

Short Communication

Salmonella* Typhimurium, Enteritidis, Infantis and Derby in pasty *dulce de leche

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In order to evaluate the fate of *Salmonella* Typhimurium, Enteritidis, Infantis and Derby in pasty *dulce de leche*, aliquots of this sweet were experimentally contaminated with these pathogenic microorganisms at 10^2 bacterial cells per gram, and later analysed to evaluate microorganism count after storage for 0, 1, 2, 3, 5, 10 and 20 days. *Salmonella* Enteritidis was able to survive for up to 20 days in *dulce de leche*, while *Salmonella* Typhimurium and Infantis for five and ten days, respectively, and serotype Derby for three days. The *Salmonella* serotypes studied showed different adaptive capabilities regarding population growth in pasty *dulce de leche*, though all representing potential hazard to the consumer's health. The results are a warning regarding the need to take the appropriate hygienic-sanitary practices during *dulce de leche* manufacture, packing and handling.

Key words: *Salmonella*, *dulce de leche*, food safety.

INTRODUCTION

Dulce de leche is the product obtained from concentration and heating of milk with the addition of sucrose (Brasil, 1997). It can be produced in pasty or tablets form. The pasty *dulce de leche* is widely consumed and commercialized in Brazil and other Latin American countries, where the fractioning into portions to be sold by retail in supermarkets and street market stalls is common. In spite of the existence of barriers against microbial growth, such as its low water activity due to the concentration of carbohydrates, the *dulce de leche* can be contaminated by microorganisms, during the manufacturing process, distribution and storage,

constituting a potential hazard to people's health (Timm et al., 2007).

Salmonellosis is a food-borne disease of frequent occurrence throughout the world. The causative agent, *Salmonella* spp., is widely distributed in nature and the main reservoir is the gastrointestinal tract of animals and man. The existence of asymptomatic carriers and persistence of *Salmonella* in the environment and in foods contributes to this organism assume a relevant role in public health (Shinohara et al., 2008). The annual occurrence of salmonellosis cases in the United States is estimated in 1.4 million, of which approximately 40,000

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Table 1. *Salmonella* counts (log MPN/g) in experimentally contaminated pasty *dulce de leche*.

Strain	Serotype	Storage days						
		0	1	2	3	5	10	20
LIPOA 2046	Typhimurium	1.5 (0.2) ^a	1.0 (0.9)	0^b	0	NR ^c	NR	NR
ATCC 13311	Typhimurium	1.3 (0.5)	0.9 (0.5)	0.2 (0.3)	0.2 (0.4)	0.2 (0.4)	NR	NR
LIPOA 2024	Enteritidis	1.9 (0.2)	0.5 (0.2)	0.2 (0.2)	1.1 (0.4)	NR	NR	NR
ATCC 14028	Enteritidis	1.9 (0.2)	0.7 (0.9)	0.5 (0.5)	2.1 (0.8)	1.3 (1.0)	1.6 (1.1)	0.1 (0.2)
LIPOA 2005	Infantis	1.3 (0.7)	2.1 (0.9)	2.6 (2.1)	1.6 (1.9)	0.7 (1.2)	NR	NR
LIPOA 2039	Infantis	1.5 (0.5)	2.0 (2.0)	1.9 (1.2)	0.9 (1.3)	2.5 (2.2)	0.1 (0.2)	NR
LIPOA 2007	Derby	1.6 (0.1)	2.0 (0.4)	1.9 (1.7)	0.3 (0.3)	NR	NR	NR
LIPOA 2035	Derby	2.1 (0.6)	1.6 (0.5)	0.1 (0.1)	0	NR	NR	NR

^a Average of three repetitions (stander deviation). ^b Log 0 = most probable number per g (MPN/g) lesser than 1. ^c NR = *Salmonella* was not recovered from any repetition. Values in bold = *Salmonella* was not recovered from all repetitions.

are confirmed by isolation of *Salmonella* (Centers for Disease Control and Prevention, 2011). In Brazil, 8,663 cases of foodborne diseases were reported to the Ministry of Health between 2000 and 2011, and *Salmonella* was the main etiological agent identified (Brasil, 2011).

The ability of *Salmonella* to survive in pasty *dulce de leche* for long periods has been reported by Andrews et al. (2011) but there are no studies about its behavior in this food. The knowledge of the fate of *Salmonella* in the environment provided by *dulce de leche* is important for the adoption of adequate control measures to avoid the infection of the consumers. The aim of this paper was to study the fate of *Salmonella enterica* subsp. *enterica* serotypes Typhimurium, Enteritidis, Infantis and Derby in pasty *dulce de leche*.

MATERIALS AND METHODS

The *dulce de leche* was prepared from standardised milk containing 3% milk fat with the addition of two hundred gram sucrose to each liter of milk. The ingredients were kept at 100-105°C and stirred for 2 h and 30 min.

Strains of *Salmonella enterica* subsp. *enterica* from serotypes Typhimurium LIPOA 2046, isolated from pasty *dulce de leche*, and ATCC 13311; Enteritidis, LIPOA 2024, from poultry sausage, and ATCC 14028; Infantis, LIPOA 2005, from pork dry-sausage, and LIPOA 2039, from bovine ground beef; Derby, LIPOA 2035, from bovine ground beef, and LIPOA 2007, from poultry ground beef, were used. The isolates were kept at -70°C and recovered when necessary.

Twenty-five gram *dulce de leche* fractions were packed in sterile plastic bags, inoculated with 0.25 mL inoculum in the concentration 10⁴ bacterial cells per mL, to obtain final concentrations of about 10² bacterial cells per gram of *dulce de leche*. The samples were homogenized, kept at a temperature between 15 and 20°C, and analyzed after storage for 0, 1, 2, 3, 5, 10 and 20 days. Twenty-five grams of non-inoculated contaminated *dulce de leche* were used for negative control. *Salmonella* were counted by the method of the most probable number (MPN). Serial dilutions of the inoculated experimentally contaminated pasty *dulce de leche* were incubated at 37°C for 20 h in tubes with buffered peptone water (BPW, Acumedia). The presence of *Salmonella* in each tube was

performed according to U.S. Food and Drug Administration - FDA recommendations (Andrews et al., 2011). The results were interpreted using the MNP table. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Several strains of *Salmonella* used to experimentally contaminate the *dulce de leche* were able to survive and to grow in this food (Table 1). The *Salmonella* ability to survive in dairy products has been reported (Hentges et al., 2010; Borges et al., 1990; Modi et. al., 2001), but the present paper is the first study about the fate of *Salmonella* in *dulce de leche*.

Silveira et al. (2012), working with wild strains of the serotypes Typhimurium and Enteritidis, found variable capacity to be able in *dulce de leche*, as our study, although we have worked with ATCC and wild strains. *Salmonella* Typhimurium was able to survive for five days in experimentally contaminated *dulce de leche*, although it was not able to grow in this medium. In despite of the strain LIPOA 2046 had been previously isolated from *dulce de leche* in a study performed by Timm et al. (TIMM), it survived for a lesser period than the strain ATCC 13311, what suggest strains LIPOA 2046 did not developed specials adaptations to this environment. It should be considered that the infective dose of *Salmonella* Typhimurium may be less than 10 bacterial cells (D'Acoust et al., 2001). Therefore, the mere presence of bacteria in food, even at low concentrations, is enough to cause concern about food security.

The two strains of *Salmonella* Enteritidis grew in *dulce de leche*, showed different behaviors as its ability of surviving. The strain ATCC 14028 showed considerable growth on the third day after the contamination, which may have contributed to the permanence of relatively high populations after ten days of storage and to the presence of some bacterial cells viable even after 20 days of the beginning of the experiment. This study suggest that strains of the serotype Enteritidis may have

high adaptability in foods with adverse conditions to its survival such as *dulce de leche*.

Both strain from the Infantis serotype showed adaptability to the environment provided by pasty *dulce de leche*, being able to survive for up to ten days of storage and increased relevantly its population in the early days. This adaptive capability justifies the concern about the presence of *Salmonella* Infantis in *dulce de leche*.

The strains of serotype Derby, showed different behavior. The strain LIPOA 2007 grew on the first day of storage, whereas the strain LIPOA 2035 showed progressive decrease in population density. However, none of the strains were recovered from the food five days after contamination, indicating that it was the serotype with lower survival capability in *dulce the leche* among the studied serotypes. This characteristic of *Salmonella* Derby is not enough to discard the serotype as important to food security, because *dulce de leche* is often consumed shortly after its acquisition in the retail market.

Conclusion

Salmonella Typhimurium, Enteritidis, Infantis and Derby showed different adaptive capabilities regarding population growth and survival in pasty *dulce de leche*, though all represent potential hazard to the consumer's health. The results are a warning regarding the need to take the appropriate hygienic-sanitary practices during *dulce de leche* manufacture, packing and handling.

Conflict of Interests

The authors have not declared any conflict of interests.

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