Heat inactivation of *Listeria innocua* in broth and food products under non-isothermal conditions

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A B S T R A C T

The objective of this work was to study the effect of three linear temperature profiles (heating rates of 1.5, 1.8 and 2.6 °C/min, from 20 to 65 °C) on *Listeria innocua* inactivation in liquid medium. The inactivation was also analyzed in artificially contaminated parsley (heating rate of 1.8 °C/min) and throughout a frying process, using a pre-cooked frozen food as case study. Inactivation showed a sigmoidal behaviour and all data was fitted with a Gompertz-inspired model. Results demonstrated that, in liquid media, *Listeria* inactivation is influenced by the temperature profile used. As heating rate increases, the shoulder decreases and the tail effect disappears. If *Listeria* was in parsley, its heat resistance increased (for identical experimental conditions in broth). Besides model adequacy was proven in all studied situations, the heating rate affected parameters' precision.

1. Introduction

Listeria monocytogenes is an actual concern in food contamination due to its ubiquitous characteristics and pathogenicity. In many situations, *Listeria innocua* is elected as the non-pathogenic specie to be used as a surrogate for *L. monocytogenes* (Kamat & Nair, 1996; Margolles, Mayo, & Reyes-Gavilán, 2000; Piyasena, Liou, & McKellar, 1998). Because of the occurrence of *Listeria* spp. in thermally processed products, studies on the evaluation of its heat resistance are of main importance. *Listeria* can grow at refrigeration temperatures and can even survive in frozen conditions. This is also a concern for pre-cooked meals.

Much work has been done at laboratory scale to clarify the *Listeria* behaviour during a heat treatment. In this situation, environmental factors (such as temperature, water activity, pH, etc.) can be easily controlled. The microbial response to these stressing factors is, in many cases, attained in broth. If the microorganism is present in a food, its behaviour will be certainly different due to food constituents' characteristics, surface topology and microbial adhesion.

When realistic food processing conditions occur (pasteurisation is one example that includes a come-up time), the temperature history affects the microbial behaviour. Bacteria submitted to non-isothermal conditions (i.e. temperature rises throughout the process time till a target value) are more heat resistant than bacteria treated isothermally. The imposed stresses will determine the protective cell response (Marechal, Martínez de Marnañón, Poirier, & Gervais, 1999).

If results obtained isothermally are used for predictions in dynamic temperature conditions, hazardous under-estimations of microbial load may occur (thus compromising safety). Studies on this topic are clearly important.

One interesting case, in which temperature rising directly affects microbial viability, is the pre-cooked foods branch. Frying is one example of the thermal processes involved, and studies on microbial inactivation via frying are an interesting field. Usually, foods are fried until its centre has received enough heat to destroy possible contaminant microorganisms and/or to change desired sensory properties. In immersion oil frying, the medium is heated to high temperatures (≅180 °C), resulting in reduced processing time. This can be a problem when the frying product is frozen (the cases of pre-cooked meals stored under frozen conditions). The time of frying may not be enough for the coldest point to reach the required temperatures. In situations of contamination in a pre-frozen stage, serious outbreaks may occur.

The heat resistance of microorganisms is often assessed by calculation of *D*-values (i.e. the time needed to reduce the population by 90%). However, this approach becomes restricted in situations where survival curves deviate from log-linearity. Depending on the environmental conditions, the survival curves

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may exhibit a sigmoidal shape with an initial delay period (or shoulder), followed by a maximum inactivation rate tending to a residual tail population. It is not consensual whether the tail is an actual observed heat resistant population or an experimental limitation of the enumeration procedure. However, in literature, some kinetic models have been applied and discussed aiming at describing conveniently such tendencies (Cole, Davies, Munro, Holyoak, & Kilsby, 1993; Juneja & Eblen, 1999; Juneja & Marks, 2003; Murphy, Marks, Johnson, & Johnson, 2000; Peleg, Penchina, & Cole, 2001). Among the models used, Gompertz-inspired functions have been successfully applied in sigmoidal inactivation kinetics, both in isothermal and time-varying temperature conditions (Bhaduri et al., 1991; Gil, Brandão, & Silva, 2006; Linton, Carter, Pierson, & Hackney, 1995).

The objectives of this study were: (i) to evaluate the influence of different time-varying temperature processes (with different heating rates) on inactivation kinetics of *L. innocua* in broth, (ii) to compare the bacterium kinetics in broth and in a food (parsley) using identical non-isothermal conditions, and (iii) to follow the survival of *L. innocua* during an entire frying process of a frozen pre-cooked product (meat pockets used as case study).

2. Materials and methods

2.1. Experimental procedures

2.1.1. Cultures

L. innocua NCTC 10528 was subcultured (30 °C, 24 h) in Tryptic Soy Broth – TSB (Lab M, Lancashire, UK) containing 0.6% yeast extract – TSBYE (Lab M). Cultures were maintained at 7 °C on Tryptic Soy Agar – TSA (Lab M) supplemented with 0.6% yeast extract – TSAYE.

2.1.2. Preparation of cultures

The second subculture of *L. innocua* was incubated at 30 °C for 20 h to yield stationary phase cultures. This cell growth phase was chosen due to its higher stress resistance than exponential phase cells (Miller, Gil, Brandão, Teixeira, & Silva, 2009).

Cells in each cellular suspension were enumerated by plating appropriate dilutions, in duplicate, on the three solid media studied.

2.1.3. Heat treatments

2.1.3.1. Temperature profiles. Heat treatments were carried out in a thermostatic bath (Julabo® FP40, Seelbach, Germany) with a temperature programmer (Julabo® HC-E07, Seelbach, Germany).

Three temperature profiles were assumed, with temperatures varying from approximately 20–65 °C at linear increasing rates; T (°C) = 21.6 + $\beta \cdot t$(min). The media temperature was then held at 65.0 °C for different periods of time, depending on the heating rate considered: (a) $\beta = 1.5$ °C min^{-1} (followed by 20 min at ~65.0 °C); (b) $\beta = 1.8$ °C min^{-1} (followed by 10 min at ~65.0 °C); (c) $\beta = 2.6$ °C min^{-1} (followed by 12 min at ~65.0 °C).

The temperature profiles correspond to actual temperatures measured in broth/food using K-type thermocouples (±0.2 °C).

2.1.3.2. Experiments in broth. 99 ml of TBSYE, used as heating medium, were dropped in an Erlenmeyer flask, which was immersed in the water bath and inoculated with 1 ml of cell suspension. The samples were continuously agitated, using a magnetic stirrer, removed at different time intervals and placed in a mixture of ice-water for posterior cell enumeration.

There was a control for each experiment, which consisted of another 99 ml of TSBYE inoculated with 1 ml of the same cellular suspension and incubated at 30 °C for the same time. The initial concentration of *L. innocua* was approximately 10^7 cfu/ml.

Three replicates of all these experiments were performed.

2.1.3.3. Experiments in foods

2.1.3.3.1. Parsley. Parsley (*Petroselinum crispum*) samples were artificially inoculated by immersion in a bacterial suspension with approximately 10^7 cfu/ml, for approximately 10 s. The samples were sealed and vacuum-packaged (Multivac A300/41/42, Wolfertschwenden, Germany) in sterilized bags. These were immersed in the thermostatic agitated water bath and subjected to a heat treatment corresponding to the temperature profile (*b*). Thermocouples were placed inside the plastic bags to measure actual temperature profile in parsley samples. Bags were positioned such that entire parsley samples were submerged and each bag was removed at different time intervals and placed in a mixture of ice-water. For each time, sterile peptone water was added to approximately 3 g of parsley and bags were pummelled for 4 min in a stomacher at normal speed. Cell enumeration was then carried out.

Uncontaminated parsley and contaminated parsley samples, which were not submitted to heat treatment, were used as controls.

2.1.3.3.2. Meat pockets. Commercially available frozen *meat pockets* (i.e. dough stuffed with sliced cooked pork and beef; it is a frozen pre-cooked product that should be fried before consumption) were artificially contaminated with *Listeria* inoculums, by injecting in the centre of the product 250 μl of the bacterial suspension with approximately 10^7 cfu/ml. The *meat pockets* were maintained at −8 °C for 1 h, prior to the heating process. Frozen meat pockets were fried in a common fryer at 180 °C (oil temperature) for a maximum of 8 min. The temperature history was monitored during the whole frying process using K-type thermocouples, placed in the centre of the samples. The values were recorded using a squirrel data logger (Grant Instruments 1023, Cambridge, England).

Samples (one sample is one *meat pocket*) were removed at different time intervals, placed in sterilized plastic bags and immersed in a mixture of ice-water. The meat of each sample was removed from the meat pocket and mixed with sterile peptone water in a stomacher bag, where it was blended by a Stomacher. Cell enumeration was then carried out.

Uncontaminated frozen meat pocket and contaminated frozen meat pocket that was not subjected to frying process were used as controls.

Three replicates of the frying process were performed.

2.1.4. Enumeration

Samples from heat treatments in liquid medium were serially diluted and plated in duplicate onto three different media: (i) TSAYE, (ii) TSAYE supplemented with 5% (w/v) sodium chloride – TSAYE + NaCl and (iii) Palcam Agar plus selective supplement (Miller, Brandão, Teixeira, & Silva, 2006). Samples from heat treatments in food products (parsley and meat pockets) were also diluted and plated in duplicate, but only in Palcam Agar plus selective supplement due to the presence of natural food microflora.

All plates were incubated at 30 °C and counted each 24 h during 5 days, or until the number of colony formation units no longer increased.

Average values of bacterial counts, from duplicate plate samples, were converted to log numbers for each combination.

2.2. Modelling procedures

2.2.1. The inactivation model

Assuming that the microbial thermal inactivation follows a sigmoidal behaviour, isothermal experimental data can be

mathematically described by a re-parameterized Gompertz-inspired model (Bhaduri et al., 1991; Char, Guerrero, & Alzamora, 2009; Gil et al., 2006; Huang, 2009; Linton et al., 1995):

$$\log\left(\frac{N}{N_0}\right)_{\text{isothermal}} = \log\left(\frac{N_{\text{res}}}{N_0}\right)\exp\left(-\exp\left(\frac{-k_{\max}e}{\log\left(\frac{N_{\text{res}}}{N_0}\right)}(L-t)+1\right)\right) \quad (1)$$

where N is the microbial cell density at a particular process time, t. The indexes 0 and res indicate initial and residual (or tail) microbial cell density, respectively. The model parameters are L (initial shoulder), $k_{\max}$ (maximum inactivation rate) and $\log(N_{\text{res}}/N_0)$ (tail).

The versatility of fitting linear data and those that contain shoulder and/or tailing effects make Gompertz one attractive model.

Microbial kinetic parameters are temperature dependent. Such dependence has been extensively studied and several models have been proposed in literature.

In relation to shoulder, the following polynomial equation was used (Ratkowsky, Olley, McMeekin, & Ball, 1982):

$$L = c(T-d)^2 \quad (2)$$

with c and d parameters.

The dependence of $k_{\max}$ on temperature was expressed by the well-known Arrhenius behaviour, using a finite reference temperature (T_{ref}) to reduce parameters' collinearity (Cohen, Birk, Mannheim, & Saguy, 1994; Haralampu, Saguy, & Karel, 1985; Van Boekel, 1996):

$$k_{\max} = k_{\text{ref}}\ \exp\left(-\frac{\text{Ea}}{R}\left(\frac{1}{T}-\frac{1}{T_{\text{ref}}}\right)\right) \quad (3)$$

where k_{ref} is the inactivation rate at a finite reference temperature, Ea the activation energy and R the universal gas constant.

For non-isothermal conditions, temperature varies with time (i.e. dynamic temperature conditions). The number of log cycles reduction (in relation to initial load), at a particular time, can be calculated by encompassing the time–temperature effect as follows:

$$\log\left(\frac{N}{N_0}\right)_{\text{non-isothermal}} = \int_0^t \frac{\mathrm{d}\left(\log\left(\frac{N}{N_0}\right)_{\text{isothermal}}\right)}{\mathrm{d}t}\mathrm{d}t' \quad (4)$$

In such conditions, L and $k_{\max}$ are time dependent and the previous expression derives to:

$$\log\left(\frac{N}{N_0}\right)_{\text{non-isothermal}} = \int_0^t \left[-k_{\max}(t')e\exp\left(-\frac{k_{\max}(t')e}{\log\left(\frac{N_{\text{res}}}{N_0}\right)}(L(t')-t')+1\right)\exp\left(-\exp\left(-\frac{k_{\max}(t')e}{\log\left(\frac{N_{\text{res}}}{N_0}\right)}(L(t')-t')+1\right)\right)\right]\mathrm{d}t' \quad (5)$$

If the temperature history $T(t)$ is known, the model valid for non-isothermal conditions can be obtained by merging Eqs. (2), (3) and (5) (Gil et al., 2006).

2.2.2. Data analysis

The parameters of the Gompertz-inspired inactivation model for non-isothermal conditions, i.e. k_{ref}, Ea, c, d and $\log(N_{\text{res}}/N_0)$, were estimated by non-linear regression analysis, fitting Eq. (5) to experimental data. The reference temperature in the Arrhenius equation was considered equal to 58.5 °C.

The simplex algorithm (Nelder & Mead, 1965) was used to minimise the sum of the squares of the residuals.

The quality of the regression was evaluated by the coefficient of determination (R^2), randomness and normality of the residuals.

Parameters' precision was evaluated by the standardised half width (SHW) at 95%, i.e. halved confidence interval divided by the estimate $\equiv$ (confidence interval$_{95\%}$/2) $\times$ (1/estimate) $\times$ 100.

All regression analysis procedures and calculations were performed in programs specially written in FORTRAN 77 language (Fortran 5.1, Microsoft Corporation®, 1990) and Microsoft® Excel 2000 (Microsoft Corporation, USA).

3. Results and discussion

The experimental inactivation data of *L. innocua* in broth (TSBYE) and fitted curves are included in Fig. 1. When the heating treatment with temperature profile (a) was applied (lower heating rate of 1.5 °C/min), the temperature of 65 °C was attained in approximately 31 min (Fig. 1(a)). At this time, and depending on the enumeration media considered, different results were observed. *L. innocua* decreased 1.7, 2.5 and 4.4 log cycles respectively, if TSAYE, Palcam Agar and TSAYE + NaCl were used.

If temperature profile (b) was applied (heating rate of 1.8 °C/min), the temperature of 65 °C was attained in approximately 25 min (Fig. 1(b)). In this case, *Listeria* cells decreased 2.5, 4.0 and 4.4 log, when enumeration was performed on TSAYE, Palcam Agar and TSAYE + NaCl, respectively.

In the case of temperature profile (c) (heating rate of 2.6 °C/min), 18 min were necessary to achieve 65 °C (Fig. 1(c)). The log cycles reductions were 1.6, 2.3 and 4.5 on TSAYE, Palcam Agar and TSAYE + NaCl, respectively.

It is notorious the effect of the heating rate on the induced heat resistance of *L. innocua*, assessed by the sigmoidal tendency of the inactivation kinetics. As heating rate increases, the shoulder decreases and the tail effect disappears. This was observed for all media considered. In Fig. 2, this effect can be better visualized for the case of TSAYE medium.

Valdramidis, Geeraerd, Bernaerts, and Van Impe (2006) obtained similar conclusions for the inactivation of *Escherichia coli*, applying heating rates from 0.15 to 1.64 °C/min (in the range 30–55 °C). These authors reported that microbial heat resistance was utmost perceived if the lowest heating rates were considered (with a corresponding come-up time higher than 30.61 min). This was also observed by Marechal et al. (1999) in the case of *Saccharomyces cerevisiae*, when linear temperature increases with heating rates from 0.21 to 1.04 °C/min (in the range 25–50 °C), were used. Ramos et al. (2001) explained these facts by the synthesis of a series of heat shock proteins (HSPs) induced by stressing temperature variations. Other microbial protective responses can be explained by modifications of the permeability of cells' membrane and by changes on cellular internal solute composition (Marechal et al., 1999). All these changes provide microbial potential ability to withstand lethal temperatures.

It was assumed that during heating-up phases no microbial growth occurred and there was a temperature limit below which no

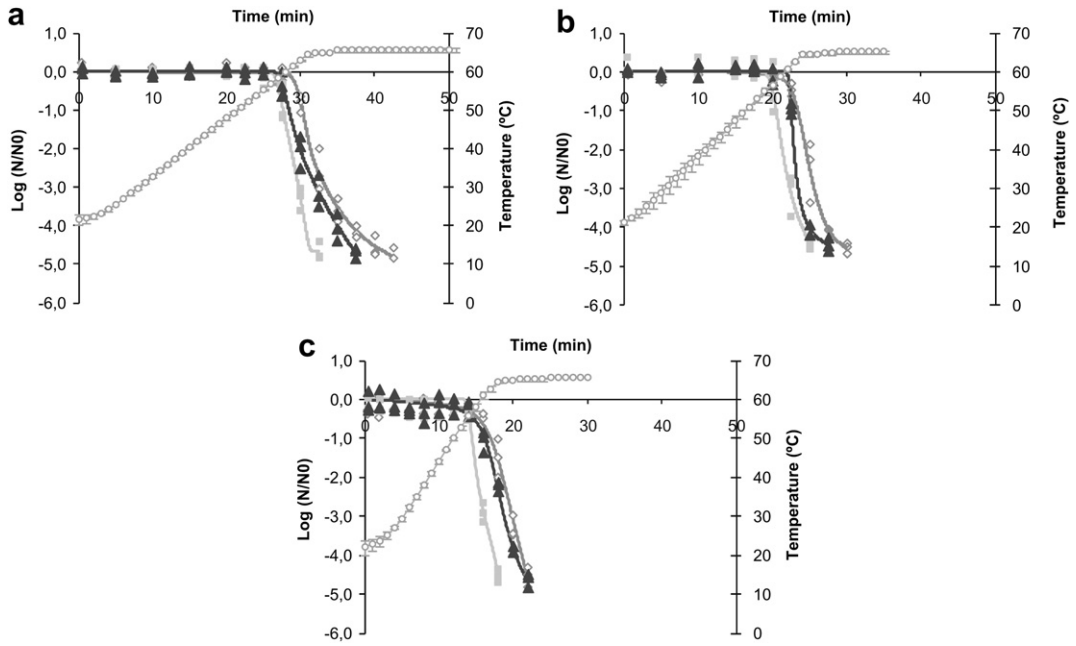

Fig. 1. Thermal inactivation data of *L. innocua*, in liquid medium, at different heating rates: (a) 1.5 °C/min; (b) 1.8 °C/min and (c) 2.6 °C/min, using three different enumeration media: (◇) TSAYE, (▲) Palcam Agar and (■) TSAYE + NaCl. Solid lines represent model fits. The bars represent the standard deviation of the temperature histories (○).

inactivation was verified. It was observed that this temperature limit was dependent on the enumeration media and on the heating rate. As the heating rate increased, this limit became lower; if TSAYE + NaCl medium was used, this was the lowest limit observed. For the lowest temperature increasing rate (of 1.5 °C/min), inactivation started at 62 °C if TSAYE was used and at 58 °C if TSAYE + NaCl was chosen. For the highest temperature increasing rate (of 2.6 °C/min), *Listeria* inactivation began at 56 °C, independently of the enumeration media selected.

It is known that selective media do not inhibit the growth of undamaged cells but prevent the growth of injured cells (Miller et al., 2006). In the case of TSAYE + NaCl, a lower count of *Listeria* means that the cytoplasmic membrane of the cells has been probably damaged. The presence of LiCl in Palcam Agar inactivates bacterial enzymes by the replacement of divalent cations by Li^+ (Mendonca & Knabel, 1994). Attending on the results presented in Fig. 1, it should be re-enforced that when temperature reached 65 °C (for the three temperature profiles used) a significant amount of *Listeria* cells had membrane damages (since 5 log-cycles of reduction were observed from TSAYE + NaCl results). This damage was at least partially repaired, since results from TSAYE only reveal a reduction of 2-log cycles for all temperature profiles studied. The same happened for the loss of enzymes functionality (but in less extension), since the difference of the results obtained for the *Listeria* cells recovered in Palcam Agar and in TSAYE was not so relevant.

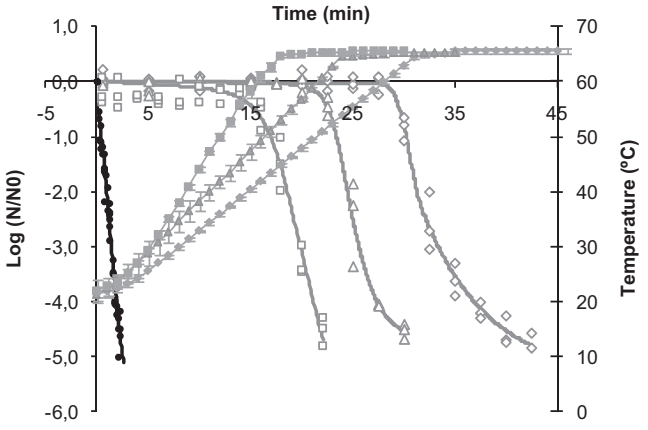

Fig. 2. Thermal inactivation data of *L. innocua*, in liquid medium, using three different temperature profiles: (◇) 1.5 °C/min, (△) 1.8 °C/min and (□) 2.6 °C/min and at a constant temperature of 65 °C (●), using TSAYE as the enumeration medium. Solid lines represent model fits. The bars represent the standard deviation of the temperature histories.

The non-isothermal inactivation processes studies were compared to an isothermal treatment, at which the temperature was maintained at 65 °C (Miller et al., 2009). At a constant temperature of 65 °C, *Listeria* suffered a 5-log cycles reduction in approximately 2.5 min. Such inactivation did not show either an initial shoulder or a tail tendency (Fig. 2). These results allow concluding that the design of a non-isothermal process should not rely on isothermal-based results.

Results of the Gompertz-inspired model parameters, estimated by fitting Eq. (5) to non-isothermal inactivation (in different media), are presented in Table 1. The adequacy of model fits was tested by residual analysis (i.e. residuals were normally distributed with means equal to zero and constant variance; residuals were random). The coefficient of determination was above 0.97 in all cases. The quality of regression analyses was therefore assessed.

The tail tendency was not evident, since considerable low values of log (N_{res}/N_0) estimates (no horizontal asymptote) were obtained. The high values for SHW at 95% could explain the difference (detected in a very first glance) between the bending towards the asymptote (which value is included in the confidence interval of the estimated parameter) and the estimated value. In such conditions, better results might be obtained if another experimental design was chosen, with more sampling points in the maximum inactivation rate period and at the last stage of the process.

The heating rate effect on parameters' precision was evident. The most precise estimates were obtained when the temperature

Table 1
Estimates of k_{ref}, Ea, c, d and tail parameters of *L. innocua* 10528, under non-isothermal treatments; Precision assessed by standard half width (SHW) at 95%.

Medium/food product	Heating rate (°C/min)	Enumeration media	k_{ref} (min^{-1})	SHW 95%	Ea ($J\,mol^{-1}$)	SHW 95%	c ($min\,K^{-2}$)	SHW 95%	d (K)	SHW 95%	Log (N_{res}/N_0)	SHW 95%
TSBYE	1.5	TSAYE	−0.506	8.38×10^{3}	1.23×10^{5}	2.81×10^{2}	0.122	4.049	350.2	6.496	−20.79	7.16×10^{2}
		TSAYE + NaCl	−0.938	2.71×10^{8}	4.22×10^{4}	6.99×10^{8}	2.341	1.17×10^{8}	334.3	5.23×10^{5}	−52.01	1.50×10^{8}
		Palcam	−0.735	1.89×10^{2}	2.33×10^{4}	4.37×10^{3}	0.579	1.02×10^{3}	338.7	1.07×10^{1}	−28.99	2.94×10^{2}
	1.8	TSAYE	−0.279	3.32×10^{8}	2.45×10^{5}	1.04×10^{8}	0.022	5.25×10^{9}	364.9	1.46×10^{6}	−14.20	1.33×10^{8}
		TSAYE + NaCl	−1.262	3.84×10^{6}	1.20×10^{3}	1.91×10^{10}	0.073	1.00×10^{8}	345.0	2.41×10^{6}	−22.70	7.46×10^{7}
		Palcam	−2.113	2.55×10^{8}	8.46×10^{4}	3.99×10^{8}	1.020	7.52×10^{6}	338.8	1.32×10^{6}	−49.29	6.48×10^{7}
	2.6	TSAYE	−0.532	1.31×10^{2}	5.59×10^{4}	4.63×10^{2}	0.015	5.44×10^{2}	362.7	2.00×10^{1}	−20.82	1.91×10^{2}
		TSAYE + NaCl	−1.592	5.46×10^{1}	6.20×10^{2}	4.13×10^{4}	0.304	1.66×10^{1}	332.4	2.18×10^{1}	−28.21	1.89×10^{2}
		Palcam	−0.670	2.49×10^{1}	6.63×10^{4}	2.01×10^{2}	0.001	1.44×10^{3}	465.3	2.11×10^{2}	−8.626	2.36×10^{2}
Parsley	1.8	Palcam	−0.229	4.34×10^{1}	5.24×10^{4}	1.04×10^{2}	0.005	9.29×10^{2}	274.3	1.02×10^{2}	−7.430	1.45×10^{2}
Meat pocket	–	Palcam	−1.329	1.13×10^{8}	1.07×10^{-1}	1.24×10^{13}	0.004	1.00×10^{8}	297.1	4.79×10^{6}	−13.09	1.68×10^{2}

profile with higher heating rate (2.6 °C/min) was used. This observation is not unexpected when a non-isothermal methodology is used in kinetic studies. Brandão and Oliveira (1997) reported the great influence of the temperature profile on parameters' accuracy and precision, in kinetics of mass transfer. This occurrence may be circumvent by the use of a convenient experimental design, selecting experimental data points and heating rates based on statistical theories (Brandão, Oliveira, & Cunha, 2001).

The difference observed for the precision of the estimates is not explained by the number of experimental points gathered. In all cases, the number of sampling points averages 30 (and is equivalent for all situations presented in Table 1), which does not limit the fit of the model used (5-parameter model).

In the design of a thermal food process, the microbial responses in media (often attained at laboratorial scale) and in foods should be a concern. To assess those differences, thermal inactivation of *L. innocua* in parsley was used as a case study. Time-varying temperature conditions were considered, and results were compared to the ones obtained in TSBYE medium (Fig. 3). The linear temperature profile of 1.8 °C/min was used (temperature ranging from 20 °C to 65 °C). Besides the sigmoidal tendency observed in both cases, inactivation behaviours were considerably different. In experiments with liquid medium, inactivation started after a shoulder period of 22 min when temperature reached 61 °C. When *Listeria* was artificially inoculated in parsley, the initial shoulder was reduced to 14 min, corresponding to a temperature of 45 °C. After the shoulder period, the rate of inactivation was higher in TSBYE medium than in the food. Although it is known that food matrixes protect bacterial cells from stressing temperature conditions, this effect was not evident throughout gradual temperature increase when *Listeria* was in parsley surface. If other kind of food was used, probably even more diverge results would be obtained. For an effective assessment of the factors that influence the microbial response under dynamic temperature conditions, further non-isothermal experiments using different temperature profiles, must be done in a wider range of foods. Food compounds, geometries, type of surfaces, microbial-surface adhesions, are potential variables affecting the microbial responses and generalized conclusions should not be withdrawn without a careful analysis of each situation.

The thermal inactivation kinetics of *L. innocua* was also followed in a frying process, where frozen *meat pockets* were used as case studied. The results of microorganism survival and the temperature history monitored at the centre of the product (i.e. the coldest point) during the entire frying process are presented in Fig. 4.

Fig. 3. Thermal inactivation data of *L. innocua*, in liquid medium (▲) and in parsley (□), using a heating rate of 1.8 °C/min. Palcam Agar was used as the enumeration medium in both situations. Solid lines represent model fits. The bars represent the standard deviation of the temperature history (○).

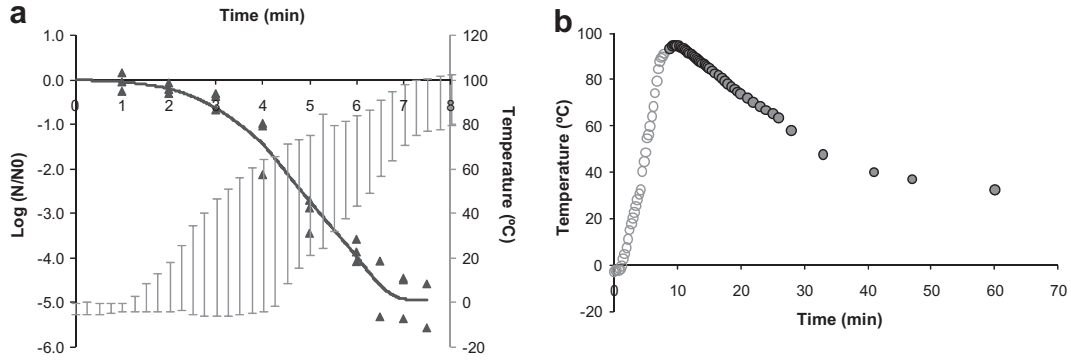

Fig. 4. Inactivation data of *L. innocua* during the frying process of a frozen meat pocket (a). Solid lines represent model fit. The bars represent the standard deviation of the temperature histories (◯). Temperature history (average values) monitored throughout 1 h (b).

Results of temperature monitorization reveal a great dispersion of the values. Besides this variation, a sigmoidal tendency of *Listeria* inactivation was observed throughout the frying process. The inactivation started after a shoulder period of approximately 3 min. At this time the temperature in the meat pocket centre varied between −2 °C and 51 °C. It is important to note that besides these are values at the central point, in external meat layers the temperature may have reached higher values and bacterium inactivation may occurred. The problems of predicting the rate of heating and the resulting changes in product quality and sterility are significant, in part because of the complexity of food materials and drawbacks in accurate physical properties estimation (Fryer & Robbins, 2005).

The effect of the cooling period on bacterial inactivation is also an important topic. Fig. 4(b) shows the temperature evolution in the meat pocket coldest point during and after the frying process (average values). As the meat pocket is removed from the fryer, the heat conduction does not stop in internal layers (unsteady state situation). After 3 min outside the fryer the temperature started to decrease, which proves that the cooling period can partially contribute to the bacteria inactivation at the food centre. Thus, it is of main importance to account for heat transfer phenomena in such processes for accurate predictions of temperature and, consequently, accurate estimations of the kinetics.

Inactivation data of *Listeria* in parsley and during the frying of meat pockets were fitted with the Gompertz-inspired model (similar to the cases in broth previously discussed). Adequacy of model fits was assessed by residual analyses and coefficient of determination (R^2 values were, respectively, 0.97 and 0.96 for parsley and meat pockets regressions). Estimates of kinetic parameters and related precision are also included in Table 1. For the case of parsley, the temperature profile used had a heating rate of 1.8 °C/min. Kinetic parameters were estimated with satisfactory precision (please focus on SHW 95% values). However, it is curious to note that this was the worse choice if experiments were carried out in broth (besides the number of experimental points was identical in both situations). This observation reinforces the role of experimental design previously discussed. In relation to meat pockets frying, although model adequacy was proven, precision of the kinetic parameters was sacrificed.

4. Conclusions

The results from the present study indicate that in liquid media *L. innocua* heat resistance under non-isothermal conditions is influenced by the heating rate. A sigmoidal tendency is observed for all the three temperature profiles. However, as heating rate increases, the shoulder period and tail decrease. Results obtained with the three enumeration media reveal that when temperature reached 65 °C (for all the temperature profiles used), a significant amount of *Listeria* cells have suffered injury. Broth experiments also highlight the importance of studying the influence of dynamic conditions on the thermal resistance of microorganisms, since the heating-up phases can contribute to an increase in cells thermotolerance.

Results in the food products demonstrated that the product greatly affects bacteria thermal resistance. Data from parsley experiments showed that, although the heat resistance of *Listeria* increased (when compared to liquid medium), the inactivation began earlier. Results during the frying process of meat pockets underline the importance of a uniform temperature distribution in the entire product. Additionally, it was concluded that, in non-isothermal treatments, not only the heating-up phases are important but also the cooling period.

The results from the present study demonstrated that the enumeration media influenced the *Listeria* recovery. Particularly for food products, special care must be taken because selective media has to be employed for the inhibition of the product endogena microflora. Hence, the results presented here for parsley and meat pockets are probably underestimated, since some microorganisms can repair themselves if plated in non-selective media.

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