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Proceeding Paper

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 an 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 (T_{ref} = 50 °C) of 2.302 (SE = 0.304), corresponding to 200 min at 50 °C to reduce ST in 1 log, and a z-value of 5.016 (SE = 0.839) °C.

Keywords: foodborne pathogens; predictive microbiology; modelling; artisanal product; D value; z value

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1. Introduction

An important contributing factor that 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 harbour *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 after stuffing.

The objective of this work, therefore, was to characterise the heat resistance parameters of *Salmonella typhimurium* in *alheira* sausage batter, utilising a log-linear primary inactivation model to estimate the D values at constant temperatures, and a Bigelow secondary model to estimate the z value.

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. Alheira batter, which is a mid-product containing cooked meat, prior to stuffing, was obtained from a local producer (Mirandela, Trás-os-Montes, Portugal). 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 a volume of 5 mL was added to 100 g alheira batter 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 was weighed into individual bags and spread out using a kitchen roll. The bags were prepared in duplicate for every time point. The duplicate bags were accommodated in a metal support and submitted to temperatures of 63°, 60°, 57° or 54 °C in an agitated immersion bath. The 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, and 50 min; and 54 °C for 0, 20, 40, 60, 80, and 100 min. After removing the bags from the water bath, each was promptly immersed into an ice water bath. Upon cooling, the quantification of *S. typhimurium* was immediately performed within two hours of the experiment by plating on Xylose Lysine Desoxycholate agar (DSHB3011, Alliance Bio Expertise, Bruz, France). Typical red colonies with black centres were counted after incubation at 37 ± 1 °C for 24 h [3].

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 logarithmic 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]. The exponential death rate, EDR [log CFU/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 °C) and z value (i.e., change in temperature that causes a 10-fold change in the D-value).

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

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

3. Results and Discussion

The inactivation curves of *S. 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. The exponential 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°, 57°, 60° and 63 °C, respectively; whereas the D values were estimated at 26.5, 7.94, 3.66 and 0.35 min at the same temperatures (Table 1).

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

Temperature (°C)	EDR (log CFU/min)	SE (EDR)	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

The D-values found in this study for *S. 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. The D values determined by these authors were in the range of 26.97 to 0.25 min at temperatures between 55 and 70 °C.

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 D values 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 min at 50 °C to reduce *S. typhimurium* in one log, and a z-value of 5.016 (SE = 0.839) °C. 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 *S. typhimurium* in sausages, the geometric centre of the *alheiras* should be kept for 9.0 s at 70 °C (D = 0.021 min).

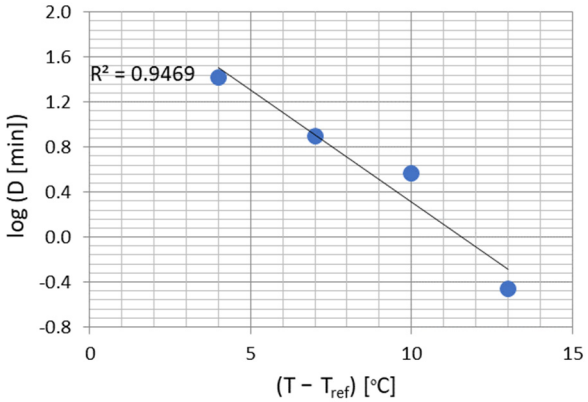

Figure 1. D values of *S. typhimurium* in alheira batter against temperature difference, showing adjusted Bigelow equation line and coefficient of determination; T_{ref} = 50 °C.

4. Conclusions

This study estimated for the first time the thermal kinetic parameters of *S. typhimurium* in 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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