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Thermal inactivation kinetics of *Salmonella* Typhimurium in *alheira* sausage batter†

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Abstract: The objective of this work was to characterise the heat resistance of Salmonella Typhimurium (ST) in *alheira* sausage batter. Two batches of *alheira* batter were obtained from a producer and inoculated with a ST overnight culture to reach ~7.0 log CFU/g in *alheira* batter. Bags containing well-spread 10-g *alheira* batter were submitted in duplicate to temperatures of 63°, 60°, 57° and 54 °C in an immersion bath. A log-linear primary model fitted to each of the inactivation curves estimated the death rates of ST in *alheira* batter with coefficients of determination ranging between 0.914 and 0.987. Through a Bigelow model, the D-value was modelled as a function of temperature, resulting in a log D* of 2.302 (SE=0.304), corresponding to 200 minutes at 50 ºC to reduce ST in 1 log, and a z-value of 5.016 (SE=0.839) ºC.

Keywords: Foodborne pathogens; predictive microbiology; modelling; artisanal product; D value

1. Introduction

An important contributing factor which leads to outbreaks of foodborne illness, including *salmonellosis*, is inadequate time/temperature exposure during thermal processing, and inadequate reheating to kill pathogens in retail food service establishments or homes [1].

Alheira is a non-ready-to-eat sausage from Northern Portugal, traditionally elaborated with a mix of pork and poultry meat. Cooked meats are shredded and mixed with salt, garlic, spices, and sliced bread soaked in hot broth to form a non-uniform paste; this paste is then stuffed into natural casings.

Although at low prevalence, artisanally-made *alheiras* have shown that they can harbor *Salmonella* spp., suggesting that if this pathogen is not inactivated during thermal treatment or if recontamination occurs before/during stuffing, *Salmonella* spp. may survive throughout maturation [2]. Thus, there is a need to implement process standardisation during the production of *alheira* sausages, in particular during the short stage of heat treatment.

The objective of this work therefore was to characterise the heat resistance parameters of *Salmonella* Typhimurium in *alheira* sausage batter, utilizing a Bigelow secondary model and a log-linear primary inactivation model.

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2. Materials and Methods

2.1. Inactivation Experiments

Salmonella Typhimurium (ATCC 43971) kept on a fresh slant was cultivated overnight in brain heart infusion broth (BHI) at 37 °C for 18 h. On the day of inoculation, the inoculum was prepared from a second subculture that had reached an absorbance (600 nm) of ~0.8. One-ml of the suspension was diluted in 4 ml of physiological water, and the volume of 5 ml was added to 100 g alheira batter (obtained from a producer in Mirandela, Portugal). This procedure produced a Salmonella concentration of 7.0-7.5 log CFU/g in the alheira mass. After homogenisation, 10 g of the batter were weighed into individual bags and spread out using a kitchen roll. Bags were prepared in duplicate for every time point. Duplicate bags were accommodated in a metal support, and submitted to temperature of 63°, 60°, 57° or 54 °C in an immersion agitated bath. Bags were sampled at 5 and 6 different time points; at 63 °C for 0, 0.5, 1, 1.5, and 5 min; 60 °C for 0, 3, 6, 9 and 12 min; 57 °C for 0, 10, 20, 30, 40, 50 min; and 54 °C for 0, 20, 40, 60, 80, 100 min. After removing the bags from the water bath, it was immersed promptly into an ice water bath. Upon cooling, the determination of Salmonella Typhimurium was immediately performed within two hours of the experiment, by plating on Xylose Lysine Desoxycholate agar (DSHB3011, Alliance Bio Expertise, France). Typical colonies were counts after incubation at 37 ± 1 °C for 24 h.

2.2. Modelling of Heat Resistance Parameters

For every survival curve, at a fixed temperature, the D value [min] (i.e., time to achieve one logaritmic reduction in microbial concentration) was estimated by adjusting the log-linear decay equation:

$$logN(t) = logN_0 - \frac{t}{D} \quad (1)$$

where N(t) is the microbial concentration [CFU/g] at time t [min]; N_0 is the initial microbial concentration [CFU/g], a parameter to be estimated. The inactivation rate, k [1/min] can be estimated as the reciprocal of D. After estimating the D values at the fixed temperature T, the Bigelow secondary model was adjusted to extract the parameters log D_{ref} (i.e., D value at the reference temperature T_{ref} of 50 ºC) and z value (i.e., change in temperature that causes a 10-fold change in the D-value).

$$log\ D = log\ D_{ref} - \frac{(T - T_{ref})}{z} \quad (2)$$

Statistical analysis was conducted in the R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

Inactivation curves of *Salmonella* Typhimurium in *alheira* batter could be well approximated to the log-linear kinetics model, as suggested by the high coefficients of determination that ranged between 0.914 and 0.987. Death rates were estimated at 0.038 (SE=0.008), 0.126 (SE=0.003), 0.273 (SE=0.063) and 2.872 (SE=0.763) log CFU/min at 54 º, 57 º, 60 º and 63 ºC, respectively; whereas D values were estimated at 26.52, 7.936, 3.663 and 0.348 min at the same temperatures (Table 1).

Table 1. Mean and standard error (SE) of the death rate (k), D values and coefficient of determination (R^2) of the log-linear primary models fitted to thermal inactivation curves of *Salmonella* Typhimurium in *alheira* batter.

Temperature (ºC)	k (log CFU/min)	SE (k)	D (min)	R^2
54	0.038	0.008	26.52	0.914
57	0.126	0.003	7.936	0.942
60	0.273	0.063	3.663	0.987
63	2.872	0.763	0.348	0.934

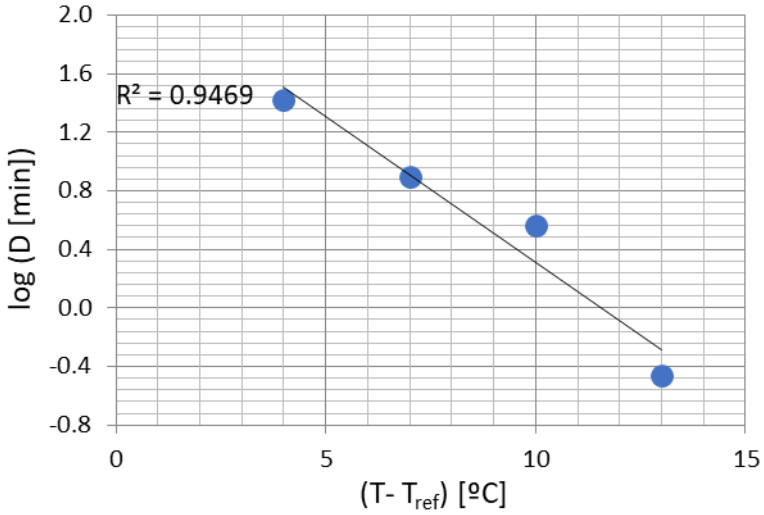

Figure 1. D values of *Salmonella* Typhimurium in allheira batter against temperature difference, showing adjusted Bigelow equation line and coefficient of determination; T_{ref}=50 ºC.

The D-values found in this study for *Salmonella* Typhimurium in a product that resembles a meat paste, were very similar to those found by Murphy et al. [4] in chicken patties, chicken tenders, beef patties and beef/turkey patties. D values determined by these authors were in the range of 26.97 to 0.25 min at temperatures between 55 and 70 ºC.

Through a Bigelow secondary model, the D value (time needed to reduce the pathogen in one log) was modelled as a function of temperature. Figure 1 shows that for the temperature interval studied, the thermal death time presented a linear behaviour with temperature, as implied by the high coefficient of determination (R^2=0.947). The fitted Bigelow model resulted in a log D_{ref} of 2.302 (SE=0.304), corresponding to D_{ref}=200 minutes at 50 ºC to reduce *Salmonella* Typhimurium in one log; and a z-value of 5.016 (SE=0.839) ºC. This model enables the prediction of D values and lethality times at other temperatures, and it is helpful to evaluate and validate the effectiveness of current thermal treatments used by industry. For instance, to reach a 7.0-log reduction lethality of *Salmonella* Typhimurium in sausages, the geometric centre of the *alheiras* should be kept for 9.0 seconds at 70 °C.

4. Conclusions

This study estimated for the first time the thermal kinetic parameters of a traditional Portuguese sausage, and will be useful to producers for designing, controlling and validating their thermal processes during *alheira* manufacture.

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