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1 **A 3D-CFD-heat-transfer-based model for the microbial inactivation of pasteurized food**  
2 **products**

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9 **ABSTRACT**

10 This study proposes to couple a 3D-CFD and heat transfer finite elements model with the  
11 microbial inactivation approach proposed by Geeraerd et al. (2000). The CFD-heat transfer  
12 model was developed using thermophysical properties for both heating fluid (water) and the  
13 processed sample (ground beef). The kinetic microbial parameters were estimated using  
14 experimental data from the inactivation of *Escherichia coli* K12 in a packaged sample. The  
15 proposed inactivation model was tested under more severe dynamic conditions than usual  
16 (heating rates from 1 to 13 °C/min). The inactivation kinetic parameters were found  
17 independent of the heating rate applied. In addition, the results reveal that the Geeraerd et al.  
18 (2000) model without shoulder is sufficient to fit the experimental data. Such a model could  
19 be beneficial in simulating microbial inactivation for food products, thus ensuring food safety  
20 by limiting, as far as possible, overtreatment.

21 *Keywords: Microbial inactivation; Modeling; 3D-CFD; Heat transfer; Pasteurization.*

**Nomenclature**

a, b	dimensions of the elliptical sample (mm)
$C_c$	critical component related to the physiological state of the cells (-)
$C_p$	apparent specific heat ( $\text{kJ kg}^{-1} \text{K}^{-1}$ )
$D_{\text{ref}}$	decimal reduction time (s)
g	gravitational constant ( $\text{m/s}^2$ )
$h_{\text{air}}$	heat transfer coefficient of air ( $\text{W m}^{-2} \text{K}^{-1}$ )
$k_{\text{max}}$	specific inactivation rate ( $\text{s}^{-1}$ )

$L$	sample length (mm)
$LL$	maximum likelihood
$n$	number of experimental data points
$N$	microbial population (CFU /g)
$N_0$	initial microbial population (CFU /g)
$N_{\text{simu}}$	simulated microbial population (CFU /g)
$N_{\text{exp}}$	experimental microbial population (CFU /g)
$p$	number of independently adjusted parameters within the model
$P$	absolute pressure (Pa)
$Q$	volumetric heat generation term ( $\text{W}/\text{m}^3$ )
$R$	radius (mm)
$T$	temperature ( $^{\circ}\text{C}$ )
$T_0$	initial temperature of product ( $^{\circ}\text{C}$ )
$T_{\infty}$	ambient temperature ( $^{\circ}\text{C}$ )
$t_i$	simulated time (s)
$T_{\text{ref}}$	microbial inactivation reference temperature ( $^{\circ}\text{C}$ )
$T_{\text{cylinder cell}}$	temperature of lateral surfaces of the cylinder ( $^{\circ}\text{C}$ )
$\vec{u}$	velocity field (m/s)
$u, v, w$	spatial components of velocity field
$V$	volume of the meat sample ( $\text{m}^3$ )
$x, y, z$	spatial coordinates in the three dimensions (m)
$z$	thermal resistance constant ( $^{\circ}\text{C}$ )
<b>Greek letters</b>	
$\rho$	density ( $\text{kg}/\text{m}^3$ )
$\mu$	dynamic viscosity (Pa s)
$\sigma$	standard deviation of experimental data
$\lambda$	thermal conductivity ( $\text{W m}^{-1} \text{K}^{-1}$ )

## 22        **1. Introduction**

23        Prediction of microbial inactivation during thermal treatment is crucial not only to ensure the  
24        safety of food products but also to avoid overtreatment (Boillereaux, Curet, Hamoud-Agha, &  
25        Simonin, 2013). Although conventional processing technologies produce safe products, they  
26        can also lead to significant changes to the sensory and nutritional attributes of foods. In

27 today's quality conscious world, much attention is given to producing foods that retain  
28 superior sensorial quality but must also remain safe (Bott, 2014; Stratakos & Koidis, 2015).  
29 Consequently, the determination of the microbial destruction level that a thermal treatment  
30 **can** deliver to a product requires both understanding of: (i) the heat transfer within the food  
31 product and (ii) the destruction kinetics of the microorganism of interest (Valdramidis et al.,  
32 2005). The necessity of such an approach is reinforced regarding the thermal heterogeneities  
33 that occur during a pasteurization process.

34 A reliable inactivation parameter estimation is essential for building predictive models (Chen,  
35 2013) and is necessary to establish **safe** minimal cooking-cooling conditions (Marcotte, Chen,  
36 Grabowski, Ramaswamy, & Piette, 2008). The determination of these parameters has been the  
37 subject of numerous studies, either under static (Ahmed, Conner, & Huffman, 1995; Smith,  
38 Maurer, Orta-Ramirez, Ryser, & Smith, 2001) or dynamic conditions (Garre et al., 2018;  
39 Hassani, Cebrián, Mañas, Condón, & Pagán, 2006; Juneja & Marks, 2003; Valdramidis,  
40 Geeraerd, Bernaerts, & Van Impe, 2006; Valdramidis, Geeraerd, & Impe, 2007). Different  
41 models have also been proposed to describe the microbial inactivation kinetics.

42 Numerous studies focusing on the estimation of microbial kinetic parameters are performed in  
43 isothermal and dynamic conditions, but under ideal laboratory conditions (capillary tubes,  
44 liquid inoculum and small sample mass). In real **time** industrial processing, food products are  
45 often submitted to time-varying temperatures. Moreover, due to several factors affecting heat  
46 transfer, i.e. geometry, thermophysical properties or external convection, significant thermal  
47 heterogeneities can occur. In the literature, the effect of capillary tube diameters on the  
48 inactivation parameters of *E. Coli*, in solid food, was investigated from a two-dimensional  
49 heat transfer approach (Chung, Wang, & Tang, 2007).

50 A recent study by Garre et al., (2018) presented a mathematical model to describe the non-  
51 isothermal microbial inactivation process. The model was dedicated to *Escherichia coli*  
52 inactivation under linear heating rates of 1, 5, 15, 35 and 40 °C/min, from 35 to 70 °C. The  
53 authors developed a mathematical model taking into account the thermotolerance of the  
54 microbial cells. The model was able to describe the experimental data using a unique set of  
55 model parameters.

56 Another investigation, Hamoud-Agha, Curet, Simonin, & Boillereaux, (2013) proposed to  
57 estimate  $D_{ref}$  and  $z$ -value of Bigelow's (1921) equation. These parameters were estimated for  
58 *E. coli* K12 inoculated into a model food under dynamic conditions (homogeneous

59 temperature distribution due to a very small solid sample). These parameters were included in  
60 the Geeraerd et al., (2000) inactivation model coupled with a 3D-heat transfer model under  
61 microwaves to illustrate the resulting inactivation heterogeneity.

62 The literature is rich concerning CFD and heat transfer modelling dedicated to pasteurization  
63 of food products (Bhuvaneswari & Anandharamakrishnan, 2014; Cordioli, Rinaldi, Copelli,  
64 Casoli, & Barbanti, 2014; Denys, Pieters, & Dewettinck, 2003; Dimou & Yanniotis, 2011).  
65 However, except in Hamoud-Agha et al., (2013), it is rare to find these models coupled with  
66 microbial inactivation equations and even less with real products under external dynamic  
67 conditions. These elements reflect the originality of this work proposal. In this study, a 3D-  
68 CFD and heat transfer approach is coupled with the Geeraerd et al. (2000) inactivation model.  
69 External temperature conditions (heating rates from 1 to 13 °C/min) are applied to  
70 *Escherichia coli* K12 inactivation in pre-packed ground beef. Model parameters are estimated  
71 from experimental data using the Levenberg-Marquardt algorithm (Marquardt, 1963).

## 72 **2. Materials and methods**

### 73 ***2.1 Experimental section***

#### 74 ***2.1.1 Sample preparation***

75 Raw ground beef (Beef Steak 5% fat, Charal<sup>®</sup>, Cholet, France) was obtained from a local  
76 retail store. The proportion of lean meat and fat was determined on the basis of information on  
77 the package. Ground beef was provided in vacuum-packed portions of 100 g. The samples  
78 were quickly frozen in a -20 °C freezer (Servathin, Carrières-sous-Poissy, France) and stored  
79 in a freezing chamber at -20 ± 1 °C. Before each experiment, a bag of the sample was thawed  
80 for 18 h in a cooling chamber (4 °C).

#### 81 ***2.1.2 Preparation of bacteria inoculum***

82 The strain of *Escherichia coli* K12 (CIP 54.117, N° 11.612) used in this study was provided  
83 by the Pasteur Institute, France, in the form of a freeze-dried sample. The strain was  
84 rehydrated in 0.2 mL and inoculated in 5 mL of Brain Heart Infusion (BHI) (Panreac  
85 Applichem – Darmstadt, Germany) at 37 °C for 18 h at 150 rpm (Incubator Shaker Series –  
86 Model Excella E24, New Jersey, USA). A pre-culture was prepared with 0.1 mL of the  
87 preceding suspension and inoculated in blood agar (Columbia + 5% Sheep blood - Bio-Rad -

88 Marnes-la-Coquette, France) at 37 °C, for 18 h. A clone of the pre-culture was then dissolved  
89 in 2 mL of sterile water (0.85% NaCl - Medium, BioMerieux). 0.1 mL of this solution was  
90 isolated in several Petri dishes of the blood agar with a sterile Pasteur pipette and incubated  
91 **under** the same conditions. The colonies of this second isolation were removed with a sterile  
92 swab and dissolved in 1 mL of the beef serum (Eurobio Serum Bovin, France) supplemented  
93 with 20% (v/v) glycerol and stored frozen at -80 °C (Thermo Scientific TSE - USA).

94 To reactivate the strain, 0.1 mL of the stocked culture was inoculated into the blood agar  
95 (Columbia agar + 5% sheep blood – 43041, BioMérieux, France) and incubated at 37 °C for  
96 18 h. Fresh colonies were carefully removed with a sterile swab and dissolved in 5 mL of  
97 medium suspension (Api 20150, BioMérieux, France) until a cellular concentration of  
98 0.5 McFarland was reached (Densitometer Densimat, BioMérieux, Italy). The optical density  
99 of 0.5 McFarland standard is comparable to that of a bacterial suspension concentration of  
100 approximately  $1.5 \times 10^8$  CFU/mL (CLSI, 2012).

### 101 *2.1.3 Inoculation of ground beef*

102 Raw ground beef samples (36 g) were removed aseptically from their refrigerated packaging  
103 (7 °C) and transferred into sterile polypropylene sampling pouches with 4 g of bacterial  
104 suspension. Ground beef was mixed manually to evenly distribute the inoculum throughout  
105 the sample, producing an initial cell concentration of approximately  $10^7$  CFU/g. For heat  
106 treatment, 3 g of inoculated ground beef samples were aseptically placed in packaging of  
107 11.6 mm wide x 60 mm **long**.

### 108 *2.1.4 Heat treatment for microbial inactivation*

109 As illustrated in Figure 1, each sample was inserted **into** the cylindrical vessel and submitted  
110 to a linear heating ramp in a programmable Peltier-based effect water bath (Hart Scientific  
111 AOIP, FC 9105, USA). After the heating treatment, the sample was quickly cooled to 20 °C  
112 by ice immersion. The temperature was measured with a thermocouple (type-K) **positioned**  
113 near the bottom of the sample. The data were collected by a data logger (AOIP Datalog,  
114 91133 Ris Orangis, France) with output recordings every 1 second. To obtain the inactivation  
115 kinetics curves, eight final temperatures (50, 52, 54, 56, 58, 60, 62 and 64 °C) were  
116 considered. Six different heating rates (1, 3, 5, 7, 10 and 13 °C/min) were applied. Triplicates  
117 were carried out for each experiment.

### 118 2.1.5 *Microbial enumeration and expression of results*

119 The method of analysis was according to ISO 16649-1 (2001). This standard procedure  
120 specifies a horizontal method for the enumeration of  $\beta$ -glucuronidase-positive *Escherichia*  
121 *coli* in products intended for human consumption. It is based on a colony-count technique at  
122 44 °C in a solid medium containing a chromogenic ingredient for detection of the  $\beta$ -  
123 glucuronidase enzyme (ISO 16649-1, 2001).

124 Untreated inoculated ground beef and treated ground beef were aseptically removed from the  
125 packaging and transferred to a sterile filtered stomacher bag (400 mL, BagPage, St. Nom,  
126 France). The samples were diluted with 1:9 (w/w) of buffered peptone water (BK131HA,  
127 Biokar Diagnostics, France) and stomached for 2 min at 230 rpm (Stomacher Lab Blender  
128 InterscienceBagMixer® - 400, Grosseron, St. Herblain, France). Decimal serial dilutions were  
129 performed in buffered peptone water. 1 mL of the initial suspension or decimal dilution was  
130 inoculated **onto** duplicate plates of tryptone-bile-glucuronic (TBX) medium (BK146HA,  
131 Biokar Diagnostics, France) and incubated between 18 h to 24 h at 44 °C. The presence of  
132 blue colonies is considered to be  $\beta$ -glucuronidase-positive *Escherichia coli*.

133 Each dish containing less than 150 typical CFU was numbered and the result was calculated  
134 as the arithmetic mean from two parallel plates. The results expressed to the base 10  
135 logarithm of the microbial population for each temperature measurement were collected. The  
136 detection limit was fixed to 1 CFU/g of the ground beef.

## 137 2.2 *Model design section*

### 138 2.2.1 *Heat transfer and computational fluid dynamics (CFD)*

139 The experimental methodology described in section 2.1.4 was considered in a CFD-heat  
140 transfer model, with the following assumptions:

- 141 • *Assumption 1:* Water in the cylindrical vessel and the packaged sample were at the  
142 same initial homogeneous temperature (7 °C).
- 143 • *Assumption 2:* The ground beef sample was considered as **being** homogeneous and  
144 isotropic.
- 145 • *Assumption 3:* Specific heat, density and thermal conductivity of the ground beef were  
146 considered as **being** constant within the temperature range (Pan & Paul Singh, 2001;  
147 Tsai, Unklesbay, Unklesbay, & Clarke, 1998).

- 148 • *Assumption 4:* The geometry of the packaged sample was an ellipsoid-cylinder and the  
 149 cross-section was considered homogeneous along the z-coordinate.
- 150 • *Assumption 5:* Axial symmetry was supposed for both thermal problems and fluid  
 151 mechanics, limiting the size to one quarter of the experimental apparatus.
- 152 • *Assumption 6:* The mass transfer and the shrinkage of the sample were assumed **to be**  
 153 negligible (in-package thermal treatment).
- 154 • *Assumption 7:* The package thickness was sufficiently thin to neglect its impact on the  
 155 heat transfer (low thermal resistance).

156 The model is aimed at predicting the temperature profile when a heating rate is **applied** to the  
 157 wall of the water bath heating cell. The model consists of two main computational domains:  
 158 the first relates to liquid water which partially fills the cylindrical cell, the second is the  
 159 ground beef sample located at the centre of the cell. As illustrated in Figure 2, the sample was  
 160 immersed in water and its upper surface was exposed to air surrounding the medium (natural  
 161 convection). The lateral surfaces of the cylinder are modelled with a first type boundary  
 162 condition ( $T = T_{\text{cylinder cell}}$ ).

### 163 2.2.2 Heat transfer modelling

164 Heat transfer is based on the general heat equation, which depends on thermophysical  
 165 properties. For the ground beef sample, the heat equation is reduced to:

$$166 \quad \rho_{(sample)} C_{p(sample)} \frac{\partial T}{\partial t} = \text{div} (\lambda_{(sample)} \nabla T) \quad (1)$$

167 For liquid water, a convective term is added to take into account the variation of the  
 168 thermophysical properties of water as a function of temperature:

$$169 \quad \rho_{(water)} C_{p(water)} \frac{\partial T}{\partial t} + \rho_{(water)} C_{p(water)} \vec{u} \nabla T = \text{div} (\lambda_{(water)} \nabla T) \quad (2)$$

170 Where  $\vec{u}$  is the velocity field at any point of the 3D sample domain ( $u, v, w$  components).

171 The initial and boundary conditions are defined with the following mathematical expressions:

$$172 \quad T = T_0, \quad t = 0, \quad \forall (x, y, z), \quad T_0 = 7^\circ\text{C} \quad (3)$$

$$173 \quad \lambda_{(sample/water)} \left. \frac{\partial T}{\partial z} \right|_{z=L} = h_{air} (T - T_\infty), \quad \forall (x, y), \forall t > 0, \quad T_\infty = 20^\circ\text{C} \quad (4)$$



174 The boundary condition expressed by equation 4 is applied to the upper surface of water and  
 175 sample.

$$176 \quad T_{cylindrical\ cell} = T_0 + \frac{dT}{dt} t \begin{cases} \text{for } (x, y) \in [0; R] \text{ and } Z = 0, \quad \forall t > 0 \\ \text{for } x, y = R, \quad \forall t > 0, \text{ for } Z \in [0, L] \end{cases} \quad (5)$$

177 Figure 2 displays the coordinate system used to describe these mathematical expressions.

178 The convective heat transfer coefficient is due to natural convection between the surrounding  
 179 air medium and the upper surface of the product. Free convection occurs only at the upper  
 180 surface of both the water and the sample (Figure 2). An empirical correlation was conducted  
 181 to compute the heat transfer coefficient on the upper cooled side of the cylinder. The air heat  
 182 transfer coefficient ( $h_{air}$ ) at the surface of the vertical cylinder with its axis running vertically  
 183 is the same as that from a vertical plate, so long as the thermal boundary layer is thin  
 184 (Churchill & Chu, 1975).

### 185 2.2.3 Fluid flow modelling

186 The fluid flow is due to natural convection occurring within the liquid water. This convective  
 187 flow is modelled from the Navier Stokes equations (Newtonian fluid with incompressible  
 188 flow). Both the continuity and momentum equations are described as follows:

$$189 \quad \begin{cases} \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0 & (\text{continuity}) \\ \rho_{water} \frac{d\vec{u}}{dt} = \rho_{water} g - \nabla P + \mu_{water} \Delta \vec{u} & (\text{momentum}) \end{cases} \quad (6)$$

190 with

$$191 \quad \frac{d\vec{u}}{dt} = \frac{\partial \vec{u}}{\partial t} + (\vec{u} \nabla) \vec{u} \quad (7)$$

192 The associated initial and boundary conditions are given as:

$$193 \quad P = P_{atm} + \rho_{water} g z \quad \text{at } t = 0, \quad \forall x, y, \quad \text{for } z \in [0; L] \quad (8)$$

$$194 \quad \vec{u} = 0 \quad \text{at } R = x, y, \quad \forall t > 0, \quad \text{for } z \in [0; L] \quad (9)$$

195 For validation purposes, coupled heat transfer and fluid flow were solved for a heating rate of  
196 10 °C /min to compare the simulation to experimental results in the same conditions.

#### 197 2.2.4 Thermophysical properties

198 Thermophysical properties of the sample used for modelling purposes are listed in Table 1.  
199 The apparent specific heat ( $C_p$ ) of ground beef (5% fat) was determined using a differential  
200 scanning calorimetry ( $\mu$ DSC VII Evo - Setaram Instrumentations, France) at a constant  
201 pressure. Samples (~ 500 mg) were weighed in aluminium cells and sealed. A hermetically  
202 sealed empty cell was used as a reference. The samples were equilibrated at 20 °C and then  
203 heated to 65 °C, at a heating rate of 1 °C/min. The measurements were made in triplicate and  
204 the average values were determined.

205 The density and the thermal conductivity of sample were taken from Pan & Paul Singh  
206 (2001). The authors reported densities of 1006-1033 kg/m<sup>3</sup> and thermal conductivities of  
207 0.35-0.41 W m<sup>-1</sup> °C<sup>-1</sup> of ground beef (4.8% fat). These values do not change significantly as a  
208 function of temperature range (between 5 and 75 °C).

209 Thermophysical properties of water were considered as temperature dependent (Green &  
210 Perry, 2007). These properties were directly implemented in the model using polynomial  
211 interpolation functions (Table 2).

#### 212 2.2.5 Computational details for CFD simulations

213 Based on the finite element method, the model solved the partial differential equations  
214 numerically, with the computational code COMSOL® Multiphysics 5.3a. In this study, the  
215 geometry consisted of irregular shapes (ellipsoid-cylinder with axial symmetry) justifying the  
216 use of the finite element method to simulate the coupled CFD-heat transfer phenomenon.

217 As shown in Figure 3, the generated mesh was designed for a 3D configuration, to ensure  
218 good accuracy for the numerical resolution (522 830 tetrahedral elements with appropriate  
219 boundary layers at the near-cell wall zone). Additional mesh sensitivity studies were  
220 performed to obtain accurate number of mesh elements in relation to the computational cost.  
221 The mesh independency to the numerical results was also verified. First, the fluid flow was  
222 solved as a stationary study in order to initialize the velocity field for a better consistent initial  
223 value.

224 Due to the temperature-dependent thermophysical properties of water, the coupled equations  
 225 (Eq. 6 and 7) within fluid and heat transfer within the meat sample (Eq. 1) and water (Eq. 2)  
 226 were solved as a time-dependent study with a direct solver (strong coupling). To ensure a  
 227 good consistent initial value, the initial time step for the resolution was fixed at  $10^{-7}$  s. Based  
 228 on these computational details, the computational time did not exceed 4 hours 9 minutes and  
 229 12 seconds on a Dell® Precision TM Workstation computer, equipped with 2×Intel® Xeon  
 230 processors (8 cores), at 2.5 GHz, with 256 GB of RAM, running on Windows®8 Professional,  
 231 64 bits.

232 Several simulations were performed depending on the heating rate being tested (from 1 to  
 233 13 °C/min).

#### 234 2.2.6 Microbial inactivation model

235 The dynamic non log-linear model developed by Geeraerd et al. (2000) was selected in this  
 236 study. The reduced model, without the tailing effect, consists of two coupled ordinary  
 237 differential equations as follows:

$$238 \quad \frac{dN}{dt} = -k_{\max} \left( \frac{1}{1 + C_c} \right) N \quad (10)$$

$$239 \quad \frac{dC_c}{dt} = -k_{\max} C_c \quad (11)$$

240 where  $N$  represents the microbial cell density (CFU/g). In equation 10,  $\frac{dN}{dt}$  is forced to zero  
 241 once the limit of detection (1 CFU/g) is reached.  $k_{\max}$  denotes the specific inactivation rate ( $s^{-1}$ )  
 242 and  $C_c$  is related to the physiological state of cells (-). A single value for  $C_c(0)$  can be used  
 243 for all experiments, since the methodology of inoculation is standardized. Consequently, a  
 244 similar initial physiological state is expected for cells

245 The temperature effect on kinetic parameters ( $k_{\max}$ ) was expressed using Bigelow's (1921)  
 246 equation:

$$247 \quad k_{\max}(T) = \frac{\ln 10}{D_{ref}} \exp \left( \frac{\ln 10}{z} (T - T_{ref}) \right) \quad (12)$$

248 where  $D_{ref}$  denotes the decimal reduction time (s) at the reference temperature  $T_{ref}$  and  $z$ -value  
 249 the thermal resistance constant ( $^{\circ}\text{C}$ ).

### 250 2.2.7 Parameter estimation procedure

251 The approach consists in estimating the model parameters of Equation 12 by minimizing the  
 252 residual sum of squares (RSS):

$$253 \quad RSS = \sum_{i=1}^n \left( \log \frac{\frac{1}{V} \int N_{simu}(t_i) dv}{N_0} - \log \frac{\overline{N}_{exp}(t_i)}{N_0} \right)^2 \quad (13)$$

254 where  $t_i$  are the times at which the thermocouple located in the sample reached the expected  
 255 the temperatures (respectively 50, 52, 54, 56, 58, 60, 62 and 64  $^{\circ}\text{C}$ ),  $n$  is the number of  
 256 experimental data points,  $N_{simu}$  is the volume-averaged value of the simulated cell density at  $t_i$ .  
 257  $N_{exp}$  is the volume-averaged value of the triplicate experimental cell density at  $t_i$ .  $V$  is the  
 258 volume of the sample and  $N_0$  is the initial cell density.

259 To minimize the RSS, the Levenberg-Marquardt algorithm (*lsqnonlin* available in  
 260 Matlab<sup>®</sup>7.10) was used. This algorithm is based on the least-squares minimization technique  
 261 and is an improvement of the Gauss-Newton algorithm. In addition, confidence intervals at  
 262 95% were calculated for the estimated parameters using *nlparci* function available in  
 263 Matlab<sup>®</sup>7.10.

### 264 2.2.8 Methodology used for model comparison and selection

265 Considering the model proposed by Geeraerd et al. (2000), it appears that parameters  $C_c(0)$ ,  
 266  $D_{ref}$  and  $z$ -value can be estimated to fit the experimental data. Nevertheless, it is necessary to  
 267 evaluate the relevance of the number of adjusted parameters. In this contribution, a non-linear  
 268 least squares approach based on minimization of the RSS (residual sum of squares) is used. It  
 269 evaluates the well-known Akaike Information Criterion (AIC) (Akaike, 1974), which  
 270 provides a means for model selection. AIC is established as follows, on the assumption that  
 271 the residues distribution is Gaussian:

$$272 \quad AIC = 2p - 2 \ln(LL) \quad (14)$$

273 Where  $p$  is number of independently adjusted parameters within the model and  $LL$  is the  
 274 maximum likelihood. If the AIC criterion is used only with the aim of comparing models, the  
 275 following simplified expression is used:

$$276 \quad AIC^* = 2 p + \frac{RSS}{\sigma^2} \quad (15)$$

277 When the residual sum of squares are almost equal for two different models, the minimum  
 278 AIC estimation selects the model with the lowest number of parameters according to the  
 279 “principle of parsimony” (Yamaoka, Nakagawa, & Uno, 1978). In addition, a variance  $\sigma^2$  was  
 280 calculated for each heating rate from the experimental results obtained in triplicate.

### 281 **3. Results and discussion**

#### 282 ***3.1 Specific heat of ground beef***

283 The apparent specific heat ( $C_p$ ) of ground beef at 5% fat was determined from 20 to 65 °C. As  
 284 can be observed in Figure 4, the  $C_p$  values were found to be quite constant on the temperature  
 285 range. The average measured value ( $C_p = 3.69 \pm 0.01 \text{ kJ kg}^{-1} \text{ K}^{-1}$ ) is close to the reported one  
 286 in the literature for meat product (Zhang, Lyng, Brunton, Morgan, & McKenna, 2004). The  
 287  $C_p$  average value was integrated directly into the heat transfer model.

#### 288 ***3.2 Heat transfer and CFD model validation***

289 To validate the modelling approach, the experimental temperatures measured as a function of  
 290 time in both the water and the sample were compared to the one simulated from the CFD-heat  
 291 transfer model. Figure 5 illustrates the case for a heat treatment at 10 °C/min. The standard  
 292 deviation of the experimental data was about  $\pm 0.3$  °C, corresponding to the measuring  
 293 accuracy of the thermocouple used. Figure 5 depicts the linear setting temperature. The  
 294 agreement between experimental and model temperatures of the water and the sample is  
 295 noticeable. Non-negligible thermal delays (about 26 s and 57 s) can be observed respectively  
 296 between the water and the sample and the set temperature and the sample, justifying the  
 297 interest of such an approach when heating rates are applied.

298 The CFD modelling enabled prediction of the velocity fields due to the natural convection  
 299 problem within a static enclosure. The Navier-Stokes equation was solved by considering a  
 300 laminar flow. The norm of the velocity field, for example at 10 °C/min, is depicted in Figure 6

301 for different processing times. Fluid motion occurs (illustrated by black arrows in Figure 6),  
302 because of the temperature dependent thermophysical properties of water. Recirculation of the  
303 heating fluid leads to velocity gradients, with a maximum value of 2.5 mm/s around the  
304 sample (norm of the velocity field). The Reynold number was calculated considering this  
305 maximum fluid velocity of 10 °C/min with a value of 9.4. This result clearly demonstrates a  
306 natural convection of water within a confined space, in which the flow regime is laminar.

307 Once the CFD approach is validated, the model illustrates the temperature gradients when  
308 heating rates are applied. Figure 7 illustrates the temperatures at the final time of simulation  
309 for the six different heating rates considered. Simulation was stopped when the simulated  
310 temperature of the corresponding point to the thermocouple position reached 64 °C.

311 For all treatments the hottest zone was located at the surface of the sample, except for the  
312 upper central part, due to contact with ambient air (cooling). The coldest zone was along the  
313 central axis of the product. The temperature gap between the hottest and coldest zones  
314 increased with the applied heating rates, so 4.3, 6.2, 8.1, 9.8, 12.4, and 15.8 °C for 1, 3, 5, 7,  
315 10 and 13 °C/min, respectively.

316 To confirm the effect of temperature heterogeneities on the volumetric microbial inactivation,  
317 equations 10, 11 and 12 were used. The simulation of the 3D local temperature distribution in  
318 the meat sample (CFD and heat transfer model solved by COMSOL® and transposed to  
319 MATLAB®) was performed to quantify the 3D local microbial inactivation. For example,  
320 under a thermal treatment at 10 °C/min, the numerical result illustrated a thermal cartography  
321 with hot and cold points. These temperature heterogeneities lead to heterogeneous local  
322 microbial inactivation represented by the hatched area in Figure 8.

323 In Figure 8, a total inactivation can be seen for the hottest point after 5.8 minutes. For the  
324 coldest point, the maximum inactivation achieved was of 2 Log<sub>10</sub>, at the end of treatment.  
325 However, it is not possible to experimentally validate these local microbial inactivation data,  
326 at every geometrical point of the domain. Consequently, the use of a mathematical model to  
327 describe the local microbial inactivation during a pasteurization process remains a relevant  
328 tool. More, it confirms the importance of considering the volumetric distribution of  
329 temperature in the meat product to accurately predict microbial inactivation.

### 3.3 Estimation of kinetic parameters of inactivation

330 Various results can be found in the literature concerning evaluation of kinetic parameters  
331 ( $D_{ref}$ ,  $T_{ref}$  and  $z$ -value) under isothermal conditions. In a study of heat resistance of  
332 *Escherichia coli* 0157:H7 inoculated into 7% fat ground beef, Ahmed et al. (1995) obtained a  
333  $D_{ref}$ , by linear regression, of 684 s (at 55 °C) and 27 s (at 60 °C), and a  $z$ -value equal to  
334 4.78 °C. In the same strain, but in ground beef with 4.8% fat, Smith et al. (2001) calculated by  
335 first-order kinetics a  $D_{ref}$  equal to 73 s (at 58 °C) and 19 s (at 61 °C), for a  $z$ -value of 3.79 °C.  
336 These variations might be attributed to differences in the strains of *E. coli* used, the fat content  
337 of meat, along with differences in recovery methods and sample size (Smith et al., 2001;  
338 Stringer, George, & Peck, 2000). In both studies, the values of  $D_{ref}$  differ greatly whereas  $z$ -  
339 value remains in the same order of magnitude.  
340

341 In this study, ground beef with 5% fat was used and the heating rates **described above** were  
342 applied. Despite these dynamical conditions, in a first approach, the parameters  $D_{ref} = 73$  s,  $z$ -  
343 value = 3.79 °C and  $T_{ref} = 58$  °C obtained by Smith et al. (2001) in isothermal conditions and  
344  $C_c(0) = 0.23$ , as reported in Hamoud-Agha et al. (2013), were used in **the** Geeraerd et al.  
345 (2000) model. Results are illustrated in Figure 9. Black circles illustrate the experimental data  
346 in triplicate, whereas the **predictions** obtained using the aforementioned parameters are  
347 represented by broken blue lines. **Note** that the model underestimates the experimental  
348 inactivations, but this underestimation has the same order of magnitude whatever the heating  
349 rate.

350 A second approach consisted in estimating the model parameters **from the dynamic**  
351 **experiments**. First, the triplet parameters  $C_c(0)$ ,  $D_{ref}$  and  $z$ -value were estimated. **Poschet et al.,**  
352 **(2005) showed that the uncertainties on  $D_{ref}$  and  $z$ -value are minimal when  $T_{ref}$  is equal to the**  
353 **middle of the lethal experimental temperature range. As  $D_{ref}$  is closely linked to  $T_{ref}$ , this value**  
354 **was fixed at 58 °C, as defined in Smith et al. (2001).** The  $C_c(0)$  value was estimated with a  
355 lower bound equal to zero. In table 3,  $C_c(0)$  is not **significantly different from zero** for all  
356 heating rates. Consequently, equation (11) can be removed and the Geeraerd et al. (2000)  
357 model becomes a classical first order inactivation model.

358 With the Bigelow model, the  $D_{ref}$  and  $z$ -values were estimated (Table 4). It should be noted  
359 that these  $AIC^*$  values are lower than the  $AIC^*$  values obtained in previous estimate procedure  
360 with three parameters (Table 3). The confidence intervals for  $D_{ref}$  increase for extreme heating  
361 rates (10 and 13 °C/min), certainly due to the large thermal heterogeneities. Another

362 **explanation** is based on Van Derlinden, Balsa-Canto, & Van Impe, (2012), where the authors  
363 discussed the link between parameter identifiability and the shape of the temperature profile.  
364 The results of  $z$ -value indicated in Table 4 are consistent with those presented by Smith et al.  
365 (2001), for a similar product at 58 °C ( $z$ -value = 3.79 °C). Indeed, this value (3.79 °C) is  
366 almost included in the confidence intervals and will thus be considered hereafter.

367 With the estimation limited to  $D_{ref}$ , the confidence intervals are widely reduced, even if they  
368 remain significant for heating rates of 10 and 13 °C /min (12.52 s and 8.75 s). These results  
369 are summarized in Table 5. Compared to Table 4, quite similar or lower values of the AIC\*  
370 were obtained. **It appears that adjusting  $D_{ref}$  is sufficient to fit the experiments without degrading  
371 the AIC\* values. Moreover, it also reduces the parameter uncertainties as verified with 95%  
372 confidence intervals.**

373 Thereby, for one estimated parameter, the  $D_{ref}$  values remain in the same order of magnitude.  
374 **Consequently, the average of the previously estimated  $D_{ref}$  values (Table 5) of 47 s was used.**  
375 The predicted inactivation curves obtained in this case are presented in black continuous line  
376 in Figure 9. They are very close to inactivation results obtained with each estimated  $D_{ref}$  (red  
377 **dotted line** is superimposed on black continuous line in figure 9). **Hence, for  $D_{ref}$  values  
378 ranging from 40 to 50 s, considering a unique value of 47 s does not significantly affect the  
379 inactivation predictions.**

380 **Finally, results reveal that the  $k_{max}$  expression with temperature dependence proposed by  
381 Bigelow is sufficient to fit experimental data obtained in dynamic conditions with large  
382 temperature heterogeneities (>15 °C for 13 °C /min), provided that these heterogeneities are  
383 considered in the prediction model. Constant values for  $T_{ref}$ ,  $z$  and  $D_{ref}$  can be considered for  
384 all heating rates in the range 1 to 13 °C /min. Additionally,  $T_{ref}$  and  $z$ -values of literature  
385 (Smith et al., 2001) were used with only a fitting of  $D_{ref}$ .**

#### 386 **4. Conclusion**

387 **The contribution of this work lies in the integration of experimental and numerical approaches  
388 dedicated to the real-food pasteurization process including both thermal and inactivation  
389 kinetics with high heating rates.**

390 The originality of this paper lies (*i*) in the integration of three-dimensional heat transfer  
391 conditions in parameter estimations applied to the inactivation model and (*ii*) in the  
392 application of extreme heating rates to challenge the validity of the Geeraerd modelling



393 approach developed for isothermal conditions. The heat transfer model was validated during  
394 blank tests, and the volume-average of logarithmic inactivation was considered to be  
395 comparable with experimental data. In addition, the experiments were carried out on real  
396 ground beef, and not in capillary tubes, as usually proposed. The inactivation model was  
397 coupled with heat transfer to design a 3D simulator to predict the spatial distribution of  
398 logarithmic inactivation of *E. coli* in a meat sample.

399 Such a 3D simulator illustrates how the temperature gradients can provoke large inactivation  
400 heterogeneities, and thus the necessity to consider them in realistic pasteurization conditions.  
401 The satisfactory agreement between model and experiments allows coupling of the modelling  
402 approach with process control procedures, allowing energy supply to reach the expected  
403 microbial inactivation while limiting temperature heterogeneities. Such an objective could be  
404 reached for example by using combined energy sources with volumetric heating to avoid  
405 overtreatment at the surface of the food product (microwaves, ohmic heating).

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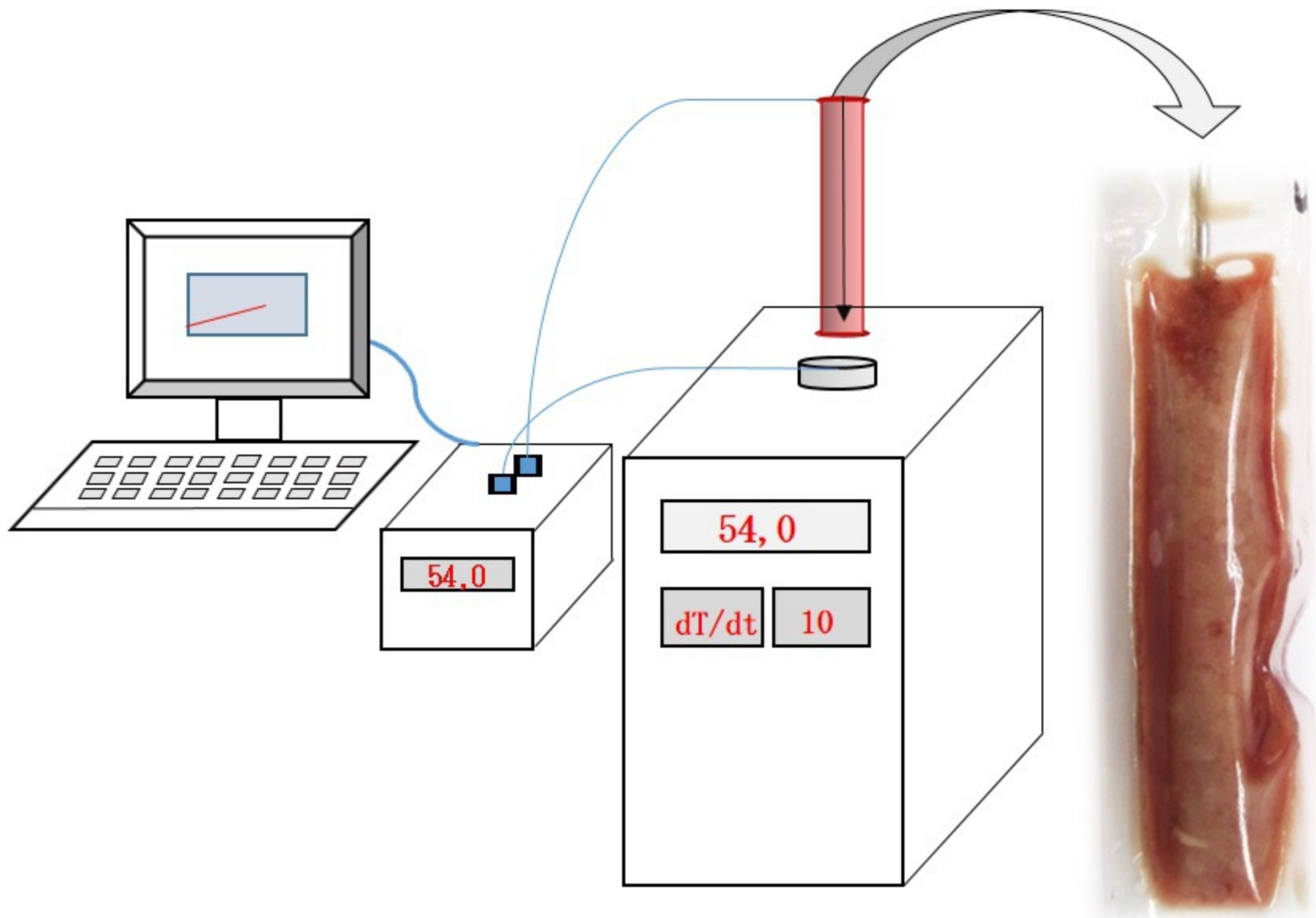
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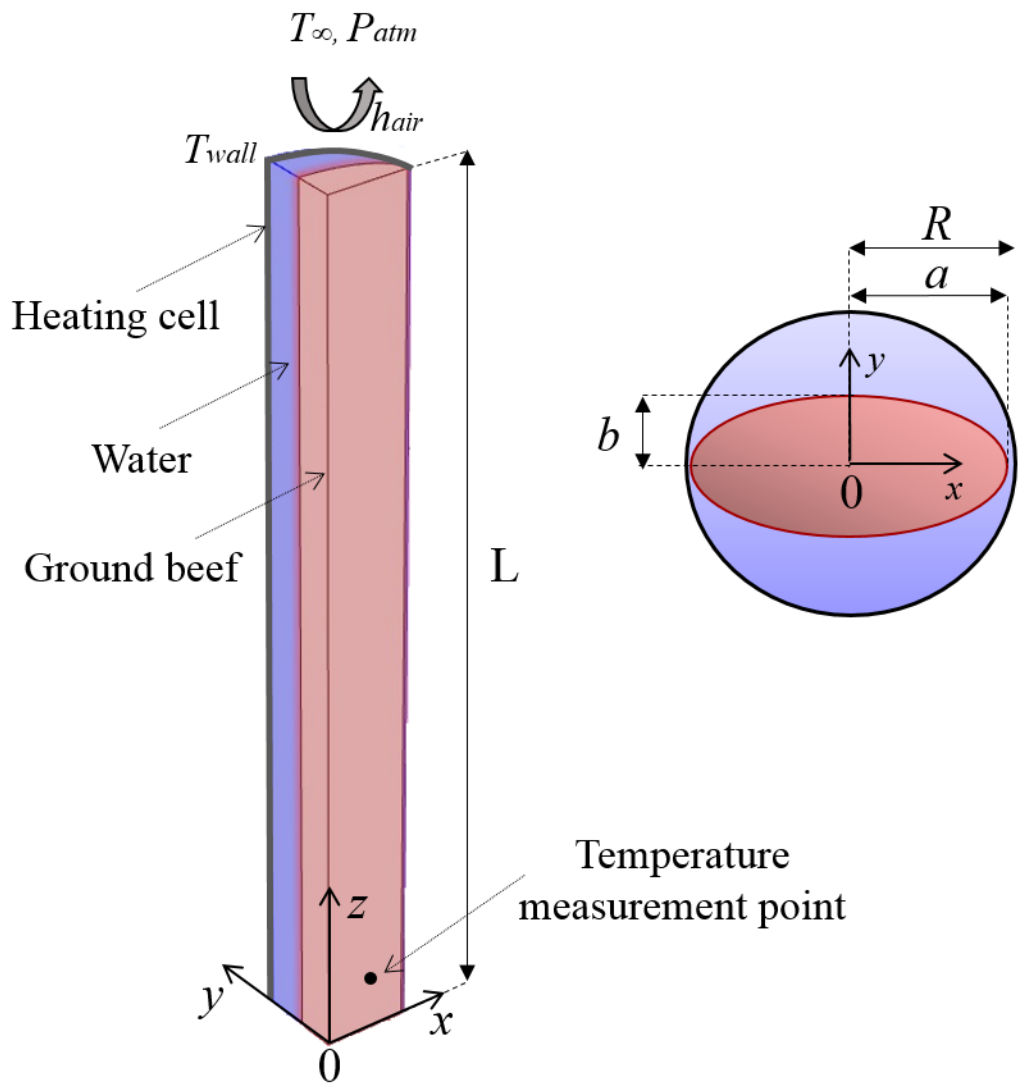
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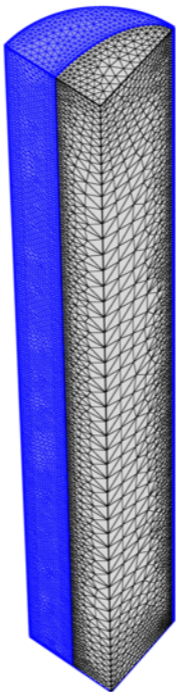
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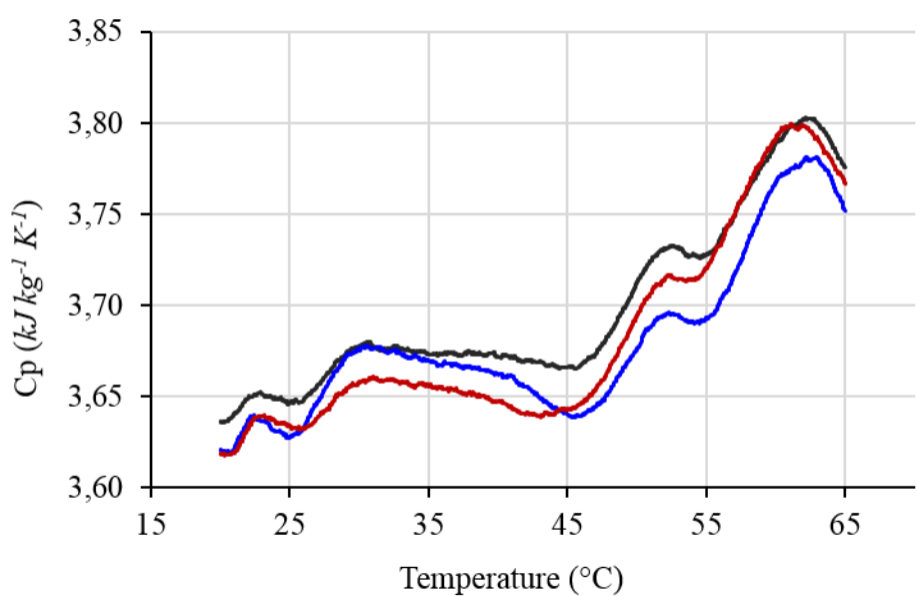
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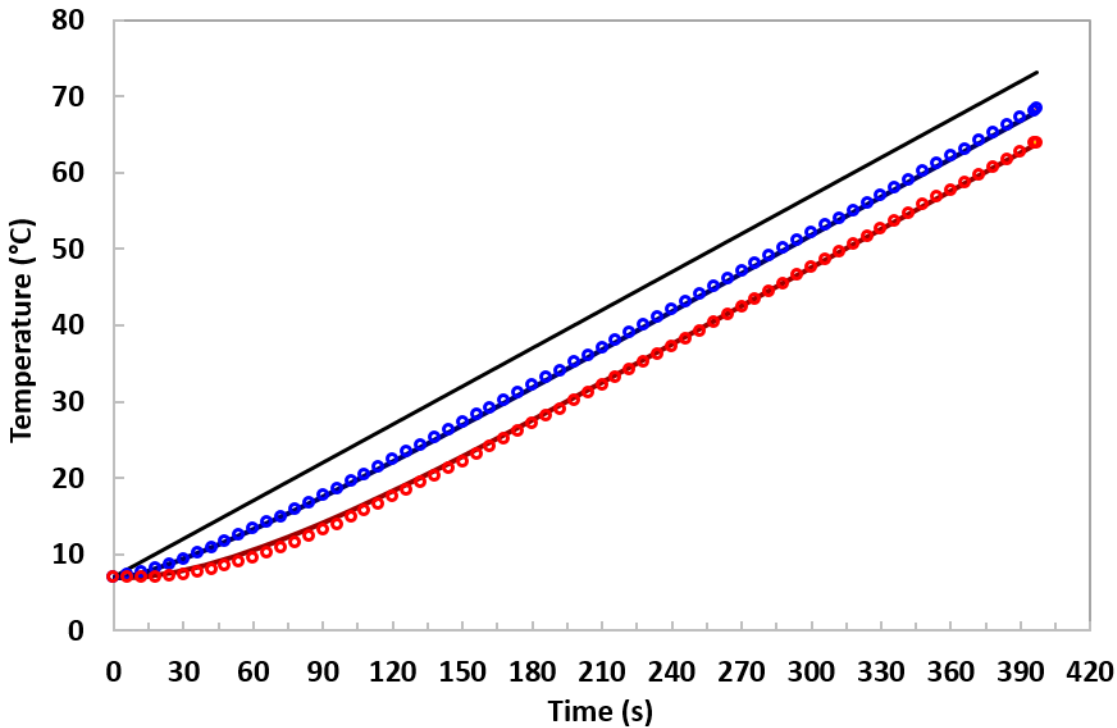




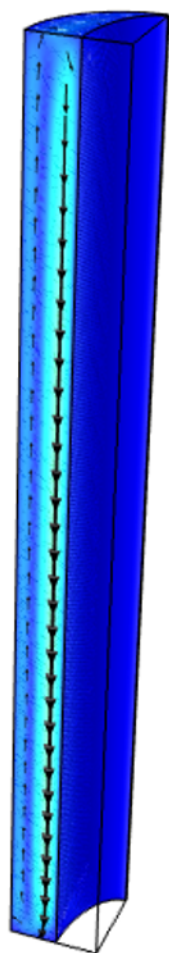
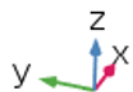








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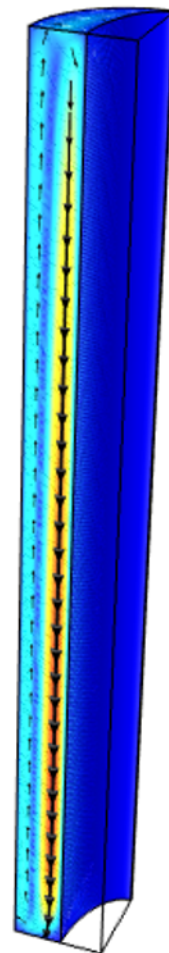
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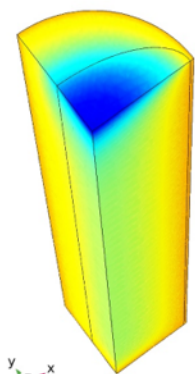
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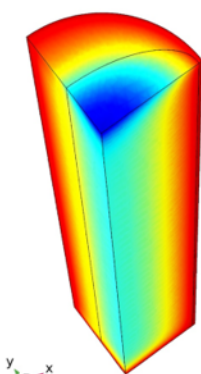
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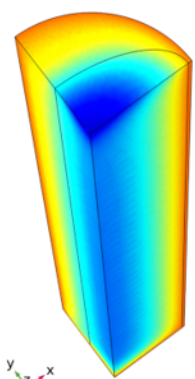
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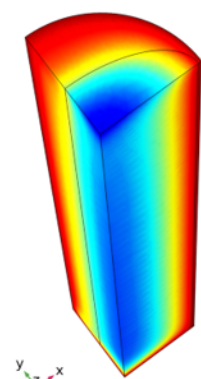
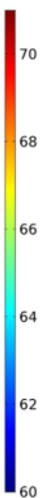
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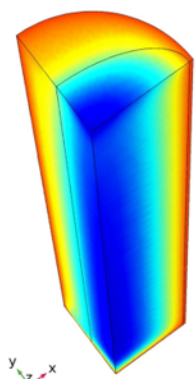
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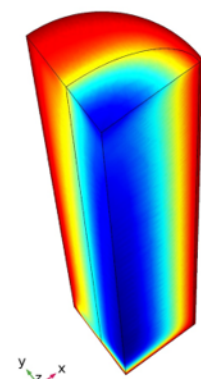
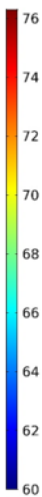
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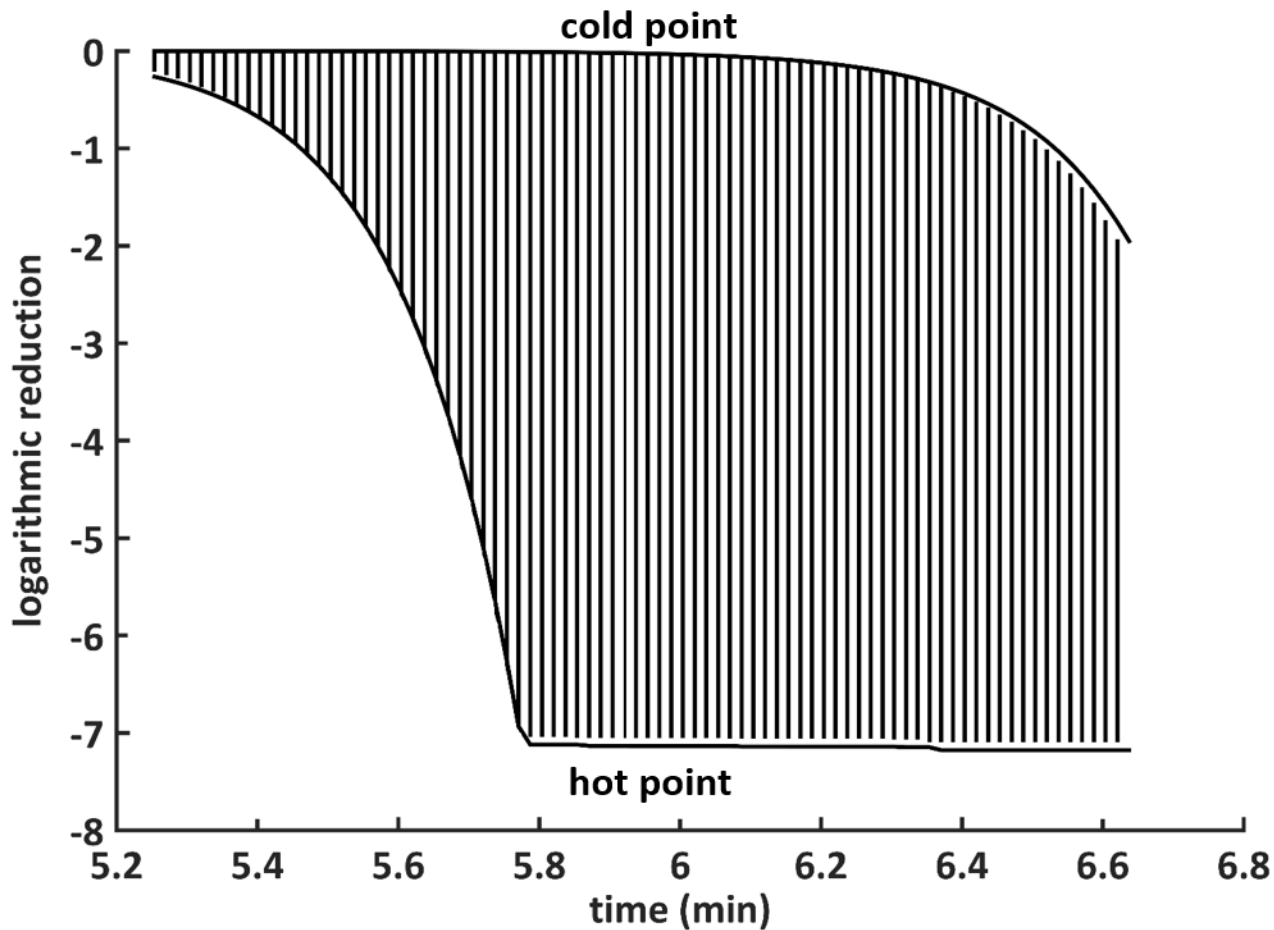
7 °C/min



10 °C/min



13 °C/min



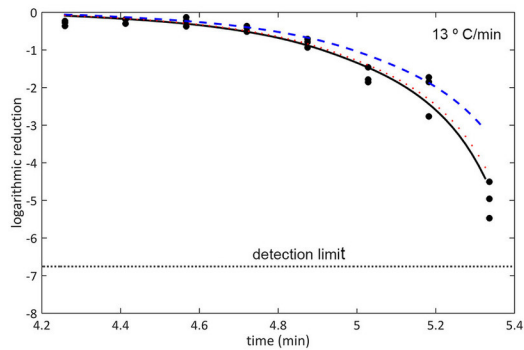
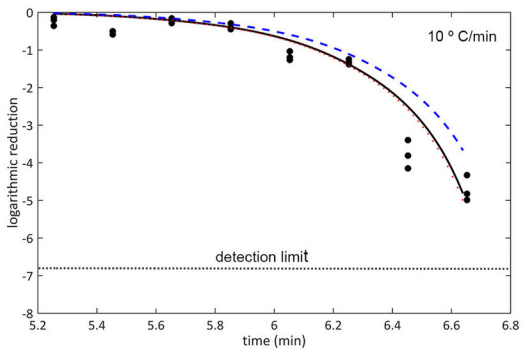
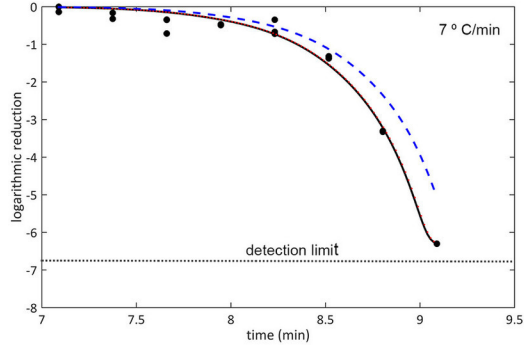
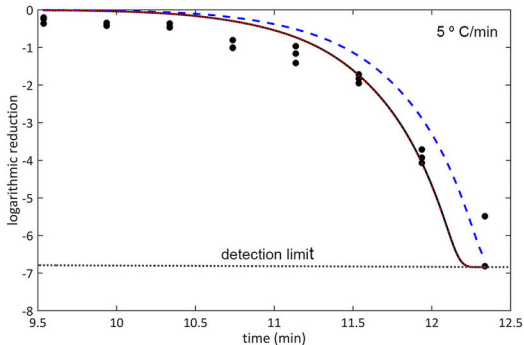
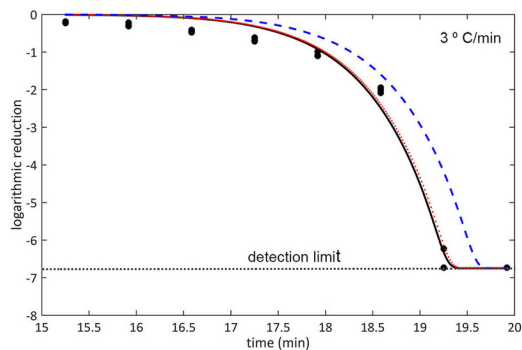
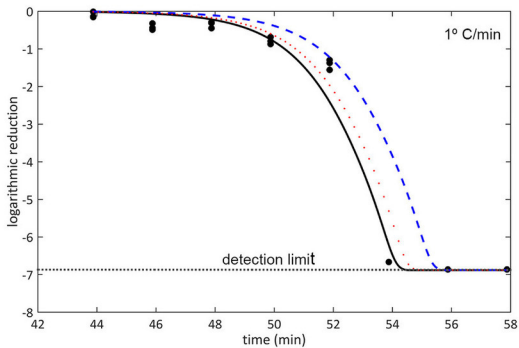


Table 1. Thermophysical properties of ground beef.

Properties	Value	Unit	Source
Apparent specific heat ( $C_p$ )	$3.69 \pm 0.01$	$\text{kJ kg}^{-1} \text{K}^{-1}$	Experimental
Thermal conductivity ( $\lambda$ )	0.35	$\text{W m}^{-1} \text{°C}^{-1}$	Pan and Singh (2001).
Density ( $\rho$ )	1006	$\text{kg/m}^3$	Pan and Singh (2001).

Table 2. Thermophysical properties of water as a function of temperature (Kelvin).

Functions of the properties*	Unit
$C_p = 12010.15 - 80.41 \times T + 0.31 \times T^2 - 5.38 \times 10^{-4} \times T^3 + 3.62 \times 10^{-7} \times T^4$	$\text{J kg}^{-1} \text{K}^{-1}$
$\lambda = -0.87 + 8.95 \times 10^{-3} \times T - 1.58 \times 10^{-5} \times T^2 + 7.97 \times 10^{-9} \times T^3$	$\text{W m}^{-1} \text{°K}^{-1}$
$\rho = 838.47 + 1.40 \times T - 3.01 \times 10^{-3} \times T^2 + 3.72 \times 10^{-7} \times T^3$	$\text{kg /m}^3$

\*Green & Perry, 2007

Table 3. Estimated values of  $D_{\text{ref}}$ ,  $z$ -value and  $C_c(0)$ , for  $T_{\text{ref}} = 58 \text{ °C}$ . Standard deviation of experimental data ( $\sigma^2$ ), 95% confidence intervals (CI) and simplified Akaike Information Criterion (AIC\*).

Heating rate ( $\text{°C /min}$ )	$\sigma^2$	$D_{\text{ref}}$ (s)	CI $D_{\text{ref}}$	$z$ -value ( $\text{°C}$ )	CI $z$ -value	$C_c(0)$	CI $C_c(0)$	AIC*
1	0.24	33.1	3.8	1.96	1.41	0	0.30	11.8
3	0.20	48.9	6.8	3.81	0.63	0	0.21	12.2
5	0.23	41.7	5.3	7.32	1.53	0.11	0.15	10.2
7	0.34	45.7	8.1	3.59	0.61	0.21	0.18	9.3
10	0.50	56.2	22.4	6.19	2.3	0.08	0.17	11
13	0.63	74.7	33.1	2.99	0.69	0.04	0.15	9.2

Table 4. Estimated values of  $D_{\text{ref}}$  and  $z$ -value ( $T_{\text{ref}} = 58 \text{ }^\circ\text{C}$ ). Standard deviation of experimental data ( $\sigma^2$ ), 95% confidence intervals (CI) and simplified Akaike Information Criterion (AIC\*).

Heating rate ( $^\circ\text{C}/\text{min}$ )	$\sigma^2$	$D_{\text{ref}}$ (s)	CI $D_{\text{ref}}$	$z$ -value ( $^\circ\text{C}$ )	CI $z$ -value	AIC*
1	0.24	33.1	3.8	1.96	1.41	8.8
3	0.20	48.9	6.8	3.81	0.63	9.2
5	0.23	40.8	5.1	6.70	1.41	7.2
7	0.34	31.4	12.4	4.80	0.81	7.2
10	0.50	62.1	23.2	5.98	2.65	8.2
13	0.63	73.1	32.6	3.02	0.71	6.6

Table 5. Estimated values of  $D_{\text{ref}}$  ( $z = 3.79$  and  $T_{\text{ref}} = 58 \text{ }^\circ\text{C}$ ). Standard deviation of experimental data ( $\sigma^2$ ), 95% confidence intervals (CI) and simplified Akaike Information Criterion (AIC\*).

Heating rate ( $^\circ\text{C}/\text{min}$ )	$\sigma^2$	$D_{\text{ref}}$ (s)	CI $D_{\text{ref}}$	AIC*
1	0.24	40.1	0.03	9
3	0.20	47.5	0.38	6.2
5	0.23	50.0	0.59	8.2
7	0.34	50.0	0.48	3.6
10	0.50	50.1	12.52	6.8
13	0.63	46.7	8.75	3.6