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# Predicting the extent of Maillard reactions in infant formula during sterilization by ohmic heating

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## ABSTRACT

This study aimed at monitoring the biochemical reactions induced by an intense thermal treatment like sterilization of a liquid infant formula. The stake is the control of the nutritional quality of the product concerning undesirable newly formed compounds, most of them being Maillard reaction products. A fluorimetric method called FAST (Fluorescence of Advanced Maillard products and Soluble Tryptophan) was used for monitoring the accumulation of Maillard products along thermal treatment. UHT sterilization was achieved by ohmic heating in a lab scale reactor specially designed for this study. The reproducibility of the thermal treatment was characterized for the three phases of the sterilization: heating, holding and cooling. The extent of Maillard reaction was monitored under two types of conditions: (i) isothermal conditions at five holding temperatures (100, 110, 120, 130 and 140°C) and (ii) nonisothermal conditions at five heating rates (0.16 to 4.19 °C.s<sup>-1</sup>). A semi-empirical model was developed to predict product temperature history during a complete sterilization treatment with good adjustment. The extent of Maillard reaction was fitted with a kinetic model of pseudo-zero order and Arrhenius parameters were identified to model the temperature effect on the chemical reaction. The physical and the chemical models were coupled to predict the extent of Maillard reaction during ohmic treatment as a function of operating parameters (temperature and electrical conditions) and product parameters (electrical conductivity...); it was successfully adjusted to the experimental results.

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*Keywords: Nonenzymatic browning; kinetics; model liquid food product; thermal treatment*

## INTRODUCTION

Sterilization of liquid infant formulas is obtained by means of severe heat treatments which affect the nutritional quality of the product either by degrading vitamins and part of the essential amino acids like lysine or by inducing the formation of undesirable biochemical compounds [1, 2]. Such degradations are mainly attributed to Maillard reaction leading to non-enzymatic browning within molecular reaction mechanisms still badly known. Nevertheless, these reactions are described better and better with the help of multiresponse modelling [3], an approach accounting for multiple reactions either successive or competitive which are starting from precursors originally present in the product and are strongly activated by temperature [4]. Another level of complexity comes from the dependence of Maillard reaction towards food environment and structure (pH, minerals, nature and concentration of substrate, water activity, amorphous or crystallized state etc.). Consequently, these types of reactions are most often studied on simple model solutions at low temperatures under isothermal conditions and there is a lack of reliable kinetic data for Maillard reaction in complex food matrix thermally treated under real process conditions, for instance HTST. However, some authors studied non-enzymatic browning in food matrixes like apple juice concentrate [5] or honey [6] heated under isothermal and dynamic conditions and proposed predictive browning models for fluctuating temperature-time protocols. Others introduced the concept of time-temperature integrator (TTI) based on the choice of compounds suitable for the assessment of a heating process of milk [7].

One of the main difficulties in studying the different pathways in the Maillard reaction lies on the multiplicity of compounds that need to be identified and quantified as markers of reaction pathways. On the contrary,

spectrometric methods can be of precious help to measure globally the rate of some reaction steps. Indeed, many advanced Maillard compounds have the property to be fluorescent and their spectral response at specified absorption/emission wavelength is proportional to their concentration. Such response is not specific but appears to be a reliable tool to monitor the extent of advanced Maillard reaction in different food matrixes [8, 9, 10]. Among them, the FAST method (Fluorescence of Advanced Maillard Products and Soluble Tryptophan), based on simple fluorescence measurements on the transparent milk supernatant obtained at pH 4.6, is well adapted for milk products. Once precisely calibrated with pertinent nutritional indicators, the FAST method appears to be of great interest for optimizing a process on the criterion of nutritional quality [11, 12].

In this study, an ohmic reactor was conceived to mimic HTST temperature conditions with characterized performances [13]. The objective was to study Maillard reaction during the sterilization by UHT treatment of a model liquid infant formula. The protein denaturation and the formation of Maillard products were monitored by means of a global indicator of fluorescence provided by the FAST index. It was thus a question of modelling the evolution of the FAST index during the heat treatment by coupling a kinetic model with a model of thermal transfer.

## MATERIALS & METHODS

*Liquid infant formula (IF)*: it was specially designed for the project and produced by an industrial partner. Its general composition in g.L<sup>-1</sup> is as follows: water 890, sugars 84, proteins 14, lipids 36 and minerals 4.6 [14]. Two batches of product were used for two serials of experiments A and B which are further described in the experimental design section.

*FAST method* [15]: 1 mL of sample was completed to 50 mL with sodium acetate buffer (0.1 M, pH 4.6) to precipitate the insoluble proteins. 4 mL of supernatant was then filtered through a 0.45 µm nylon filter (VWR, France) and placed in a disposable 4 faces acryl cuve (Sarstedt, France). The fluorescence was measured with a spectrofluorimeter (CaryEclipse Varian, France) at excitation/emission wavelengths of 290/340 nm for tryptophan ( $F_{trp}$ ) and 330/420 nm for advanced Maillard products ( $F_{amp}$ ). The FAST index is given by the ratio  $(F_{trp}/F_{amp}) * 100$ . Maximum variation coefficients of 3.5% were obtained for  $F_{trp}$ ,  $F_{amp}$  and FAST index. Tryptophan fluorescence ( $F_{trp}$ ) enables to calculate the soluble protein content. It was calibrated with soluble whey proteins (Prolacta 90 from Lactalis, Laval, France) solutions from 0.2 to 20 g.L<sup>-1</sup> [14].

*Ohmic reactor*: a lab scale reactor was specially designed for the project, to make reproducible sterilization treatments with fully characterized thermal history [13]. It is made of a 100 mL cylindrical treatment cell (internal diameter: 3.55 cm; length: 10.1 cm), closed hermetically at both extremities by two plane DSA (Dimension Stable Anodes) electrodes (Figure 1). The reactor is equipped with a sampling device composed of five independent manual valves connected to five sampling cylinders of 15 mL capacity each. Voltage (0-320 V; ± 0.25%) and current (0-20 A; ± 0.5%) are delivered by a 5 kW power source. Temperature is monitored by means of K-type thermocouples (NiCr/NiAl; diameter 1.5 mm, precision ± 1.5 °C) placed in both the cell and first sampling cylinder and it is regulated by monitoring equipment. Temperature, voltage and current are recorded at 0.2 s frequency. Nitrogen is used to maintain pressure in the treatment cell in order to reach temperature levels above 100°C (maximum 6 bars, 180°C). Reproducibility of a thermal treatment was estimated on the basis of thermal history and extent of Maillard reaction: 2.3% relative variation was obtained for the heating phase, 1% for holding and 20% for cooling; an average standard deviation of 3.8% was obtained for FAST index during a thermal treatment.

*Experimental design*: sterilization of the infant formula consisted in a three phase's thermal treatment, heating, holding and cooling; samples were extracted from the reactor for analysis, at different treatment times of the heating and holding phases. Two serials of experiments were conducted (Table 1), with the objective of acquiring kinetic parameters for two distinct situations: i) by sampling under isothermal conditions, i.e. during the holding phase of the thermal treatment (serial A) at five different holding temperatures (100, 110, 120, 130 and 140°C) and ii) by sampling under nonisothermal conditions, i.e. during the heating phase (serial B) at five different heating rates (0.16, 0.38, 0.86, 1.19 and 4.19 °C.s<sup>-1</sup>). All thermal treatments were done in triplicate whereas the sampling times were not repeated and were rather distributed so as to cover the whole time range.

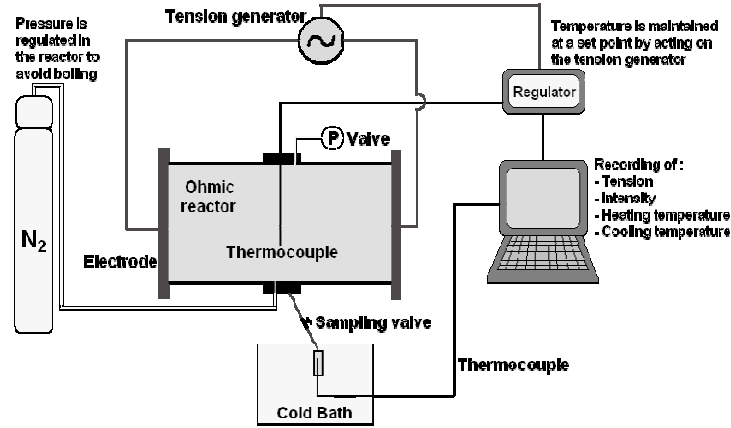


Figure 1. Diagram of the instrumented ohmic reactor [13].

Table 1. Experimental design

Serial	Heating phase			Holding phase	
	Heating rate* (°C.s <sup>-1</sup> )	Duration (min)	Sampling (°C)	Temperature (°C)	Sampling (min)
A	1.41	1.15	/	100	0.5 - 1
		1.22		110	0.25 - 2
		1.29		120	0.25 - 0.5
		1.36		130	0.17 - 0.25
		1.42		140	0.17 - 0.25
B	0.16	12.5	10	140	0.5
	0.38	5.26			
	0.86	2.33			
	1.19	1.68			
	4.19	0.48			

\* Mean heating rate calculated on the 20-140 °C domain

## RESULTS & DISCUSSION

*Predicting product temperature:* heating and holding phases can be modelled by a semi-empirical equation balancing heat production by Joule effect and heat losses through the cell's walls [13]:

$$\frac{dT}{dt} = \alpha \left( \frac{1 + aT_0}{a} + T - T_0 \right) - \frac{1}{\tau} (T - T_c) \quad (1)$$

with:  $T$  product temperature (°C);  $t$  time (s);  $a$  product characteristic constant (°C<sup>-1</sup>);  $T_0$  product initial temperature (°C);  $T_c$  external temperature (20 ± 5 °C).  $\alpha$  (s<sup>-1</sup>) is a simplifying constant based on process and product parameters and  $\tau$  (s) is the time constant of the heat transfer model:

$$\alpha = \frac{U^2 \sigma_0 a}{e^2 \rho c_p (1 + aT_0)} \quad (2) \quad \text{and} \quad \tau = \frac{\rho V c_p}{KA} \quad (3)$$

with:  $U$  electrical tension (V);  $\sigma_0$  product initial electrical conductivity (S.m<sup>-1</sup>);  $e$  distance between the two electrodes (m);  $\rho$  density of the product (kg.m<sup>-3</sup>);  $c_p$  specific heat capacity (J.kg<sup>-1</sup>.K<sup>-1</sup>);  $V$  product volume (m<sup>3</sup>);  $K$  global exchange coefficient (W.m<sup>-2</sup>.K<sup>-1</sup>);  $A$  contact surface with the product (m<sup>2</sup>).

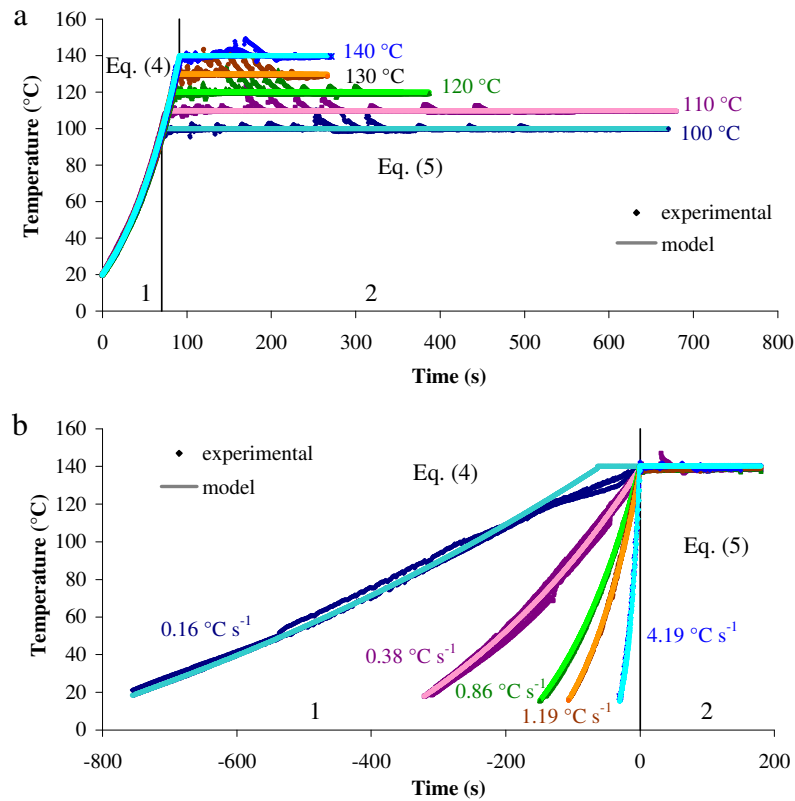
The *heating* equation can be obtained by integrating Eq. (1) with boundary conditions given by:  $T(0) = T_0$ .

$$T(t) = \frac{\alpha\tau + aT_c}{a(\alpha\tau - 1)} \left[ \exp\left(\frac{\alpha\tau - 1}{\tau}t\right) - 1 \right] \quad (4)$$

During *holding*, the absence of temperature variation allows simplifying Eq. (1) and extracting  $T(t)$ :

$$T(t) = \frac{1}{1 - \alpha\tau} \left[ \frac{\alpha\tau}{a} + T_c \right] \quad (5)$$

These models were fitted (Microsoft Excel's Solver) to the experimental data by adjusting the time constant  $\tau$  in order to minimize the sum of squares of the differences between experimental and modelled values. It was expressed as the Root Mean Square Error (RMSE) relatively to the mean value of the predicted data. The adjustment was good with RMSE around 3% for heating and 1% for holding (Figure 2) [13]. The cooling phase was shown to have no contribution to the sterilization value [14]; it was thus considered negligible in terms of chemical reaction and it was not taken into account even if modelling is possible [13].



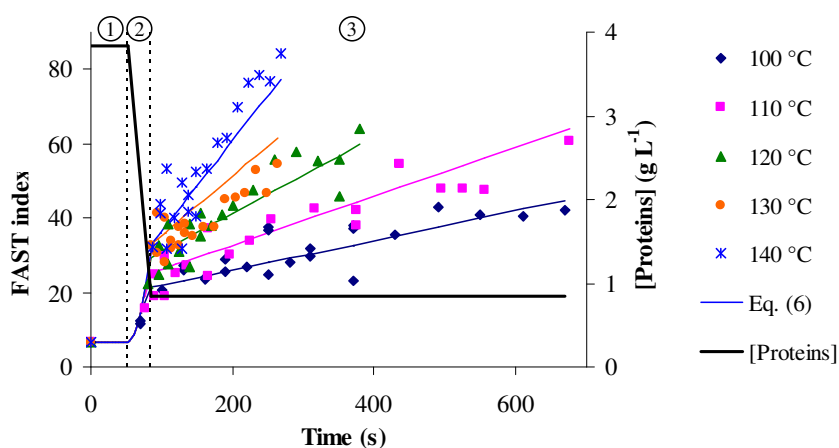
**Figure 2.** Temperature modelling of data acquired for serial A experiments (a) and serial B experiments (b). 1 corresponds to the heating phase modelled by Eq. (4) and 2 corresponds to the holding phase modelled by Eq. (5). Serial B experiments are centred on the beginning of the holding phase; negative times correspond to heating and positive times to holding.

*Kinetic model:* the extent of Maillard reaction was monitored by means of the FAST index and fitted with a kinetic model of pseudo-zero order. Arrhenius parameters were identified to model the temperature effect on the chemical reaction [13]:

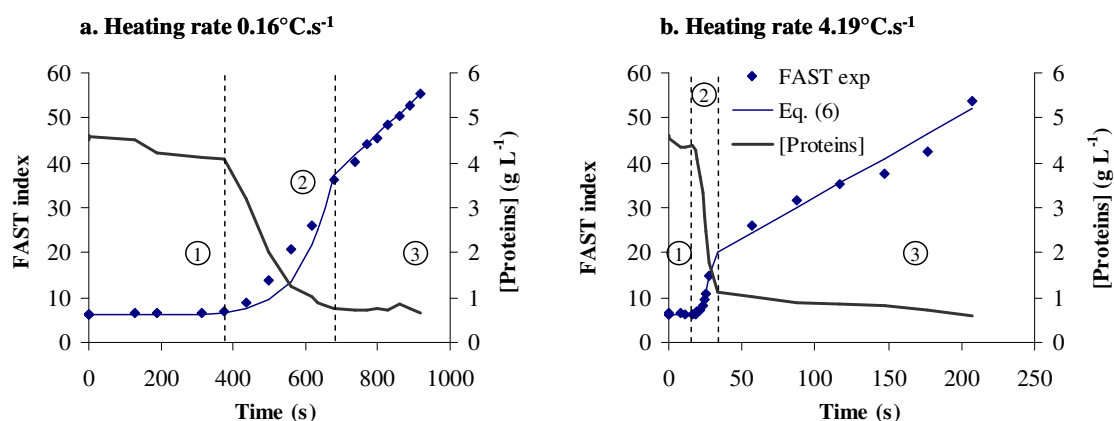
$$FAST(t) = k(T) \cdot t + FAST_0 = k_0 \exp\left(-\frac{E_a}{RT}\right) \cdot t + FAST_0 \quad (6)$$

where  $k(T)$  is the reaction rate constant,  $k_0$  the pre-exponential factor (concentration unit. $s^{-1}$ ),  $E_a$  the activation energy ( $\text{kJ}\cdot\text{mol}^{-1}$ ),  $R$  the perfect gases constant ( $8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ ) and  $T$  the temperature (K).  $k(T)$ ,  $FAST_0$ ,  $k_0$  and  $E_a$  can be determined as the slope and intercept of linear regressions of respectively FAST index against time and of  $\ln(k(T))$  against  $1/T$ .

*Coupling heat transfer and kinetic models:* Eq. (4) and Eq. (5) were used to predict temperature during heating or holding respectively and used in Eq. (6) to model the extent of Maillard reaction as marked by FAST index during ohmic treatments. The thermo-kinetic model given by Eq. (6) is a function of operating parameters (temperature and electrical conditions) and product parameters (electrical conductivity...). By definition, FAST index is bound to protein concentration represented by the tryptophan fluorescence ( $F_{trp}$ ). Eq. (6) was thus adapted according to the observation of protein behaviour. Three phases were identified (figure 3): ① a latency phase which lasts until the product reaches  $70^\circ\text{C}$ . During the latency phase, no chemical reaction could be detected and  $k_0$  and  $E_a$  were put to zero. ② A brutal decrease in protein content indicates a second phase, during which  $k_0$  and  $E_a$  were identified by fitting Eq. (6) to the experimental data of serial A and B experiments. ③ A final stable phase corresponds to isothermal conditions, where  $k_0$  and  $E_a$  parameters were identified from serial A experiments. Good adjustment of Eq. (6) to serial A experiments was obtained except for  $130^\circ\text{C}$  (probably due to experimental errors) as well as to serial B experiments (figure 4). So, the thermo-kinetic model enables to describe the heating as well as the holding phase with RMSE values lower than 15% whereas the relative error due to variability of the thermal treatment on the FAST index was reported to be maximum 3.8% [13].



**Figure 3.** Kinetics of the FAST index and proteins concentration monitored under isothermal conditions (serial A) at 5 holding temperatures and modelled by Eq. (6). ① Latency phase, ② Quick reaction phase and ③ Final stable phase.



**Figure 4.** Kinetics of the FAST index monitored under non isothermal conditions (serial B) up to  $140^\circ\text{C}$  at 2 extreme heating rates (a.  $0.38^\circ\text{C}\cdot\text{s}^{-1}$  and b.  $4.19^\circ\text{C}\cdot\text{s}^{-1}$ ) and modelled by Eq. (6). ① Latency phase, ② Quick reaction phase and ③ Final stable phase.

## CONCLUSION

The results confirm the significant promoting role of temperature in Maillard products formation. For a targeted sterilization value, high temperature short time treatments are in favour of limiting Maillard reaction development. Besides, it appears that the heating phase has noticeable contribution in Maillard product formation that cannot be neglected in kinetic studies. A simple thermo-kinetic model based on pseudo zero order kinetic of a global marker like FAST index with an Arrhenius type of temperature dependence enabled to describe a complex pool of reactions occurring in a liquid infant formula thermally treated under UHT conditions. The only required parameters are the initial thermo-physical and electrical properties of the product and the ohmic operating conditions. The dynamic phase as well as the isothermal phase of the thermal treatment by ohmic heating could be faithfully described thus allowing prediction of possible contamination by processed induced contaminants during sterilization of a liquid food. Such a predictive model could be used as a tool for optimizing the nutritional quality of a given formulation by monitoring the thermal conditions during processing.

## ACKNOWLEDGEMENTS

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