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Artigo Científico

Quantification of *Lactobacillus* sp in fermented milks marketed in Fortaleza-CE[1]

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Abstract: This research aimed to evaluate the quantification of *Lactobacillus* sp in three brands of fermented milks collected in the supermarkets of Fortaleza-CE. At the time of purchase of the products, it was observed that none of the lots were at an ideal cooling temperature (up to 5 °C) according to Brazilian legislation (2002). All the evaluated products declared to have bacteria of the genus *Lactobacillus*, so the viability/ quantification of the probiotic cultures was monitored as a function of the total count of lactic acid bacteria in MRS agar at 37 °C under anaerobic conditions for 72h (IDF, 1995). The samples were previously diluted in peptone water (0.1%) and plated using the Spread Plate technique (ICMSF, 1978), and the results were expressed in Colony Forming Units per mL of product (UFC/mL). It is observed that of the three brands evaluated, only one showed a significant amount of cells for the product to be considered probiotic.

Key Words: Probiotics; Lactic Acid Bacteria; Acidophilic milks

Quantificação de *Lactobacillus* sp em leites fermentados comercializados em Fortaleza-CE

Resumo: Realizou-se a quantificação de *Lactobacillus* sp em três marcas de leites fermentados adquiridos em supermercados de Fortaleza-CE. No momento da compra dos produtos, observou-se que nenhum dos lotes estavam em temperatura ideal de refrigeração (até 5 º.C), como preconizado pela a Agência Nacional de Vigilância Sanitária (2002). Todos os produtos avaliados declaravam possuir bactérias do gênero *Lactobacillus* sp, assim a viabilidade/quantificação das culturas probióticas foi monitorada em função da contagem total de bactérias ácido lácticas em ágar MRS a 37 °.C sob anaerobiose durante 72 h. As amostras foram previamente diluídas em água peptonada (0,1%) e plaqueadas utilizando-se a técnica de Spread Plate, sendo os resultados expressos em Unidades Formadoras de Colônia por mL de produto (UFC/mL). Constatou-se que, das três marcas avaliadas, somente uma apresentou quantidade de células significativamente adequada para que o produto venha a ser considerado probiótico.

Palavras-Chave: Probióticos; Bactérias Ácido Lácticas; Leites acidófilos

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1. Introduction

It is observed a growth of the food market with functional claims due to a greater awareness of the population regarding the relationship between health and food (Silva e Martins, 2018; Silva et al., 2017; Monteiro, 2017; Silva et al., 2016; Cruz et al., 2013; Ozen et al., 2012).

Thus, the production of functional foods, including probiotic bacteria, it is an area that conquered space in the food industry in the last years. The consumers are more conscious of the relationship between food and health, and for this reason it has been increasing the search for foods that, besides nurturing, provide benefits to the consumers' health (Fagundes et al., 2018; Guarner, et al., 2017; Oliveira et al., 2017).

To satisfy this new market, bacteria considered probiotic have been incorporated into a wide variety of foods and beverages that are part of the normal diet (Menezes et al., 2013). In this way, the consumer can enjoy sensorially pleasant meals, while at the same time gaining beneficial effects to his health (Coman et al., 2012).

In order for a microorganism to be used as a probiotic, it must be able to express its activity, which is considered beneficial to the health of the host. Some examples of activities performed by probiotics are: stabilization of the intestinal microbiota after the use of antibiotics; promotion of gastrointestinal resistance to colonization of pathogens; reduction of the population of pathogens through the production of acetic and lactic acids, of bacteriocins of different antimicrobial compounds; promotion of lactose digestion in individuals intolerant to this carbohydrate; stimulation of the immune system; relief of constipation, in addition to increased absorption of minerals and vitamins (Oliveira; Batista, 2012; Saad, 2006).

Thus the claim for products containing probiotics should point out the species of the microorganism (probiotic) present that contributes to the balance of the intestinal microbiota. It should also be argued that consumption of such products should be associated with balanced diet and healthy living habits as a potent value-adding factor.

According to the Brazilian Legislation (2008), the minimum viable quantity to effectively be classified as a probiotic should be in the range of 10^8 to 10^9 Colony Forming Units (CFU) in the daily recommendation of the product ready for consumption. Smaller values can be accepted as long as the company proves the effectiveness of the product.

To observe the efficacy of a probiotic food, the number of bacteria present must be viable, active and abundant until the end of the shelf life of the product. In this sense, in foods such as fermented milks, processing and storage under refrigeration are fundamental for this viability (Saad, 2006; Zhao et al., 2008).

According to ANVISA (2008), it is important that probiotic bacteria remain in a high number of viable cells during the shelf-life of the product until its consumption. Several factors affect the survival of microorganisms in the food product, such as the culture used, the inoculum concentration, the interaction between the bacteria present in the food, the acidity of the medium, the level of dissolved oxygen, the availability of nutrients, the temperature and incubation time, storage temperature, etc. (Moroti et al., 2015).

The aim of the work was verify the viability / quantification of Lactobacillus sp present in some brands of fermented milks commercialized in the city of Fortaleza-CE.

2. Materials and Metods

Three brands of fermented milks, which declare the presence of probiotic microorganisms, marketed in the city of Fortaleza-CE, were evaluated. From each brand, three batches were analyzed, chosen by the criterion of validity of the product, thus totaling 18 samples. These were acquired in medium-sized markets of the city, being considered as criterion of product selection, the claim of functional property in the label. At the time of collection in open gondolas, the storage temperature was checked and the expiration date of the registered product.

The cooling temperatures of the gondolas and the samples were verified at the moment of purchase of the products, through a digital thermometer (Akso). After checking the temperature, the samples were conditioned and

transported in isothermal boxes, containing ice cubes, to the Laboratory of Microbiology of Food of the Center University of the State of Ceará, in order to be submitted to the microbiological analyzes. It should be noted that the samples were stored under refrigeration until the time of analysis.

All the evaluated products declared to have bacteria of the genus *Lactobacillus*, thus the viability/quantification of the probiotic cultures was monitored as a function of the total count of lactic acid bacteria in MRS agar at 37 °C under anaerobic conditions for 72 hours (IDF, 1988). The samples were previously diluted in peptone water (0,1 %) and then plated using the Spread Plate technique (ICMSF, 1978). The results were expressed in Colony Forming Units per mL of product (CFU/mL).

3. Results and Discussion

It was found in the three brands evaluated that both the temperature of the cooling gondolas and the evaluated products did not conform to the standards established by ANVISA (up to 5 °.C) (Table 1).

Table 1 Condition of the temperatures of expositors and of different brands of fermented milks (2018, Fortaleza-CE)

Brands	Gondola (Temp. °C)	Product (Temp. °C)
A	6.6	12.4
B	5.6	10
C	5.6	9

Franco e Landgraf (2004) report that the ideal temperature of the gondolas should be below 5 °C, since this temperature can inhibit the growth of possible pathogenic microorganisms existing in the refrigerated products sold in supermarkets.

Bramorski e Vasconcellos (2005) observed that there may be differences between the temperature recorded by the refrigeration chambers and that measured by the thermometer, evidencing the tendency to lack of calibration and periodic maintenance of the equipment.

These results may be related to the lack of maintenance / calibration of the gondolas used in the commercialization of the product, which can lead to sudden changes in temperature and, consequently, losses in the quality and attribution of functional property. In addition, it should be noted that the gondolas that commercialized the fermented milks were opened, which may have contributed to the temperature changes found.

Montanhini e Parades (2015), observed that closed gondolas exhibits less temperature variations during the day than open gondolas, which favors the maintenance of food quality within the established values during the shelf life of the product.

Petrus et al. (2010), observed that the ideal temperature for this type of food is 4 ºC, as this value contributes to the microbiological quality until the end of the shelf life. Santos et al. (2011), reported that a 2 °C rise in storage temperature may favor a 50% reduction in product stability over its shelf life.

Regarding the quantification of lactic bacteria, it was verified that of the three brands evaluated, only one presented counts of bacteria compatible with the current legislation (Table 2).

It was found that the brand A showed the highest count for probiotic microorganisms, more specifically *Lactobacillus* sp. The other two brands presented counts below the recommendation recommended by the current legislation, which may favor a reduction in their alleged beneficial and functional properties.

Table 2 Average quantification of *Lactobacillus* sp in different brands of fermented milks (2018, Fortaleza-CE)

Brands	Viable cell count (CFU/mL)*
A	$1,4 \times 10^8$
B	$7,2 \times 10^6$
C	$5,05 \times 10^6$

Source: Research data; *According to RDC legislation, January 2, 2002, the number of viable cells for a food to be considered probiotic is 10^8 to 10^9 UFC / mL

According to the Fermented Milks and Lactic Acid Beverages Association, in countries such as Japan, the minimum required quantity is 10^7 CFU of probiotic micro-organisms in the product by the end of shelf life (Stanto et al., 2005).

In Brazil, the minimum required quantity is higher, in the range of 10^8 to 10^9 CFU/mL in the product. Therefore, as already mentioned, two brands evaluated in this study were not in compliance, which may compromise the

functional food claim advocated by the Brazilian Resolution (Brasil, 2007).

According to a technical regulation on the identity and quality of fermented milks, the minimum established count of lactic bacteria to ensure the sensorial quality of the product is 106 CFU/mL (Brazil, 2000). In this sense, considering this requirement, all evaluated brands were in accordance with the requirements of the current legislation, which contributes to the sensorial characteristics of the product, but not to claim functional food.

According to (2003), frequents analyzes such as the one of the present study are of fundamental importance, since they demonstrate the need for a greater control in relation to the quantity of microorganisms added to exercise the probiotic function, in order to minimize the commercialization of those that have counts lower than the established limit and which may possibly not have the beneficial effect suggested for such foods.

Changes in the composition of fermented milks can influence the viability of the microorganisms propagated by them. However, it is a great challenge to keep the count of probiotic microorganisms in the products since most of them are sensitive to oxygen, heat and exposition to acid (Stanto et al., 2005).

Thus, it is of fundamental importance that the cultures to be used remain viable during the shelf-life of the product, since factors such as pH and the presence of preservatives may affect the survival rate of microorganisms (Yerlikaya, 2014).

Therefore, low counts of lactic acid bacteria in fermented milks, with a probiotic claim, may demonstrate the risk that some foodstuffs may present because they do not adequately promote the beneficial effects alleged to consumers.

For this reason, effective inspection in the industry and sales points becomes necessary to prevent the commercialization of products that present low amount of lactic acid bacteria in the product.

4 Conclusion

Of the three samples evaluated, only one brand of fermented milk showed lactic acid counts within the established standard for the probiotic claim;

It is observed the necessity of a greater control in the temperature of commercialization, since the temperature conditions to which the fermented milks have been submitted, can affect the survival and viability of the probiotic microorganism, during the shelf-life of the product;

Taking these aspects into account, it is suggested that there should be a greater oversight of the points of sale in order to ensure the desired bacterial contribution and that it has the alleged beneficial effect on consumer health.

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