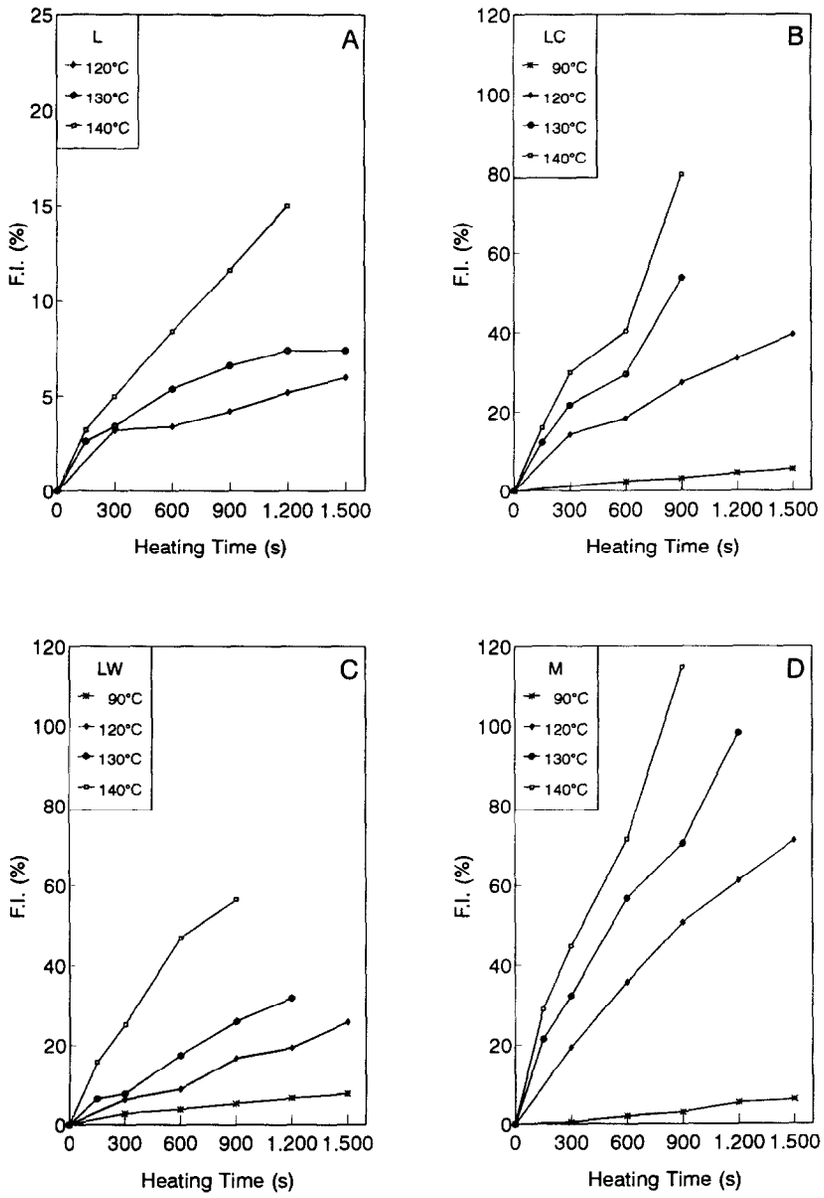

Fig. 1. Changes of relative fluorescence intensity (FI, %) during heating at 90°C, 120°C, 130°C and 140°C in synthetic milk ultrafiltrate systems: lactose system (L), lactose/casein system (LC), lactose/serum proteins system (LW), whole raw milk (M). Quinine sulphate solutions of 0.1 μg ml^{-1} for system L and 10 μg ml^{-1} for systems LC, LW and M were used for calibration.

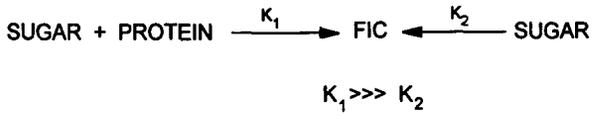

Fig. 2. Suggested network for the formation of free fluorescent intermediary compounds (FIC) in heated milk and milk-resembling systems.

formed in previous stages (early stage of the MR and Strecker degradation reactions) that are involved in its genesis. A suggested schematic pathway for the formation of fluorescent compounds is shown in Fig. 2. Of course, this is a simplification of the unknown route for FIC formation during the MR. Our data indicate that the reaction constant of the values of free FIC yielded from the MR (k_1) is higher than the reaction constant rate of free FIC formed from sugar degradation (k_2) as the only route. Moreover, the results of free FIC accumulation in system L were between 500 and 1000 times lower than for systems with protein.

One should be aware that chemical changes in milk are often the result of many separate reactions, each with its own energy of activation (E_a). All these reactions may depend in different ways on the reaction conditions. Consequently, an activation energy obtained from the temperature dependence of a reaction rate should be considered as an apparent, average one (Van Boekel & Walstra, 1995). Formation of free FIC could be described by zero-order kinetics in systems with proteins (LC, LW and M) whereas in system L first-order kinetics fitted better. Baisier & Labuza (1992) described zero-order kinetics for fluorescence in model systems with glucose (0.4 M) and glycine (0.05–0.4 M) in phosphate buffer (0.1 M, pH 7). Petriella *et al.* (1985) stated that the reaction order for fluorescent compounds formed in a glucose/lysine system was between zero-order and first-order, probably 0.6. The extent of formation of FIC is mainly related to the intensity of the thermal process, where temperature and heating time appear to be important parameters.

Table 3. Kinetic parameters for formation of intermediary fluorescent compounds

	L	LC	LW	M
K_T				
90°C	ND	0.0037	0.0049	0.0044
		(0.996)	(0.972)	(0.970)
120°C	0.0006	0.0252	0.0168	0.0473
	(0.948)	(0.974)	(0.986)	(0.983)
130°C	0.0009	0.0551	0.0266	0.0764
	(0.965)	(0.967)	(0.991)	(0.983)
140°C	0.0014	0.0817	0.0626	0.1201
	(0.962)	(0.959)	(0.966)	(0.986)
A_0	7.86×10^4	7.69×10^8	1.73×10^6	5.75×10^9
Q_{10}	1.57±0.08	1.86±0.12	1.60±0.08	1.94±0.13
Z	50.76±4.52	37.69±3.52	49.70±4.64	35.30±3.30
E_a	61.3±1.1[a]	78.6±3.4[b]	59.6±8.7[a]	83.9±5.5[b]
P	<0.05	<0.05	<0.05	<0.05
CI	±13.97	±14.62	±37.43	±23.66

Zero-order reaction for systems LC, LW and M; first-order reaction in system L. Reaction constant (K_T, FI s^{-1} for LC, LW and M; s^{-1} for L); r^2 curve value in parentheses ($P<0.001$). Arrhenius factor (A_0, FI s^{-1} for LC, LW and M; s^{-1} for L), Q_{10} value, Z value (°C) and apparent activation energy of process (E_a, kJ mol^{-1}) with standard deviation (different superscript letters indicate significant differences, $P\leq0.05$). P value, two-tailed; CI, 95% confidence interval.
L, lactose; LC, lactose/casein model system; LW, lactose/whey protein model system; M, milk. ND, not determined.

Table 3 shows values of reaction rates (K_T) calculated for each temperature as well as apparent activation energies (E_a) calculated in the range 90–140°C for the LC, LW and M systems and 120–140°C for the L system. Other frequently used kinetic parameters, such as Q_{10} and Z, are also reported. Temperature dependence of a reaction can be expressed as Q_{10}, which represents the factor by which the rate of a reaction is increased when the temperature is raised by 10°C; the Z value is defined as the temperature rise (°C) needed to increase the rate of a reaction by a factor of 10 (Walstra & Jenness, 1984). The Z value and Q_{10} were calculated according to eqns 1 and 2, respectively:

$$Z(C) = \frac{2.303RT^2}{E_a} \quad (1)$$

$$Q_{10} = 10^{\frac{10}{Z}} \quad (2)$$

The Q_{10} and Z values summarized in Table 3 are the average values calculated for the range of temperature studied since they are temperature-dependent. In this case, the M and LC systems are more sensitive to temperature change.

The Arrhenius plot of the logarithm of the rate constant as a function of the reciprocal of the absolute temperature (eqn 3) enabled the activation energies to be calculated (Fig. 3):

$$K_T = A_0 \exp^{(-E_a/RT)} \quad (3)$$

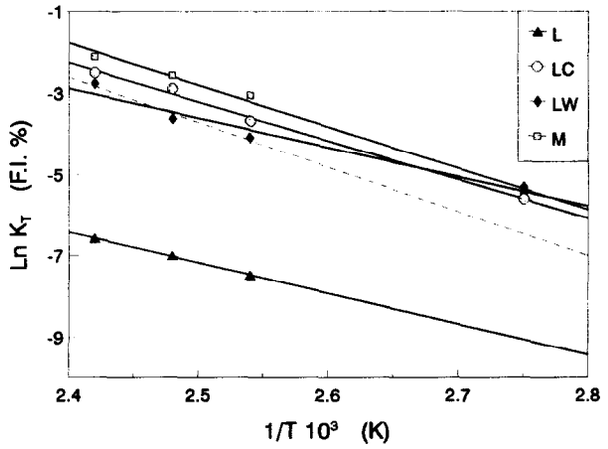

Fig. 3. Arrhenius plot for the formation of free intermediary fluorescent compounds in L, LC, LW systems and milk. Dotted line represents LW system for 120–140°C; see text for more information.

where K_T is reaction rate constant, A_0 is the pre-exponential Arrhenius factor, R is the ideal gas constant, T is the absolute temperature, E_a is the apparent activation energy of the process.

The rate of free FIC formation in the LC and LW systems and M at temperatures of 90–140°C follows an Arrhenius relationship with E_a of 78.6, 59.6 and 83.9 kJ mol^{-1}, respectively. Apparent E_a values calculated for LC and M were close, while LW system had a lower apparent E_a value. The behaviour of fluorescence accumulation in the LW system indicates that it could have two different stages, at around 90°C and 120°C, which means two different E_a. If we take into account the higher temperatures, $E_{a(120-140)}$ is 88.9 kJ mol^{-1}, which is close to the other E_a values in LC and M. Further experiments at temperatures lower than 90°C are currently being carried out to determine this behaviour with more accuracy.

No literature data are available with which to compare our results because most other studies have been carried out under storage conditions (Simonson & Tarassuk, 1952); moreover, the mechanism and therefore the rate of the MR depend on the kind of sugar and protein used and on the different ratios between them (Lerici *et al.*, 1990). Patton & Chism (1952) demonstrated that fluorescence formation showed an induction period followed by a period of increasing concentration until a maximum was reached, after which the concentration decreased, and Cerrutti *et al.* (1985) stated that the induction period for formation of coloured products is not identical to that for fluorescent compound, being longer for fluorescence. We have not observed any lag period in our systems. This observation may be due to the much higher temperature conditions used in our experiment.

In milk and milk products, browning is perhaps most significant in evaporated milk, although sweetened condensed milk, UHT milk and dried milk products are also subject to this defect. Methods have been developed to follow the browning reactions in different stages, but only few literature data can be found about accumulation of fluorescent intermediary compounds. Above, we indicated the possibility of using free FIC values as a heat-induced index to evaluate the heat damage in processed food as well as the well-known browning index at 420 nm (Palombo *et al.*, 1984).

The validity of the free FIC index to characterize heat-treated milk was tested in a group of commercial Spanish milk samples. In Fig. 4 a frequency histogram of levels of free FIC in UHT-treated Spanish milk is depicted. Data are expressed as percent relative fluorescence (FI%). The distribution more or less resembles a gaussian curve with a mean value of 28.4 ± 3.8 FI% (corrected for blank). In addition, free FIC levels were determined for pasteurized milk and in-bottle sterilized milk. The average values were 19.77 ± 1.69 ($n = 9$, 17.9–22.0) for pasteurized milk and 44.08 ± 4.65 ($n = 6$, 37.1–50.4) for in-bottle sterilized milk. It seems that the free FIC index can distinguish between different industrial heat treatments, but we should take into consideration

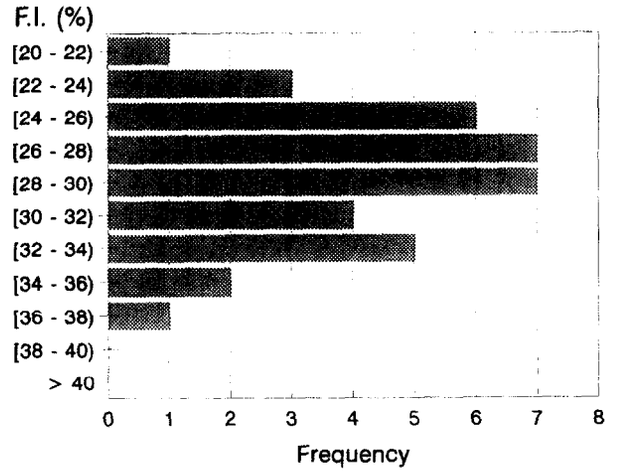

Fig. 4. Frequency histogram of levels of free intermediary fluorescent compounds in UHT-treated Spanish commercial milk.

that all the commercial milk samples were purchased less than 1 month after they were processed. In the same batch of samples, total HMF was also measured according to the RP-HPLC procedure of Morales *et al.* (1992). HMF is useful as an index for determining the severity of heat treatment in milk (Fink & Kessler, 1986) and in food in general. A significant correlation between HMF and free FIC was obtained for commercial UHT samples: free FIC (FI%) = 1.987 HMF (μmol $litre^{-1}$) + 18.201 ($r = 0.935$, n = 36, $P < 0.005$). Some experiments should be conducted to determine the stability of the free FIC index during the shelf life of the processed milk since it is known that the level of furosine, as an index of Amadori products, during storage of UHT milk is time- and temperature-dependent (Nangpal & Reuter, 1990).

In conclusion, accumulation of fluorescent compounds in milk and milk-resembling systems seems to have two different reaction routes. From a quantitative point of view, the most important route is the Maillard reaction, but fluorescent compounds are also formed in trace amounts by Lobry de Bruyn–Alberda van Ekenstein transformation. The results show that in M and the LC system, more FIC are formed than in the LW system during prolonged reaction times under these experimental conditions. In any case, fluorescence accumulation is clearly dependent on the time and temperature of the treatment in all the protein content systems. This observation could be a starting point to take into account the measure of free FIC as a heating index in heat-processed milk products. Obviously, this is an introductory study and more research should be done to see if free FIC indices are useful parameters to distinguish industrial processes in milk and milk products.

ACKNOWLEDGEMENTS

This work was supported by project ALI-93/0810, provided by the Comision Interministerial de Ciencia y

Tecnologia. The authors wish to thank Dr. Ir. M. A. J. S. van Boekel of the Department of Food Science (Wageningen Agricultural University, The Netherlands) for his comments on the kinetic aspects of this work.

REFERENCES

Adrian, J. (1975). Les traitements thermiques appliqus aux produits laitiers et leurs consquences dans le domaine azot. *Lait*, **1**, 24–40.

Baisier, W. M. & Labuza, T. P. (1992). Maillard browning kinetics in a liquid model system. *J. Agric. Food Chem.*, **40**(5), 707–713.

Baltes, W. (1982). Chemical changes in food by the Maillard reaction. *Food Chem.*, **9**, 59–73.

Burton, H. S., McSweeny, D. J. & Biltcliffe, D. O. (1963). Nonenzymic browning. Development of chromophores in the glucose–glycine and sucrose–glycine systems. *J. Food Sci.*, **28**, 631–639.

Cerrutti, P., Resnik, S. L., Seldes, A. & Fontan, C. F. (1985). Kinetics of deteriorative reactions in model food systems of high water activity: glucose loss, 5-hydroxymethylfurfural accumulation and fluorescence development due to nonenzymatic browning. *J. Food Sci.*, **50**, 627–630.

Choi, R. P., Koncus, A. F., O'Malley, C. M. & Fairbanks, B. W. (1949). A proposed method for the determination of color of dry products of milk. *J. Dairy Sci.*, **32**, 580–586.

Davidek, T., Velisek, J., Davidek, J. & Pech, P. (1992) Amino acids derived 13-disubstituted imidazoles in nonenzymatic browning reactions. *Sbornik.*, **54**, 165–183.

Ferretti, A. & Flanagan, P. (1972). Steam volatile constituents of stale nonfat dry milk. The role of the Maillard reaction in staling. *J. Agric. Food Chem.*, **20**(3), 695–697.

Fink, R. & Kessler, H. G. (1986). HMF values in heat treated and stored milk. *Milchwissenschaft*, **41**, 638–641.

Fink, R. & Kessler, H. G. (1988). Comparison of method for distinguishing UHT treated and sterilization of milk. *Milchwissenschaft*, **43**(5), 275–280.

Henle, T. & Klostermeyer, H. (1993). Determination of protein-bound 2-amino-6-(2-formyl-pyrrolyl)-hexanoic acid ('pyrraline') by ion exchange chromatography and photodiode array detection. *Z. Lebensm. Unters. Forsch.*, **196**, 1–4.

Henle, T., Walter, H. & Klostermeyer, H. (1991). Evaluation of the extent of the early Maillard reaction in milk products by direct measurement of the Amadori-product lactuloselysine. *Z. Lebensm. Unters. Forsch.*, **193**, 119–122.

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *J. Agric. Food Chem.*, **1**, 928–943.

Jenness, R. & Coulter, S. T. (1948). Measurement of fluorescent materials in milk and milk products. *J. Dairy Sci.*, **31**, 367–381.

Jenness, R. & Koops, J. (1962). Preparation and properties of salt solution which simulates milk ultrafiltrate. *Neth. Milk Dairy J.*, **16**, 154–164.

Klein, E., Ledl, F., Bergmueller, W. & Severin, T. (1992). Reactivity of Maillard products with a pyrrole structure. *Z. Lebensm. Unters. Forsch.*, **194**(6), 556–560.

Kramholler, B., Ledl, F., Lerche, H. & Severin, T. (1992). HPLC separation of some characteristic components in reaction mixtures of disaccharides with amines—model systems for milk and cereal products. *Z. Lebensm. Unters. Forsch.*, **194**, 431–433.

Ledl, F., Hiebl, J. & Severin, T. (1983). Formation of coloured β-pyranones from hexoses and pentoses. *Z. Lebensm. Unters. Forsch.*, **177**, 353–355.

Lerici, C. R., Barbanti, D. & Cherubin, S. (1990). Early indicators of chemical changes in foods due to enzymatic or nonenzymatic browning reactions. *Lebensm. Wiss. Technol.*, **23**, 289–294.

Lüdemann, G. & Erbersdobler, H. F. (1990) Model experiments on the formation of *N*-carboxymethyllysine (CML) in foods. In *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*. Birkhäuser Verlag, Basel, Switzerland.

Madson, M. & Feather, M. S. (1981). An improved preparation of 3-deoxy-D-erythro-hesus-2-ulose via the bis(benzohydrazone) and some related constitutional studies. *Carbohydr. Res.*, **94**, 183–191.

Matsuda, T., Kato, Y. & Nakamura, R. (1991). Lysine loss and polymerization of bovine β-lactoglobulin by amino carbonyl reaction with lactulose (4-*O*-beta-galactopyranosyl-D-fructose). *J. Agric. Food Chem.*, **39**(7), 1201–1204.

Milic, B. L. & Piletic, M. V. (1984). The mechanism of pyrrole pyrazine and pyridine formation in non-enzymic browning reaction. *Food Chem.*, **13**(3), 165–180.

Morales, F. J., Romero, C. & Jiménez-Pérez, S. (1992). An enhanced liquid chromatographic method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia*, **33**(1-2), 45–48.

Morales, F. J., 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. *J. Food Protect.*, **58**(3), 310–315.

Nangpal, A. & Reuter, H. (1990). Reference diagram for furosine content in UHT milk. *Kiel. Milchwirtsch. Forschungsber.*, **42**, 77–86.

Narayan, K. A. & Andreotti, R. E. (1989). Kinetics of lysine loss in compressed model systems due to maillard reaction. *J. Food Biochem.*, **13**, 105–125.

Pagliarini, E., Peri, C. & Abba, S. (1990). Kinetics study on colour changes in milk due to heat. *J. Food Sci.*, **55**, 1766–1767.

Palombo, R., Gertler, A. & Saugy, I. (1984). A simplified method for determination of browning in dairy powders. *J. Food Sci.*, **49**, 1609–1613.

Patton, S. (1950) Studies of heated milk: I. Formation of 5-HMF. *J. Dairy Sci.*, **33**, 324–328.

Patton, A. P. & Chism, P. (1952). Paper chromatography of browning reactions of fluorogens. *Nature*, **167**, 406.

Potter, P. E. & Patton, S. (1956) Evidence of maltol and HMF in evaporated milk as shown by paper chromatography. *J. Dairy Sci.*, **39**, 978–982.

Petriella, C., Resnik, S. L., Lozano, R. D. & Chirife, J. (1985). Kinetics of deteriorative reactions in model systems food systems of high water activity: 1. Color changes due to nonenzymatic browning. *J. Food Sci.*, **50**, 622–626.

Resmini, P., Pellegrino, L. & Battelli, G. (1990). Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci.*, **3**, 173–183.

Shaw, P. E. & Berry, R. E. (1977). Hexose–amino acid degradation involving formation of pyrroles, furans and other low molecular weight products. *J. Agric. Food Chem.*, **25**(3), 641–644.

Shigematsu, H., Kurata, T., Kato, H. & Fujimaki, M. (1971). Formation of 2-(5-hydroxymethyl-2-formylpyrrol-1-yl)alkyl acid lactones on roasting alkyl-α-amino acid with D-glucose. *Agric. Biol. Chem.*, **35**, 2097–2105.

Tarassuk, N. P. & Simonson, H. D. (1950). The browning and fluorescence of evaporated milk. *Food Technol.*, **4**, 88–92.

Troyano, E., Olano, A., Jimeno, M. L., Sanz, J. & Martinez-Castro, I. (1992). Isolation and characterization of 3-deoxypentulose and its determination in heated milk. *J. Dairy Res.*, **59**, 507–515.

Van Boekel, M. A. J. S. & Walstra, P. (1995). Use of kinetics in studying heat-induced changes in foods. In *Heat-induced Changes in Milk*, 2nd edn. I.D.F., pp. 22–50.

Walstra, P. & Jenness, R. (1984). Heating. In *Dairy Chemistry and Physics*. Wiley-Interscience, New York.