Effect of heating rates on the thermal resistance of *Cronobacter sakazakii* DPC 6529

Efecto de la velocidad de calentamiento en la termorresistencia de *Cronobacter sakazakii* DPC 6529

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Abstract

Cronobacter sakazakii **is an opportunistic pathogen causing meningitis, septicaemia and enterocolitis in neonates and it is usually related to the consumption of contaminated Powdered Infant Formula (PIF). Industrial thermal treatments involve three distinct stages: heating, holding and cooling, and all three stages may contribute to the microbial inactivation. In this study the effect of heating rates on the inactivation of *C. sakazakii* DPC 6529 was determined and the results showed that *C. sakazakii* presents an unusual behavior under non-isothermal treatments compared to the behavior of other non-spore-forming microorganisms.**

Keywords: *Cronobacter sakazakii*; heat resistance; powdered Infant Formula; non-isothermal treatments.

Resumen

Cronobacter sakazakii **es un patógeno oportunista que causa meningitis, septicemia y enterocolitis en neonatos. Está frecuentemente relacionado con el consumo de fórmula infantil en polvo. Los tratamientos térmicos industriales consisten en tres etapas diferentes: calentamiento, mantenimiento y enfriamiento, y todos ellas contribuyen a la inactivación microbiana. En este estudio el efecto de la velocidad de calentamiento sobre la inactivación de *C. sakazakii* DPC 6529 fue determinado y los resultados mostraron que *C. sakazakii* presenta un comportamiento inusual bajo tratamientos no isotérmicos comparado con el comportamiento de otros microorganismos no formadores de esporas.**

Palabras clave: *Cronobacter sakazakii*; resistencia térmica; fórmula infantil en polvo; tratamientos no isotérmicos.

1. INTRODUCTION

Cronobacter sakazakii is a Gram-negative and ubiquitous enterobacteria [1]. It is considered an opportunistic pathogen whose major risk group are pretern, low-birth-weight or immune-compromised infants. Outbreaks of this microorganism have been epidemiologically linked to the

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consumption of Powdered Infant Formula (PIF). PIF is considered a non-sterile product, and after its reconstitution could be considered a rich medium which allows bacterial growth.

C. sakazakii has been proposed to have one of the highest thermal resistances among the members of the *Enterobacteriaceae* family [2]. Several studies have been done on calculating the thermal resistance of this microorganism, among variety of strains and on different heating media, under isothermal conditions [3]. However, industrial thermal treatments involve three distinct stages: heating, holding and cooling, and all three stages may contribute to the microbial inactivation. There are no studies on the effect of heating rates and the behaviour of *C. sakazakii* under non-isothermal conditions. This poses the study of the microbial inactivation kinetics under non-isothermal conditions in order to obtain results under more realistic situations [4, 5].

The aim of this study was to determine the effect of heating rates and complete thermal treatments on the inactivation of *C. sakazakii* DPC 6529, to compare the thermal resistance values obtained under isothermal treatments conditions with those obtained from the non-isothermal results, in Peptone Water (PW) and PIF.

2. MATERIALS AND METHODS

2.1 Bacterial strain and culture conditions

Cronobacter sakazakii DPC 6529 used in this study was obtained from the University College Cork culture collection. The culture was routinely grown overnight (~16 h) at 37 °C in Luria-Bertani broth (LB).

2.2 Heat resistance determinations

Heat resistance determinations were performed in a thermoresistometer Mastia [4]. The isothermal heat treatments were performed at 58 and 62°C. The non-isothermal treatments were performed at constant heating rates, 1 °C/min and 20 °C/min. Furthermore, more complex heat treatments (heating, holding and cooling periods) were also performed at a heating rate of 30 °C/min. The heating media used were Peptone Water (PW) and Powdered Infant Formula (PIF). Once the treatment temperature was reached and stable, the medium was inoculated with 0.2 mL of the culture suspension. Samples were collected into sterile test tubes at preset intervals, immediately diluted, plated in LB agar and incubated at 37 °C for 24 h.

2.3 Heat resistance data analysis

The isothermal inactivation processes have been described using the cumulative form of the Weibull distribution [6]. The behaviour of the microorganism under non-isothermal heat treatments was described with the model proposed for the description of dynamic inactivation processes, based also in the Weibull distribution [7].

3. RESULTS AND DISCUSSION

3.1 Isothermal resistance of *Cronobacter sakazakii* DPC 6529

The survival curves obtained in both heating media were non-linear for all the tested temperatures, showing the presence of tails. Moreover, the p values of the Weibull model obtained were below 1 (Table 1), supporting the tailing phenomenon. This phenomenon could be due to the rapid inactivation of the weak members of the population and the ability of the remaining survivors to adapt to the applied lethal agent, becoming more resistant [8]. The results obtained showed that at a low treatment temperature the thermal resistance of *C. sakazakii* was higher in PIF tan in PW while at a higher treatment temperature the thermal resistance of the microorganism was similar in both heating media. It has been reported that differences in the thermal resistance of *C. sakazakii* can be due to differences in the composition of the heating media [9].

3.2 Determination of the thermal resistance and the effect of heating rates under non-isothermal treatments based on isothermal results

When predictions were done by using the estimated parameters under isothermal treatments (Table 1), it can be observed a difference between the predictions and the experimental data for both heating rates and for the complex thermal treatment (Figure 1). For both constant heating rates, experimental values were above the predictions. However, for the complex treatment, they were below the predictions (Figure 1), which suggest that there is no clear effect of the heating rates on the thermal inactivation of *C. sakazakii*. These results are in disagreement with those of other authors, who found that higher heating rates led to faster inactivation of vegetative cells [4, 5, 10]. The different behaviour among bacterial species exposed to different heating rates could depend on how microbial cells respond to the effect of the heating rates. It has been reported that at slow heating rates the bacterial cell has time to prepare itself to the challenge and survive, probably due to the induction and expression of Heat Shock Response (HSR) genes and proteins [10].

4. CONCLUSIONS

Under non-isothermal treatments, the heating rates do not have a clear effect on the inactivation of *C. sakazakii* DPC 6529. This microorganism showed an usual stress response to thermal treatments. Under complete thermal treatments, the results suggest that the unusual thermal stress response of *C. sakazakii* could subserve this microorganism to survive to complex thermal treatments, such as pasteurization.

5. ACKNOWLEDGMENTS

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6. REFERENCES

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Tables and Figures

Table 1. Heat resistance values of *Cronobacter sakazakii* DPC 6529 obtained under isothermal treatments in Peptone Water (PW) and Powdered Infant Formula (PIF), derived from the Weibull model.

	δ_{60}		*Z*		*P*		
	estimate	std	estimate	std	estimate	std	**RMSE**
PW	0.09	0.02	4.72	0.23	0.76	0.08	0.51
PIF	0.10	0.01	3.98	0.10	0.68	0.04	0.32

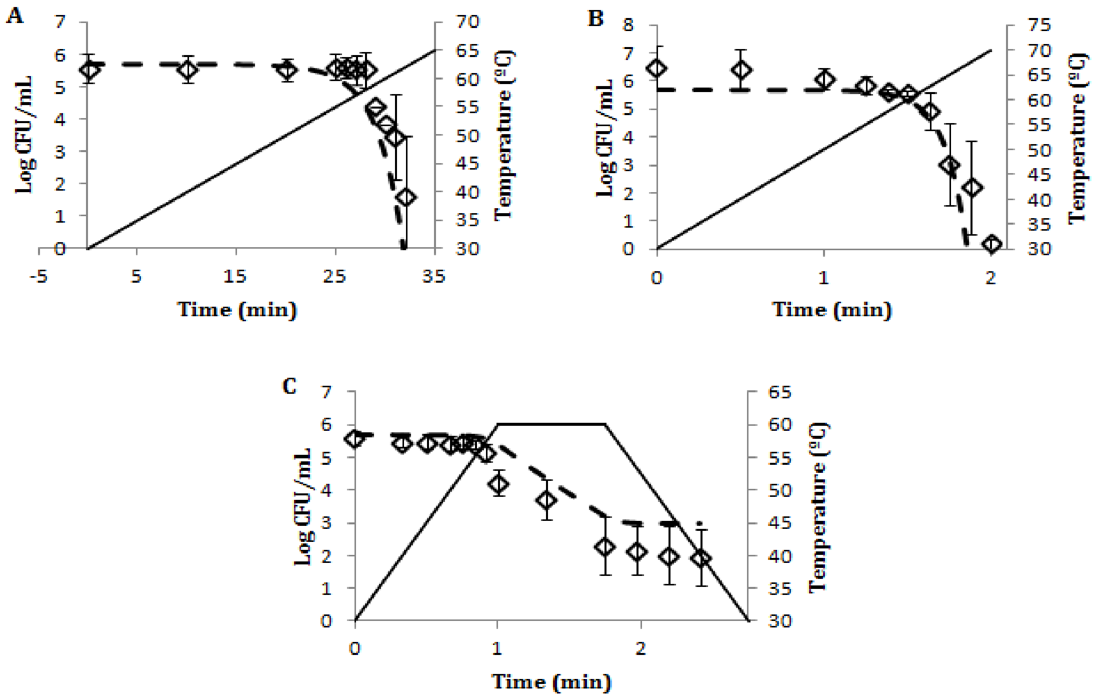

Figure 1. Effect on the heating rate on the survival of *Cronobacter sakazakii* DPC 6529 in Powdered Infant Formula. Experimental results (◊), Weibull model predictions based on the isothermal data (dashed lines), and temperature (continuous lines). At 1 °C/min (A), 20 °C/min (B) and complete thermal treatment at 30 °C/min (C).