

## ***B. cereus* INOCULATED WHOLE MILK PACKAGES STUDY**

<sup>1</sup>Cristiana de Paula Pacheco Sanchez e <sup>2</sup>Pilar Rodriguez de Massaguer

### **ABSTRACT**

The rate of defects was studied in Brazilian continuous thermal process applied to whole milk (pH 6.6) packed in carton packages. The process conditions were established considering *Bacillus cereus* 0486 kinetic parameters ( $D_{121C}=0.23$  min;  $z=19.81C$ ) obtained in dynamic system. Its spores were selected as Brazilian target since incidence of strains from bacilli group have been observed in UHT milk. To verify the theoretical defect rate of *B. cereus* 0486 spores, four thermal processes at 1.24L/min, in duplicate, were carried out in an aseptic pilot unit and tested by direct inoculation packages. The first thermal treatment simulated 1.35% of defects (150.5s 121C). The second one, simulating 1.35% of defects, was equivalent to less severe Brazilian commercial process (24.3s 134C). The third treatment, for 24.3s at 134C, was inoculated with  $1E+02$  spores/mL. Finally the fourth thermal process, (36.3s a 134C) was inoculated with  $1E+02$  esporos/mL. The thermal processes at 134C did not allow the survival of *B. cereus* 0486 when  $1E+02$  spores/mL was inoculated. In the other processes, 1 and 1.33% of defects founded was close than the theoretical ones. The utilization of kinetic parameters determinate in dynamic systems allows better simulation of thermal process in UHT region for milk with accurate prediction of defect rates. The less severe Brazilian UHT milk thermal process, promote sterile packages free from the pathogen *B. cereus* when its initial contamination is  $1E+02$  spores/mL.

Keywords: UHT milk, *Bacillus cereus*, Kinetic parameters, Continuous process.

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<sup>1</sup> Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Cidade Universitária, CEP: 13083-862, Campinas, SP, Brasil. [pacheco@fea.unicamp.br](mailto:pacheco@fea.unicamp.br); [esteril@unicamp.br](mailto:esteril@unicamp.br).

## INTRODUCTION

For product development in aseptic lines, challenge tests are recommended with inoculated packages. In conducting such in-plant tests, the product is batch inoculated with the appropriate test organism. At least, 100 inoculated containers per processing variable should be prepared for incubation because detection of non-sterility with certainty requires many samples, since only a low number of packages may be non-sterile (1). It is usually necessary to run a protocol of 4 or 5 processes variables by changing temperatures and maintaining a constant product flow rate; one of these processes is equivalent to the one which is going to be verified.

The incidence of *Bacillus* in UHT Brazilian milk was studied and the results showed 6.25% of samples with more than 100 CFU/mL of milk (2). *Bacillus cereus* is the most important *Bacillus* species, in the dairy industry, in terms of food hygiene and public health and has been isolated, sporadically, from bulk milk tank (3). It is associated with cattle feed throughout year and being more common in raw milk during the summer months. Even with low concentrations of *B. cereus* in raw and pasteurized milk pre-incubated at ambient temperature, *B. cereus* can dominate the *Bacillus* population, reaching levels associated with the enterotoxin production (4).

## METHODS

*B. cereus* 0486 spore suspension was prepared (5) and standardized (6). Brazilian whole milk was characterized by pH measurement, aerobic plate counts, mesophilic aerobic sporeforming count, mesophilic anaerobic sporeforming detection (7) and *B. cereus* spores count (8). Inoculation level (No/package) was calculated as:  $\text{Log}(\text{No}/\text{package}) = F / D_T + \text{Log}N$ , where : N=% of contaminated packages, D = time to destroy 90% of population, F = Residence time in hold section and T= temperature, considering *B. cereus* 0486 kinetic parameters,  $D_{121C}=13.90s$  and  $z=19.76C$ , obtained in dynamic system by (9). The processes, estimated using Equivalent Point

Method (10), were tested based on less severe Brazilian commercial processes: **(A)** 150.5 s at 121C, inoculated with 5.0E+06 spores/mL of milk to cause 1.35% of defects, **(B1)** 24.3 s at 134C, inoculated with 7.0E+03 spores/mL of milk to cause 1.35% of defects, **(B2)** 24.3 s at 134C, inoculated with 1.0E+02 spores/mL of milk simulating common contamination level of raw milk. **(C)** 36.3 s at 134C, inoculated with 1.0E+02 spores/mL of milk simulating the commercial contamination level of raw milk. All processes, in duplicate, were carried out in an *Microthermics* UHT/HTST Lab-250DH aseptic unit available at UNICAMP. Flow rate of 1.24 L/min was applied in all cases. After each heat process 300 sterile glass bottles were aseptically filled and sealed at 25C. All bottles were incubated for 14 days at 30C (11). Milk physical chemical characterization and *B. cereus* phenotypical confirmation were done.

## RESULTS AND DISCUSSION

*B. cereus* suspension was 5.0E+09 spores/mL of milk. The microbiological counts reported in table 1 were higher than those reported in literature and/or Brazilian standard for pasteurized milk because for aerobic colony the standard count is 1E+06 CFU/mL of milk and for mesophilic aerobic sporeforming the respective count exceeds in 50% the upper limit permitted in Brazilian legislation. The initial pasteurized milk contaminated with *B. cereus* was considered in total inoculum's level for thermal processes. As seen in table 2, the predicted rate of defects, based on dynamic kinetic parameters showed a good performance since, for processes A and B1, with survivors confirmed as *B. cereus*, both the predicted (1.35%) and experimental (1-1.33%) rate of defects were close; besides that, the predicted rate was higher than the experimental offering safety margin for processors. Figure 1 shows duplicate processes profiles with theoretical 1.35% rate of defects. At 134C, profile for repetition 1 shows a slightly higher temperature at the retention section, justifying higher lethality (1% of defects). At 121C, profiles for repetition 1 and

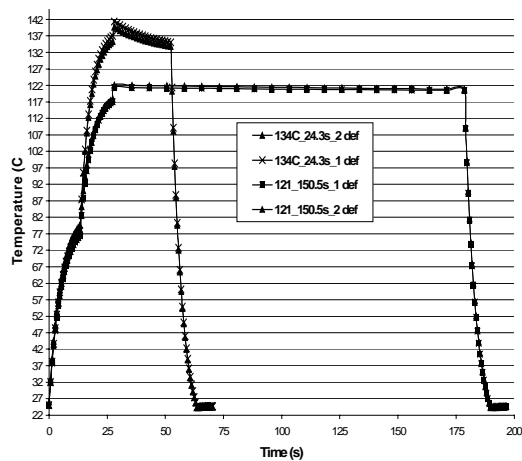
2, were superposed and the 1.33% rate of defects was observed. Figure 2 shows duplicate processes profiles with  $1E+02$  spores/mL. At 134C, profiles for repetition 1 and 2, were also superposed and the 1.33% rate of defects was observed.

**TABLE 1.** Brazilian Whole Milk Characterization

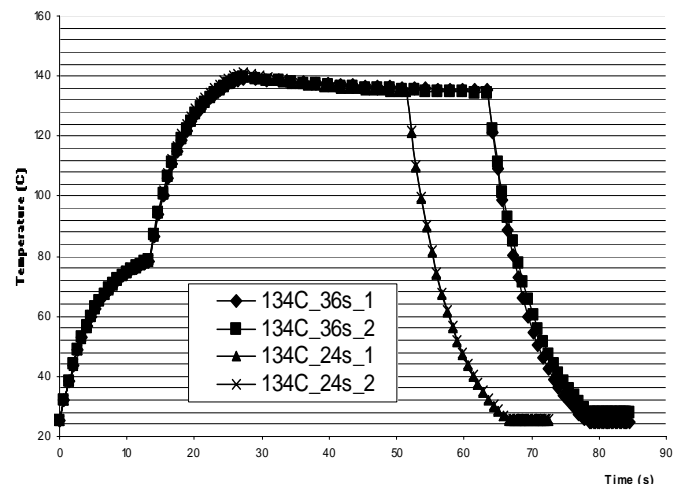
Characterization	Value
pH	6.92
Aerobic Plate Count	$1.22E+07$ UFC/mL of milk
Mesophilic Aerobic Sporofomers Count	$2.00E+02$ spores/mL of milk
Mesophilic Anaerobic Sporofomers Detection	33.33%
<i>B. cereus</i> Spore Count	$3.00E+02$ spores/mL of milk

**TABLE 2.** Direct inoculation heat treatment with *B. cereus* spores

Conditions	Process			
	A	B1	B2	C
Decimal reduction expected	10.8	7.9	7.9	11.8
Predicted rate of defects (%)	1.35	1.35	$1.29E-04$	$1.60E-08$
<b>1° repetition</b>				
Experimental rate of defects (%)	1.33	1	<1	<1
Error (Predicted-experimental) (%)	0.02	0.35	-	-
<b>2° repetition</b>				
Experimental rate of defects (%)	1.33	1.33	<1	<1
Error (Predicted-experimental) (%)	0.02	0.02	-	-



**FIGURE 1.** Time-temperature profile for processes A and B1 with 1.35% of defects



**FIGURE 2.** Time-temperature profile for processes B2 and C with  $1E+02$  esp/mL

## CONCLUSIONS

Kinetic parameters in dynamic systems allow better prediction of thermal process rate of defects, with *B. cereus* as target, outside and inside UHT region for milk.

The equivalent processes **B2** and **C**, from 24.3 to 36.3 s at 134C, did not allow the survival of *B. cereus* 0486 when the contamination level was 1.0E+02 spores/mL of milk. Even with no survival of *B. cereus*, at the 1.0E+02 spores/mL of milk inoculation level, the equivalent Brazilian commercial process less severe (134C/24.3s) must be redesign, with dynamic kinetic parameters, to promote, at least, 9 log reductions of mesophilic aerobic sporulated population, more resistant than *B. cereus*, to protect the consumers and avoid survivors when raw contamination is higher than 1.0E+02 spores/mL of milk. Despite the Brazilian regulation indicates that UHT milk thermal treatment is between 130 -150C for 2-4 s; experimentally only thermal processes for Brazilian milk at 134C for 36.3s or higher, could safety assure the absence of *B. cereus* considering the microbial quality of Brazilian milk.

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