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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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 10^3 bacterial cells per mL, to obtain final concentrations of about 10^2 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’Aoust 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

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Storage days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>LIPOA 2046</td>
<td>Typhimurium</td>
<td>1.5 (0.2)</td>
</tr>
<tr>
<td>ATCC 13311</td>
<td>Typhimurium</td>
<td>1.3 (0.5)</td>
</tr>
<tr>
<td>LIPOA 2024</td>
<td>Enteritidis</td>
<td>1.9 (0.2)</td>
</tr>
<tr>
<td>ATCC 14028</td>
<td>Enteritidis</td>
<td>1.9 (0.2)</td>
</tr>
<tr>
<td>LIPOA 2005</td>
<td>Infantis</td>
<td>1.3 (0.7)</td>
</tr>
<tr>
<td>LIPOA 2039</td>
<td>Infantis</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td>LIPOA 2007</td>
<td>Derby</td>
<td>1.6 (0.1)</td>
</tr>
<tr>
<td>LIPOA 2035</td>
<td>Derby</td>
<td>2.1 (0.6)</td>
</tr>
</tbody>
</table>

^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.
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.

REFERENCES