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Comparative thermal impact of two UHT technologies, continuous ohmic heating and direct steam injection, on the nutritional properties of liquid infant formula

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Abstract:
A continuous pilot plant for liquid sterilization was used to compare ohmic heating and steam injection on liquid infant formula under the same conditions of pre-heating and holding. Samples were collected at different holding times and temperatures and analyzed for reactions of thermal degradation. Two substrates were measured: soluble proteins and vitamin C and different intermediate or advanced products of Maillard reaction were monitored: furosine, carboxymethyllysine (CML), FAST index (Fluorescence of Advanced Maillard products and Soluble Tryptophan) and color ($\Delta L^*$, $a^*$ and $b^*$). Pseudo-zero order kinetics was established for the Maillard products or global markers and Arrhenius parameters could be calculated. Equivalent markers contents were obtained after ohmic heating and steam injection showing equivalent quality of the infant formula for both sterilization technologies.

Key words: Maillard reaction; thermal degradation; kinetics; thermal treatment

Highlights:
1. Ohmic heating technology is appropriate for sterilization of heat sensitive products
2. Ohmic heating is comparable to steam injection for UHT sterilization
3. Equivalent nutritional quality of the liquid infant formula is obtained
4. Accumulation of Maillard products can be described by a simple Arrhenius model
1. Introduction

Milk based infant formulas (IF) which composition is close to mature human milk, are designed to cover the nutritional needs of healthy infants. Product stability and microbiological safety are usually achieved by atomization or heat sterilization. However, severe heat treatments have deleterious effects on the sensory, biophysical and nutritional properties of milk products (Finot et al., 1981). Major changes are detected in thermally treated milks like protein denaturation and aggregation (Oldfield et al., 1998, 2000), lipid-protein and protein-protein interactions (Fenaille et al., 2006), sugar isomerization (Berg and Van Boekel, 1994) and a wide range of chemical reactions, the Maillard reaction being the main involved (Hiller and Lorenzen, 2010; Pellegrino et al., 1995; van Boekel, 1998). It is a complex series of reactions in which, in milk, a condensation reaction first occurs between the carbonyl group of lactose and the ε-amino group of lysine. The new compounds produced by Maillard reaction are likely to affect the quality, nutritional value and safety of the product (Birlouez-Aragon et al., 2006).

Two classical modes of high temperature short time (HTST) heating - indirect and direct UHT - are commonly used for sterilization of milk and milk products. In the indirect mode, the milk is separated from the heating fluid (steam or warm water under pressure) by a wall while in the second case it is mixed with the warming fluid (steam). Some of the steam is condensed, giving up its latent heat of vaporization to the product and giving a much more rapid rate of heating than is available with any indirect system. After holding at the sterilizing temperature, the product is expanded in a cooling vessel, where the pressure is at a level below atmospheric. It immediately boils and gives up water in the form of water vapor which is removed from the vessel and condensed. Steam injection (SI) has been observed to be the best technology to limit thermal damage of milks (Birlouez-Aragon et al., 1998; van Asselt et al., 2008). Although it is a very efficient heating method, where the product can reach temperatures above 150 °C in less than a second, the main disadvantage of SI relies on increased complexity and costs (Burton, 1994). Firstly, the water used for vapor production needs to be of “culinary” grade: it fulfills very strict hygiene standards and the use of chemical sterilizing agents that might end in the food product is a critical point. Boilers and steam generation equipment have to be operated in a way to prevent foaming, priming, carryover and excessive entrainment of boiling water into the steam (Canadian Food Inspection Agency, 2014). Secondly, the dilution of the product caused by steam condensation (up to 11 % of the product) needs to be compensated by a vaporization step to recover the exact initial solid content of the milk; the recovered vapor requires expensive devices to be recycled in comparison with an indirect heat exchanger allowing 80 to 90% heat recovery (Burton, 1994). Thirdly, elevated costs of heat and water are involved in direct UHT: the steam distribution through a network of pipes down to the injector comes along with heat losses and large amounts of water are required for the operating of the condenser. Furthermore, the residence time distribution can be quite dispersed within the steam nozzle causing thermal heterogeneity. Finally, fouling in the downstream area of the SI heater is an important operating problem, threatening the quality of product and affecting plant operation (Truong et al., 2002).

Another direct way of warming up a food product is ohmic heating. It is based on a simple mechanism – the Joule effect – which consists of raising the product temperature by passing an electrical current directly through the food when placed between two electrodes. Ohmic heating (OH) offers the big advantage over conventional indirect heating methods, of being based on pure-volume heating: the heat is directly dissipated in the product which behaves as a resistance thanks to the presence of free ions, resulting in fast and uniform heating. Even though it is based on the
use of electricity which is less and less appreciated today, the electrical conversion into heat energy is close to 100% (Ghnimi et al., 2007) and the very low thermal inertia makes possible fast and precise regulation. Heat transfer is a function of the electrical and thermal parameters of the product and equipment which makes the process easy-to-drive (Goullieux and Pain, 2014; Roux et al., 2010). However, fouling on electrode surfaces constitutes one of the main problems of OH (Ayadi et al., 2004, 2005; Fillaudeau et al., 2007; Stancl and Zitny, 2010). Besides production shut down for cleaning, fouling induces quality losses of the product and presence of electric arcs. Two big ways have been explored to limit fouling in OH: the one goes through cooling the walls of the ohmic reactor (Pain et al., 2013); and the second is based on the use of a fluid jet subjected to the electric field (Ghnimi et al., 2008). The first approach was used in this study to limit fouling problems during sterilization of the liquid infant formula.

The question of quantifying the healthiness of a product, which is crucial for infant formulas, and the possibility of optimizing food processing with respect to health aspects were addressed by van Boekel and Jongen (1997) who observed among others, the (anti)mutagenicity of Maillard products in heated milk. Different authors have used various markers of heat damage in milk and infant formulas in order to evaluate the effect of a given technology on the product quality: the combination of furosine and lactulose values was used by Pellegrino et al. to build information about the thermal history of sterilized milk (Hiller and Lorenzen, 2010). Rufián-Henares et al. (2004) monitored the extent of Maillard reaction by measuring furosine and color in model infant formula mixed and heated at lab scale. In addition to furosine, hydroxymethylfurfural (HMF) and pyrraline were studied by Contreras-Calderon et al. (2008) as indicators of thermal damage together with available lysine as nutritional indicator, to evaluate heat damage to ingredients used in commercial infant formulas. Morales et al. (2004) proposed to use the ratio of maltose to maltulose and furosine as quality parameters for infant formula. Rapid fluorimetric methods were investigated to assess the quality of heat-treated food products: the FAST index (Fluorescence of Advanced Maillard products and Soluble Tryptophan) was first designed for liquid milk products (Birlouez-Aragon et al., 1998) and extended to liquid infant formulas (Birlouez-Aragon et al., 2004; Damjanovic Desic and Birlouez-Aragon, 2011); it is based on the fluorescence of Maillard products observed in the soluble extract of the food and corrected for the protein concentration of the obtained solution. Front face fluorescence finger prints were used as rapid predictor of the nutritional quality of a bigger range of food products, by correlating the fluorescence response to the concentration of specific Maillard products like carboxymethyllysine (CML) in IF (Birlouez-Aragon et al., 2005), HMF in milk (Schamberger and Labuza, 2006), or the concentration of vitamin C, protein denaturation and accumulation of Maillard products in IF (Diez et al., 2008). Finally, Feinberg et al. (2006) demonstrated that no tracer can universally discriminate pasteurization from high pasteurization, direct UHT, indirect UHT, and sterilization of commercial milks. He recommended a multivariate approach by combining at least five tracers, the most discriminative ones being those which globally measure the structural modifications of the milk protein rather than those which specifically quantify the metabolites of the Maillard reaction.

In this study two types of continuous direct UHT treatments were applied to sterilize a model infant formula. The first objective was to compare the performances of OH to SI which is considered the best technology for UHT treatments of milk and milk products. Both technologies were applied at pilot scale, switching from one to another on the same pilot plant to generate identical time-temperature histories of the sterilized IF. They were compared on the basis of thermal degradation of proteins or vitamin C and production of Maillard products. The second objective was to generate kinetic data under real HTST processing conditions, including heating, holding and cooling phase, by modulating the holding time and temperature.
2. Materials and methods

2.1. Liquid infant formula

The liquid infant formula was specially designed for these experiments by industrial partners of ICARE project. It was produced at industrial scale using microfiltrated skim milk supplemented with various other ingredients in order to fulfil the nutritional target of the designed product (Table 1). The liquid product was packed into vacuum bags of 1 ton (2 units, one for each trial), transported and stored at 4 °C for 4 days.

Table 1. General composition and physical properties of the liquid infant formula.

<table>
<thead>
<tr>
<th>Composition</th>
<th>(g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>887</td>
</tr>
<tr>
<td>Sugars</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>84</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>11.5</td>
</tr>
<tr>
<td>Whey</td>
<td>2.5</td>
</tr>
<tr>
<td>Lipids</td>
<td>36</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>14.6</td>
</tr>
<tr>
<td>Mono-unsaturated fatty acids</td>
<td>12.8</td>
</tr>
<tr>
<td>Poly-unsaturated fatty acids</td>
<td>7.1</td>
</tr>
<tr>
<td>Minerals</td>
<td>4.6</td>
</tr>
<tr>
<td>Iron</td>
<td>0.009</td>
</tr>
<tr>
<td>Vitamins</td>
<td>0.43</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.00063</td>
</tr>
</tbody>
</table>

Physical properties

- Density at 20 °C\(^{(a)}\) (g/L) 1026 ± 4
- Viscosity\(^{(b)}\) (mPa.s) \(\exp(0.633 - 0.016\times T + 1.78E-5\times T^2)\)
- Specific heat capacity\(^{(c)}\) (J/kg/K) \(3939 + 0.16\times T + 0.0041\times T^2\)
- Thermal conductivity\(^{(c)}\) (W/m/K) \(0.534 + 0.0016\times T - 6.37E-6\times T^2\)
- Electrical conductivity\(^{(d)}\) (S/m) \(0.1082 + 0.008142\times T\)

\(^{(a)}\) Measured \((n = 192)\), decrease of about 6% between 20 and 140 °C (second order equation)\(^{(c)}\)
\(^{(b)}\) According to McCarthy (2011), considered equivalent to milk (0.03-15% fat, 70-135 °C), \(T\) in °C
\(^{(c)}\) According to Singh and Heldman (2001), temperature range of 0-150 °C, \(T\) in °C
\(^{(d)}\) According to Roux et al. (2010), temperature range of 20-140 °C, \(T\) in °C

2.2. Analytical methods

2.2.1. FAST method

The analytical method used to monitor the Fluorescence of Advanced Maillard products and Soluble Tryptophan (FAST) was the one previously developed and patented (Birlouez-Aragon, 2002; Birlouez-Aragon et al., 2002): first, a soluble extract of the IF was produced by bringing 1 mL sample to pH 4.6 using 50 mL of sodium acetate buffer (0.1 M, pH 4.6). 4 mL of
supernatant were then filtered through a 0.45 µm nylon filter (VWR, France) and placed in a disposable 4 faces acryl cuve (Sarstedt, France). The fluorescence of two complementary indicators was measured in the same sample, using a spectrofluorimeter (CaryEclipse Varian, France); at excitation/emission wavelengths of 290/340 nm, measurement of tryptophan fluorescence ($F_{trp}$) enabled to calculate the soluble protein content and at 330/420 nm measurement of advanced Maillard products ($F_{amp}$) were performed in the acid soluble supernatant. The FAST index was then calculated from both fluorescence signals, giving an indication of the nutritional damage undergone during the thermal treatment:

$$\text{FAST index} = \frac{F_{amp}}{F_{trp}} \cdot 100$$  \hspace{1cm} (1)

Maximum variation coefficients of 3.5% were obtained for $F_{trp}$, $F_{amp}$ and FAST index. $F_{trp}$ was calibrated with soluble whey proteins (Prolacta 90 from Lactalis, Laval, France) solutions of varying concentration $C_{\text{soluble proteins}}$ from 0.2 to 20 g/L. The calibration curve given by Eq. (2) showed linearity between these two quantification limits:

$$F_{trp} = (223.75 \pm 0.89) \cdot C_{\text{soluble proteins}} - (42.983 \pm 5.691) \hspace{1cm} (2)$$

2.2.2. Furosine and carboxymethyllysine analysis

Furosine and carboxymethyllysine (CML) were analyzed in triplicate according to the method proposed by Charissou et al. (2007): the samples were defatted with ethanol and dichloromethane before centrifugation. A proteinic ring was formed, transferred to another tube and hydrolyzed with concentrated HCl. After vacuum drying, the mix was further dissolved in water, filtered (nylon, 0.22 µm) and dried again. Then, a derivatization step comprised esterification of the carboxylic functions (methanol under strongly acidic conditions) and acylation of the amine functions (anhydrous trifluoroacetic acid). Finally, CML and furosine were quantified by selected ion monitoring on a gas chromatograph coupled with tandem mass spectrometry (Thermo Electron Corporation, Waltham, USA). In order to relate the results to the protein concentration, protein quantification was obtained using the rapid fluorescamine method (Yaylayan et al., 1992): 20 µL of filtered hydrolysate was made up to 3 mL by borate buffer (0.2 M; pH 8.5) and mixed with 100 µL of fluorescamine (0.15 g/L of acetone). The fluorescence was measured at excitation/emission wavelengths of 390/475 nm on a spectrofluorimeter (Fluoromax-Spex, Jobin-Yvon, Longjumeau, France).

To save time, the concentration of furosine and CML was also predicted by frontal fluorescence. Frontal fluorescence measurements were made by using a sensor developed for ICARE project. The method used by Rizkallah et al. (2008) for biscuits was applied to samples of thawed infant formula and the model to predict concentration was constructed by reference to the GC-MS-MS method described above. The values predicted by frontal fluorescence were mixed to those obtained by GC-MS-MS and no difference between the values obtained by either chemistry or spectrometry will be made in the rest of the study. The limits of quantification were estimated around 1 mg/100 g proteins for furosine and 1 µg/mg proteins for CML.

2.2.3. Vitamin C

Vitamin C was analyzed by HPLC with fluorescence detection according to the method developed by Tessier et al. (1996) and adapted to dairy product by Gliguem and Birlouez-Aragon

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1 ICARE : Impeding neo-formed Contaminants Accumulation to Reduce their health Effects; Collective research project (COLL-CT-2005-516415). Sixth Framework Program of the European Community (2002-2006)
Relative contents (in percentage) were expressed by dividing the peak area by the peak area of the control sample. The quantification domain is comprised between areas of $3 \times 10^5$ and $3.5 \times 10^6$ with coefficient of variation of repeatability lower than 5%.

2.2.4. Color

Color measurements were done directly on 5 mL fresh or defrost samples with a colorimeter (Luci 100, Dr Lange, Villeurbanne, France) and expressed in the CIELAB system with $L^* a^* b^*$ coordinates. Samples were illuminated with D65 artificial daylight (10° standard angle). Relative variations of 1.5%, 2.5% and 2.0% respectively, were obtained for $L^*$, $a^*$ and $b^*$ parameters due to colorimeter uncertainty ($n = 10$). To better characterize the variations in color, each parameter was expressed in Δ values meaning that the initial value of the parameter was subtracted to the measured ones.

2.3. Continuous sterilization equipment

The pilot plant was located in the Stazione Sperimentale per l’Industria delle Conserve Alimentari (SSICA) in Parma, Italy. The complete sterilization device was divided into four sections dedicated to preheating, heating, holding and cooling (Fig. 1). A flexible assembling of the different sections of the pilot plant enabled interchanging the heating section from ohmic heating to steam injection without changing the rest of the device. The infant formula (4 °C) was directly poured out of the vacuum pouches into a feeding tank and sent by a pressure pump to a first plate heat exchanger for preheating up to 50 °C. The product then entered the heating section, either ohmic or steam injection, to be heated up to the target temperature (120-140 °C). The target temperature was maintained in the holding section which was equipped with seven valves enabling sampling the product at different treatment times (Table 2). The thermal treatment finally ended in the cooling section with a second plate heat exchanger fed with cold water (almost 10 °C). The whole system was maintained under pressure (4 bars) by means of a counter pressure valve placed downstream to the process.

The ohmic cell used was patented (Pain et al., 2013) and specially designed to limit fouling. It was based on the principle developed by Pain et al. (2003) and improved regarding energy savings. The ohmic cell was constituted by two coaxial tubes. The warmer liquid circulating through the inlet path is cooled by the colder liquid circulating through the outlet path without any need for a secondary cooling fluid (Fig. 2). The product was continuously heated by electrodes made of titanium and placed along the longitudinal flowing axis.

The steam injection equipment consisted in a direct inlet of steam into the continuous flow of product obtained by connecting an additional pipe to the principal one. The steam flow was manually set to ensure that the product reached the target temperature.

No PID was used to stabilize the temperature: for OH, the temperature of the product was adjusted by delivering the adequate power with a manual setting and for SI by manually increasing or decreasing the injected steam flow rate. Once the appropriate settings were found, a few minutes were spent to confirm the temperature stability before samples could be taken. The temperature was monitored with sensors of maximum +/-1°C accuracy and remained stable all along the experiments at each targeted temperature.
Fig. 1. Schematic diagram of the continuous sterilization device.

Table 2. Average treatment times and sterilization values corresponding to the seven sampling points after ohmic heating or steam injection continuous sterilization of the liquid infant formula.

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Average treatment time (s)</th>
<th>Sterilization values ($F$ (s))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH</td>
<td>SI</td>
</tr>
<tr>
<td>S1</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>S2</td>
<td>2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>S3</td>
<td>5.9</td>
<td>4.8</td>
</tr>
<tr>
<td>S4</td>
<td>13.7</td>
<td>12.5</td>
</tr>
<tr>
<td>S5</td>
<td>39.5</td>
<td>38.3</td>
</tr>
<tr>
<td>S6</td>
<td>113.0</td>
<td>111.9</td>
</tr>
<tr>
<td>S7</td>
<td>154.3</td>
<td>153.2</td>
</tr>
</tbody>
</table>

Fig. 2. Schematic diagram of the ohmic cell with coaxial symmetry according to the principle proposed in Pain et al. (2013).
Sampling was made by simply opening the valve without any specific pressure control, inducing partial vaporization of the product because of pressure drop. Knowing the flow of liquid product – 150 L/h – and the length and diameter of the tubes, seven treatment times could be calculated for the ohmic heating device from 1.6 s to 154.3 s and for the steam injection one from 0.4 s to 153.2 s (Table 2). These times enable to calculate the sterilization values corresponding to the holding phase:

\[
F = 10^{\frac{\Delta T - T_{ref}}{z}} \times t
\]

(3)

where \( F \) (unit of time) is the sterilization value, \( T \) (°C) is the treatment temperature, \( T_{ref} \) (°C) is the reference temperature for sterilization (i.e. 121.1 °C), \( z \) (°C) is the temperature elevation that reduces the decimal reduction time by one log (i.e. 10 °C) and \( t \) (unit of time) is the duration of the treatment.

2.4. Experimental design

Two separate trials were done: first a resilience trial was dedicated to check the presence or absence of fouling in the ohmic cell. It consisted in running 1.5 h at a holding temperature of 120 °C and then increase temperature to 130 °C and 140 °C and run at 140 °C for 2.5 more hours. During the holding phase at 140 °C, samples were regularly taken at different residence times – S1, S4, S5, S7 (Table 2) – and measured for FAST index and color for comparison with untreated infant formula.

The second trial was dedicated to the kinetic study, during which three holding temperatures were applied – 120, 130 and 140 °C – and samples were taken at seven different heating times (Table 2) after stabilizing the system; each experiment was made in triplicate. All samples were analyzed for six chemical markers of the thermal treatment: FAST index, soluble proteins, color, vitamin C, furosine and CML.

3. Results and discussion

3.1. Process comparison between the two technologies and energetic considerations

The treatment of the product required enough energy to heat the infant formula from its initial temperature \( T_0 \) (50 °C) until the target temperature \( T_f \) (120 - 140 °C). This quantity of energy can be evaluated using Eq. (4) and knowing the flow rate, thermo-physical parameters (Table 1) and temperature difference between the initial and the final states of the product:

\[
\dot{Q} = \dot{m}_{IF} \times C_{pIF} \times (T_f - T_0)_{IF}
\]

(4)

where \( \dot{Q} \) (W) is the energy necessary to increase the temperature of the infant formula (IF) from its initial to its final temperature; \( \dot{m}_{IF} \) (kg/s) is the mass flow of infant formula and \( C_{pIF} \) (J/kg/K) its specific heat capacity.

Under such temperature conditions and with infant formula parameters calculated at a median temperature between \( T_0 \) and \( T_f \), Eq. (4) gives an energy value of 11 to 15 kW. This energy had to
be delivered either by electricity for the OH or by steam condensation and cooling for the SI. Knowing the temperature of the injected steam ($T_s = 145 \degree C$) it is then possible to calculate its flow rate for each final product temperature using Eq. (5):

$$\dot{Q} = \dot{m_s} \times \Delta H_{(145\degree C)} + \dot{m_s} \times C_{pW} \times (T_s - T_f)$$ (5)

where $\dot{m_s}$ (kg/s) is the mass flow of steam (or water); $\Delta H_{(180\degree C)}$ (J/kg) is the enthalpy of steam condensation at 145 $\degree$C; $C_{pW}$ (J/kg/K) is the specific heat capacity of the water.

Eq. (5) gives steam flow rates of 0.005 and 0.007 kg/s for final product temperatures of 120 and 140 $\degree$C respectively, using the $\dot{Q}$ value given by Eq. (4) and $\Delta H_{(145\degree C)}$ and an estimated value of $C_{pW}$ at a median temperature between $T_s$ and $T_f$, according to Bergman et al., (2011). Such a steam flowrate allows predicting a non negligible dilution of the SI samples of 12 to 17%. The vaporization during sampling is expected to compensate, at least partly, the product dilution due to steam condensation. Furthermore, vaporization during sampling, although uncontrolled, is expected to be the same for OH and SI technology, which means that OH samples may be more concentrated than SI samples in this study, but to an unknown extent. Despite that, it was chosen not to correct the measured concentration of newly formed compounds (NFC) and to keep in mind that OH samples are probably slightly more concentrated than SI samples for accumulating NFC and slightly more diluted for transition markers. This uncertainty would be worth being removed in further studies.

The sterilization values ($F$) calculated for the holding phase show that the treatment becomes realistic compared to industrial standards ($F > 152$ s, corresponding to a 12 log reduction of a Clostridium botulinum population) for the samplings S2-S3 at 140 $\degree$C and S5 at 130 $\degree$C. For 120 $\degree$C, the treatment should have been continued a little longer after S7 (42-43 seconds).

### 3.2. Resilience trial

The resilience trial aimed at detecting any occurrence of fouling during the time covering the kinetic study. A first holding phase, set at 120 $\degree$C, lasted 1.5 hour and a second phase, set at 140 $\degree$C lasted 2.5 more hours. During the resilience trial, no temperature decay could be observed which indicates that no fouling occurred. Indeed, when a fouling layer appears on the electrodes, it acts like an insulation layer that provokes a loss in the quantity of energy delivered in the product and consequently temperature decay.

Besides, the use of chemical markers (FAST index and color) was also tested to check fouling during the duration of the 140 $\degree$C resilience test. Samples were taken from the S1, S4, S5 and S7 sampling points. The sampling point S1 (1.6 s) reflected the product state at the entrance of the holding section as well as the exit of the ohmic treatment cell (Fig. 1). The FAST index of S1 samples gave stable values around 20 during the 2.5 h running at 140 $\degree$C, above the value of 6.1 of the untreated sample. This indicates that no fouling occurred in the ohmic applicator while it most probably occurred in the holding section: the FAST index measured on S4 to S7 samples increased during the trial all along the holding tube (S4: 22.7 to 25.2; S7: 66.1 to 75.5). The color measurements were not sensitive enough to detect any change during the 2.5 h resilience trial at 140 $\degree$C. Such results confirm the good performances of this new ohmic applicator regarding fouling.
3.3. Determination of the kinetic parameters of the observed chemical markers

The evolution of the chemical markers concentration with time can be separated into two categories: the ones with linear increase of concentration during the isothermal phase of the thermal treatment (Fast index, color, furosine and CML) and those with constant concentration (vitamin C and soluble proteins). The first category regroups specific markers of Maillard reaction like furosine and CML while FAST index and color (divided in its 3 components ΔL*, Δa* and Δb*) are global markers of the same reaction. The kinetic behavior of such markers has been successfully represented in a previous article by Roux et al. (2009) using a model of pseudo-zero order (Eq. (7)), where \( k(T) \) parameter could be estimated using linear regression applied to the isothermal part of the curves (Table 3):

\[
C = C_0 + k(T) \times t \tag{7}
\]

\( C \) is the reactant concentration at time \( t \) and \( C_0 \) its initial concentration; \( k(T) \) is the reaction rate depending on temperature \( T \).

Arrhenius parameters – kinetic rate constant \( k(T_{ref}) \) at a reference temperature \( T_{ref} \) chosen to be the middle of the studied temperature interval and activation energy \( E_a \) (Eq. (8)) – could then be estimated by fitting Eq. (8) on all experimental data. This reparametrized Arrhenius equation enables to be more accurate for the \( k(T_{ref}) \) determination than for a determination of the pre-exponential factor \( (k_0) \) classically used but very far away from the temperature domains generally studied (van Boekel, 2009).

\[
k(T) = k(T_{ref}) \times \exp \left( -\frac{E_a}{R} \times \left( \frac{1}{T} - \frac{1}{T_{ref}} \right) \right) \tag{8}
\]

Table 3. Kinetic parameters (mean ± standard deviation) of the different markers of Maillard reaction observed during the thermal treatment of an infant formula by ohmic heating or steam injection. \( k(T) \) were estimated using linear regression on the data obtained during the holding phase. \( k(T_{ref} = 130 \degree C) \) and \( E_a \) were estimated by a global fitting of all experimental data according to Eq. (8).

<table>
<thead>
<tr>
<th>Reaction rate of a pseudo-zero order type of kinetic (U/s) (^{(a)})</th>
<th>Parameters calculated from Arrhenius equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{1(120 \degree C)} )</td>
<td>( k_{1(130 \degree C)} )</td>
</tr>
<tr>
<td><strong>FAST</strong></td>
<td></td>
</tr>
<tr>
<td>SI(^{(b)})</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>OH(^{(b)})</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>( \Delta L^* )</td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>0.001 ± 0.002</td>
</tr>
<tr>
<td>OH</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>( \Delta a^* )</td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>0.0008 ± 0.0005</td>
</tr>
<tr>
<td>OH</td>
<td>-0.0501 ± 0.0736</td>
</tr>
<tr>
<td>( \Delta b^* )</td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>0.0012 ± 0.0013</td>
</tr>
<tr>
<td>OH</td>
<td>0.0035 ± 0.0007</td>
</tr>
<tr>
<td><strong>Furo.</strong></td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>OH</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td><strong>CML</strong></td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>OH</td>
<td>6.7 ± 0.9</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Unit of the marker: arbitrary unit for FAST index, \( \Delta L^* \), \( \Delta a^* \) and \( \Delta b^* \), mg/100 g proteins for furosine, ng/mg proteins for CML.

\(^{(b)}\) SI: steam injection; OH: ohmic heating
Comparable values of $k(T)$, $k(T_{\text{ref}})$ and $E_a$ were obtained for steam injection and ohmic heating regarding FAST index. In the case of color ($\Delta L^*$, $\Delta a^*$ and $\Delta b^*$), very little variations were detected all along the trials, resulting in very low kinetic rate constants that could not be well identified (high standard deviations). But the Arrhenius parameters showed no major differences between both technologies. Finally, furosine and CML presented quite high differences on the $k$ values but activation energies were close. The results presented in Table 3 are fairly comparable to the kinetic data obtained in a previous work for a model infant formula treated by ohmic heating in a lab-scale batch reactor (Roux et al., 2009).

3.4. Comparison of the kinetic behavior of the markers between ohmic heating and steam injection

Thanks to the device presented in Fig. 1, the residence time of the liquid formula could be considered approximately the same for ohmic heating and steam injection. The thermal markers could thus be compared by plotting them for each technology, the closer to the bisector, the better the equivalence between both technologies (Fig. 3). Confirming the proximity of the kinetic parameters shown in Table 3, the results obtained on FAST index emphasized the equivalence of both technologies. No significant difference could be detected on soluble proteins content between ohmic heating and steam injection. Even though furosine and CML had comparable $k_0$ and $E_a$, they seemed to be generated in higher amount by ohmic heating. However, this is only noticeable at high treatment times whereas industrial treatments would only last several seconds, i.e. in the equivalence zone. As regards color, slight differences could be detected in line with the kinetic parameters. Most of the $\Delta L^*$ points stayed on the zero position for ohmic heating while darkening was observed for steam injection. $\Delta a^*$ and $\Delta b^*$ (data not shown) were the same for both technologies except for extreme time/temperature conditions (sampling times 6 and 7 at 130 and 140°C) where it showed higher values for ohmic heating than steam injection. Therefore, if we exclude the extreme holding times which are not realistic, almost no color differences were generated by both technologies and they would probably not be detectable on the final industrial product. Finally, vitamin C seemed to be better preserved by ohmic heating than steam injection: the data points are clearly situated above the bisector, showing significantly higher residual vitamin C after a continuous sterilization by ohmic heating than steam injection. However, such results should be confirmed because they can, at least partially, be due to the fact that OH samples are not diluted during the treatment contrary to SI ones.
Fig. 3. Comparison of FAST index, soluble proteins, furosine, carboxymethyllysine (CML), color ($\Delta L^*$) and residual vitamin C of a liquid infant formula thermally treated under the same holding temperature conditions of sterilization by steam injection or ohmic heating; the full symbols correspond to predicted values of furosine and CML by front face fluorescence and the empty ones to chemical analysis by GC-MS-MS.
4. Conclusion

Continuous ohmic heating applied at pilot scale gave very satisfactory results in terms of preservation of the nutritional quality of a liquid infant formula sterilized under UHT conditions. Chemical markers like FAST index, furosine or carboxymethyllysine indicated an equivalent extent of Maillard reaction during sterilization by ohmic heating in comparison with steam injection which is classically considered the best performing technology. The product sterilized by ohmic heating appeared to be slightly richer in Maillard products like furosine and CML than by steam injection but this only for extreme time/temperature settings which were not representative of real industrial conditions. The color seemed to remain more constant with ohmic heating than with steam injection. The extent of soluble protein denaturation was comparable for both technologies while vitamin C appeared to be even better preserved by ohmic heating. Keeping in mind that the markers concentrations for SI are probably slightly underestimated, the conclusion of equivalence between the two treatments makes even more sense. Furthermore, since no fouling was detected in the ohmic applicator after two and a half hours processing at 140 °C, continuous ohmic heating could thus be considered as a very promising technology for industrial thermal treatment of fragile dairy products. Such conclusions would of course deserve to be confirmed by trials with representative durations of treatment of the industrial conditions.

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