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Pathogenic microorganisms survival in *ambrosia*

Sobrevivência de micro-organismos patogênicos em ambrosia

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Abstract

Ambrosia is a kind of *dulce de leche* homemade with milk, eggs and sugar. It is usually sold in free markets and it is largely consumed in South America. Food contamination by pathogenic microorganisms can occur during the food processing, in distribution centers, in retail markets or in the consumer's homes. The aim of this study was to evaluate the survival in *ambrosia* of main pathogenic microorganisms eventually transmitted by dairy products. *Ambrosia* fractions were experimentally contaminated with *Salmonella enterica* subsp. *enterica* serotype Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*. Analysis to evaluate the microorganisms' viability were made after storage for 0, 1, 2, 3, 5, 10, 20 and 30 days. *Salmonella* and *L. monocytogenes* were recovered from all samples during the 30 days of study. *E. coli* O157:H7 was isolated until the tenth day and *S. aureus* until the third day. It was demonstrated that important pathogenic microorganisms are able to survive up to 30 days in *ambrosia*, which makes this product a potential carrier of food-borne diseases. This work is the first study about the possibility of *ambrosia* transmitting relevant public-health danger pathogenic microorganisms.

Key words: *Ambrosia, dulce de leche, Salmonella, Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus*

Resumo

Ambrosia é um tipo de doce de leite preparado artesanalmente com leite, ovos e açúcar, comumente comercializado em feiras livres e muito consumido na América do Sul. A contaminação de alimentos por microrganismos patogênicos pode ocorrer durante as etapas de processamento, nos centros de distribuição, no mercado varejista ou na casa do consumidor. O trabalho teve como objetivo avaliar a sobrevivência em ambrosia dos principais microrganismos patogênicos eventualmente transmitidos por leite e derivados. Alíquotas de ambrosia foram experimentalmente contaminadas com *Salmonella enterica subsp. enterica* sorotipo Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes* e *Staphylococcus aureus*. Foram realizadas pesquisas dos microrganismos inoculados após 0, 1, 2, 3, 5, 10, 20 e 30 dias de estocagem. *Salmonella* e *L. monocytogenes* foram recuperadas de todas as amostras durante os 30 dias de estudo. *E. coli* O157:H7 foi isolada até o 10º dia e *S. aureus* foi recuperado até o terceiro dia de estocagem. Foi demonstrado que microrganismos patogênicos importantes em saúde pública são capazes de sobreviver por até 30 dias em ambrosia, o que faz deste doce um potencial veículo de doenças transmitidas por alimentos. O presente trabalho é o primeiro estudo sobre a possibilidade da ambrosia veicular microrganismos importantes em saúde pública.

Palavras-chave: Ambrosia, doce de leite, *Salmonella, Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus*

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Dulce de leche is a product obtained from the concentration and heating of milk with the addition of sucrose, developing a characteristic colour, texture and taste due to non-enzymatic browning reactions (HOUGH et al., 1991). This food is largely consumed in South America, especially in Brazil, Uruguay and Argentina, however there have not been much study about it. The *ambrosia* is a very popular kind of *dulce de leche* homemade with milk, eggs and sugar, usually sold in free markets.

Food contamination by pathogenic microorganisms is a potential hazard to people's health. Food can be contaminated by inadequate practices on the farm, during the manufacturing process, in the distribution centres, in the retail markets or in the consumers' homes. According to Timm et al. (2007) the possibility of *dulce de leche* carrying pathogenic bacteria cannot be excluded, in spite of its low water activity due to the high concentration of carbohydrates. The risk of *ambrosia* contamination is increased by homemade manufacture, many times under improper manipulation and environment exposition. There are few papers on *dulce de leche* in scientific bibliography. Most studies were developed in Argentina or Brazil and focus on the manufacturing process or the physical and chemical characteristics of the product.

The aim of this work was to evaluate the survival of the pathogenic microorganisms *Salmonella enterica* subsp. *enterica* serotype Typhimurium, *L. monocytogenes*, *E. coli* O157:H7 and *S. aureus* in *ambrosia*.

Salmonella enterica subsp. *enterica* serotype Typhimurium LIPOA 2046, which have been isolated from *dulce de leche* (TIMM et al., 2007), *Escherichia coli* O157:H7 ATCC 43895 resistant to nalidixic acid, *Listeria monocytogenes* LIPOA 3002 and *Staphylococcus aureus* LIPOA 4001, which have been isolated from sausage and cheese, respectively, were used. To induce resistance to nalidixic acid, the *E. coli* O157:H7 strain was successively cultured in Plate Count Agar (PCA, Acumedia, Michigan, USA) containing increasing concentrations of nalidixic acid until it grew in PCA with 100 µg nalidixic acid per mL medium. The isolates were kept at –70ºC in Brain Heart Infusion (BHI, Acumedia) cultures mixed with an equal volume of glycerol at 80% in phosphate-buffered saline (PBS 0.01 M, pH 7.4) and recovered when necessary.

The strains were recovered by culture in BHI at 37ºC for about twenty hours and grown until stationary phase. Inocula with about 10^4 bacterial cells per mL were prepared from serial dilutions of the cultures in order to obtain final concentrations of about 10^2 bacterial cells per g, after *ambrosia* experimental contamination.

The *ambrosia* used for experimental contamination with pathogenic microorganisms was prepared from standardised milk containing 3% milk fat. Twelve chicken eggs (yolk and albumen) and 500 g refined sugar was added to each liter of milk. The ingredients were homogenized and kept at 100-105ºC, without stirring, in an open pan on low fire for two hours.

Twenty-five gram *ambrosia* fractions were packed in sterile plastic bags, experimentally contaminated with 0.25 mL inoculum, homogenized and kept at a temperature between 15 and 20ºC. Analysis to evaluate the microorganisms' viability were made after storage for 0, 1, 2, 3, 5, 10, 20 and 30 days. *Salmonella* and *L. monocytogenes* recovery followed U.S. Food and Drug Administration (FDA) recommendations (ANDREWS; JACOBSON; HAMMACK, 2011; HITCHINS; JINNEMAN, 2003). To recover *E. coli* O157:H7, 225 mL Buffered Peptone Water (BPW, Acumedia) was added to the experimentally contaminated *ambrosia* and incubated at 37ºC for 24 hours. Then 1 mL was transferred to 9 mL MacConkey broth (Difco, Maryland, USA) and incubated for 24 hours at 37ºC. From this culture, streaking on MacConkey agar (Difco) containing 100µg nalidixic acid per mL

medium was performed and it was then incubated for 24 hours at 37ºC. To recover *Staphylococcus aureus,* each *ambrosia* contaminated fraction was incubated at 37ºC for 24 hours in 225 mL Trypticase Soy Broth (TSB, Acumedia) containing 1% (w/v) sodium pyruvate and 10% (w/v) sodium chloride. Afterwards, streaking on Baird-Parker agar (Acumedia) was carried out from the culture and the plates were incubated for 24 hours at 37ºC to obtain colony isolation and identification according to FDA recommendations (BENNETT; LANCETTE, 2001). Twenty-five grams of non- experimentally contaminated *ambrosia* was tested to check the presence of the studied microorganism species and serotypes for negative control. The experiment was performed in triplicate.

Salmonella and *L. monocytogenes* were recovered from all aliquots analysed. *E. coli* O157:H7 was recovered until the 10th day after the experimental contamination and *S. aureus* until the 3th day of storage. No microorganisms of the species and serotypes studied were recovered from the negative controls.

Ambrosia aliquots were experimentally contaminated with the main pathogenic microorganisms eventually transmitted by milk and dairy products. Although largely consumed, there are not reports about the role of this food in the food-borne diseases epidemiology. To our knowledge, the present paper is the first study about the putative transmission of relevant public-health hazardous microorganisms in *ambrosia*.

There are not reports about studies on microbiological evaluation and the hygienic-sanitary quality of *ambrosia*. Even works about other kinds of *dulce de leche* were rare. The main study was made by Hentges et al. (2010) using pasty *dulce de leche* experimentally contaminated with pathogenic microorganisms. This researchers recovered *Salmonella*, *E. coli*, *S. aureus* and *L. monocytogenes* from *dulce de leche* experimentally contaminated at 10^3 bacterial cells per g after storage for 30 days. Nevertheles, at 10^1 bacterial cells per g concentration, *E. coli* and *S. aureus* did not survive beyond the 5th and 10th day, respectively. Our results are very similar to theirs, suggesting that the environment provided by pasty *dulce de leche* and *ambrosia* is not very distinct in regard to microorganisms survival.

Salmonella is one of the most common pathogens involved in food toxinfection outbreaks, and dairy products are important *Salmonella* carriers (CDC, 2011). On the other hand, *L. monocytogenes* occurrence in dairy products has been reported especially in cheese (BORGES et al., 2003; PARK et al., 2002; RULDOLF; SCHERER, 2001). The infectious dose of *Salmonella* Typhimurium may be lower than 10 bacterial cells (D'AOUST; MAURER; BAILEY, 2001), and Inoue et al. (2000) consider food with a *L. monocytogenes* concentration higher than 100 CFU/g as high risk food. Thus, the very presence of these pathogens in food is enough to raise concern on food safety. This fact, together with the survival ability of *Salmonella* and *L. monocytogenes* in this food, makes the *ambrosia* a product to be considered in the food-borne salmonellosis and listeriosis occurrence.

The *Salmonella* Typhimurium strain used in this work was isolated from *dulce de leche* in previous study (TIMM et al., 2007), therefore it is possible that the microorganism has adapted to the environment provided by this food. According to D'Aoust, Maurer e Bailey (2001), *Salmonella* is able to survive for long periods in food with high lipid concentrations, which protect the pathogen from unfavorable factors found in some foods. It is possible that the lipids of the whole milk and eggs used to prepare the *ambrosia* have contributed to the *Salmonella* survival during the 30 days of storage. Timm et al. (2007) consider that the common practice of opening the *doce de leite* containers in retail markets to sell in little portions increase the hazard of contamination and transmission of undesirable microorganisms to consumers. This circumstance may also occur because the handmade

conditions of the preparation and packing of the *ambrosia.*

E. coli O157:H7 occurrence in food, as well as that of *Salmonella* and *L. monocytogenes*, is unacceptable. *E. coli* O157:H7 have been associated to several outbreaks of food-borne diseases and the dairy products have been reported as carriers for these bacteria (HUSSEIN; SAKUMA, 2005). The results obtained shows that this pathogen has limited ability to survive in the environment provided by *ambrosia*. However, survival up to 10 days can be dangerous, considering the severity of some clinical cases caused by *E. coli* O157:H7 and the very low infective dose to susceptible groups of 10 to 100 bacterial cells or even lower (ARMSTRONG; HOLLINGSWORTH; MORRIS, 1996).

S. aureus is very spread in the nature, and it is one of the more common agents of food intoxication outbreaks (BALABAN; RASOOLY, 2000). The microorganism is found in the nasal fossae and on the skin of 50% of healthy people. Food industry workers were usually implicated in the intoxication outbreaks, but equipment and environment also may be sources of contamination by *S. aureus*. The intoxication dose of enterotoxin is less than 1.0 microgram. This toxin level is reached when *S. aureus* populations exceed 10^5 organisms/g in food (HAIT, 2012). Considering that the microorganism was only recovered from the experimentally contaminated samples up to three days after inoculation, it seems that even populations larger than those used in this study do not increase but may eventually decrease in the *ambrosia* environment. This, together with the bacterial concentration needed to produce enough enterotoxin to cause food poisoning in humans, suggests that *ambrosia* is not such a high hazard to consumers regarding food-borne *S. aureus*.

The simple fact of a food to be homemade produced does not mean that it does not have hygienic-sanitary quality to be a safe food to human consumption. However, when the products are not surveyed by official agencies, there are not guarantee of standardization of the hygiene processes nether inspection of the good manufacturing practices, which increase the hazards of food contamination and transmission of pathogenic microorganisms to humans.

In this study, it was demonstrated that relevant public-health danger pathogenic microorganisms were able to survive up to 30 days in *ambrosia*, which makes this product a potential carrier of food-borne diseases. The results are an alert regarding the adoption of appropriate hygienic-sanitary proceedings during the production, handling and packing of the *ambrosia* and the attention by the official agency of food inspection to homemade *ambrosia*.

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