

Occurrence of *Bacillus sporothermodurans* in UHT Milk Commercialized in the State of Rio Grande Do Sul, Brazil

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Abstract: Milk produced with Ultra High Temperature process is known as UHT. Milk is the most widely accepted by Brazilian consumers. The presence of mesophilic microorganisms in high levels in UHT products will be able to cause the deterioration and/or the reduction of the shelf life. In this study we determined the counting of viable aerobic mesophilic microorganisms and verified the presence of *Bacillus sporothermodurans* (BST) in 511 samples of UHT milk. Eleven different brands have been collected during the period of August 2005 to July 2008, from different cities of the State of Rio Grande do Sul, Brazil. According to the results obtained, it can be concluded that 100% of the total samples of UHT milk analyzed, independent of the brand, were in accordance with the effective legal standard for the viable aerobic mesophilic microorganisms (maximum of 10^2 UFC/ml). It was observed that 55 (10.8%) of the total samples showed characteristic colonies of BST. This allows to affirm that BST is one mesophilic well adapted, exceptionally resistant, being able to survive UHT milk treatments.

Key words: Milk UHT • *Bacillus sporothermodurans* • Brazil

INTRODUCTION

Milk is considered a rich food in nutritional components, constituting an excellent culture medium for various microorganisms. When obtained or processed under inadequate sanitary conditions, may become an important vehicle of transmission of pathogens for humans [1].

The microflora of milk varies considerably in number and species, depending on how it becomes contaminated. When coming from healthy animals, milked aseptically, contains few organisms, but may suffer contamination from the environment and human practices, depending on methods used in the treatment of animals, which determine the microbial load and the species involved in the process [2,3].

According Panetta [4], the processes of benefiting to ensure quality of milk will be efficient if they respect time and temperature, so that the microorganisms are removed and preserved the sensory characteristics and nutritional value of the product.

Commercially, different types of UHT are available as is the case of pasteurized milk [5]. UHT milk is

the homogenized milk subjected to a temperature of 130-150°C for 2 to 4 seconds by thermal processing stream, immediately cooled to temperatures below 32°C and filled under aseptic conditions in sterile containers and sealed [6].

In certain circumstances, highly resistant spores can survive UHT processes subsequently germinating and growing in stored milk. These bacteria spores are described as highly resistant to heat, being named of *Bacillus sporothermodurans* (BST) [7].

According to Petterson *et al.* [7,8], the BST is presented in the form of long rod shape ($> 30 \mu\text{m}$) and stringy, with approximately 0.7 mm in diameter, which produce uneven color when subjected to Gram stain or granular appearance and a string of pearls. The cells also exhibit motility by flagella peritrich and are aerobic strict microorganisms. The range of growth temperature ranges from 20 to 52°C, but with better development between 35 to 42°C and pH 5.7.

There are few studies and technical data about this organism in the literature, involving behavior, physiological and biochemical characteristics, heat resistance and levels of contamination.

The present study aimed to quantify the viable aerobic mesophilic microorganisms and to verify the presence of BST in different brands of UHT milk commercialized in the State of Rio Grande do Sul, Brazil.

MATERIAL AND METHODS

A total of 511 samples of UHT milk (integral, semi-skimmed or skimmed) of 11 different brands commercialized in the State of Rio Grande do Sul, had been examined in this study. Samples have been collected from different cities during the period of August 2005 to July 2008 and the analyses were conducted at the Food Microbiology Laboratory from Univas.

The procedure for the enumeration of viable aerobic microorganisms in liquid UHT dairy products followed the methodology of the Normative Instruction Nr. 62, of the Ministry of Agriculture, Livestock and Supply (MAPA), Brazil [6].

RESULTS AND DISCUSSION

From August 2005 to July 2008, 11 different brands of UHT milk commercialized in different locations in the State of Rio Grande do Sul have been analyzed. Table 1 shows the number of samples analyzed for each type of UHT milk (integral, semi-skimmed and skimmed) and the number of samples of UHT milk confirmed for BST (Figure 1). Depending on the brand analyzed, not always the three different types of UHT milk were available on the market.

Of the 511 samples of UHT milk subjected to analysis, no samples showed visible changes such as bloating, casting, coagulation, after incubation at 36°C for 7 days. All the samples of UHT milk submitted to the analysis were in accordance with the effective legal standards for viable aerobic mesophilic microorganisms (maximum 10²CFU/ml). However, 55 (10.8%) of the total analyzed showed characteristics of BST colonies, being these proceeding ones from seven brands of UHT integral milk (marks C, E, F, G, H, J, K) (Table 1).

According to Pettersson *et al.* [7] the UHT treatment used, should result in destruction of vegetative cells and endospores in raw material, which is not confirmed in this study, where results showed characteristic colonies in seven brands subjected to analysis (Table 1). Only four brands (A, B, D and I) did not reveal BST, which affirms that the result is expressive for integral UHT milk.

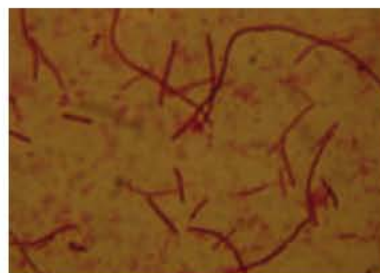


Fig. 1: Morphology of BST isolated from UHT milk. Long rods shapes (> 30 μm), Gram-positive, 3 mm diameter after cultivated for 3 days at 36°C on BHI agar. Source: Food Microbiology Laboratory-increase of 1000 times.

Table 1: Number of samples for each type of UHT milk (integral, semi-skimmed and skimmed) and number of positive samples for BST of 11 different brands of UHT milk commercialized in the State of Rio Grande do Sul

Brand	Nr. of samples	Type of UHT milk	Nr. of positive BST (%)
A	5	Integral	-
	-	Semi-skimmed	-
	5	Skim	-
B	2	Integral	-
	-	Semi-skimmed	-
	-	Skim	-
C	20	Integral	4 (20)
	1	Semi-skimmed	-
	-	Skim	-
D	4	Integral	-
	-	Semi-skimmed	-
	-	Skim	-
E	64	Integral	8 (13)
	-	Semi-skimmed	-
	5	Skim	-
F	16	Integral	13 (81)
	-	Semi-skimmed	-
	1	Skim	-
G	259	Integral	11 (4,2)
	1	Semi-skimmed	-
	10	Skim	-
H	13	Integral	8 (62)
	1	Semi-skimmed	-
	3	Skim	-
I	3	Integral	-
	1	Semi-skimmed	-
	1	Skim	-
J	20	Integral	7 (35)
	6	Semi-skimmed	-
	5	Skim	-
K	62	Integral	4 (6,4)
	2	Semi-skimmed	-
	1	Skim	-

Research conducted in the 60's has already tried to improve the quality of milk, studying the spores of *Bacillus* resistant processing UHT [8,10].

According to Busatta *et al.* [11] 11 samples of UHT milk marketed in the city Alto Uruguai, Rio Grande do Sul State determined the presence of BST on 6 different brands (54.5%). REZENDE *et al.* [2] analyzed 120 samples of four different brands of UHT milk in the region of Ribeirão Preto, SP. *Bacillus cereus* had been found in 34.1% of the samples studied. They also noticed that the total samples analyzed, 36 (30.0%) did not followed the standards established by Brazilian legislation for viable mesophilic microorganisms.

In a study conducted by Coelho *et al.* [12] where 80 samples of UHT integral milk have been analyzed, it was found that 41.2% had populations above the allowed by BST. The researchers mentioned that the quality of raw material used for the production of UHT milk and the type of processing the product should be taken into account when evaluating the results.

In this study, the presence of BST was in higher proportion in UHT integral milk, unlike the results obtained by Pettersson *et al.* [7], who observed higher rate of contamination in lots of skimmed milk than for lots of integral milk. According to the researchers, because the BST are strict aerobic bacteria, oxygen probably widespread in the milk is larger, allowing a development with ease before starting a new sporulation.

According to Franco and Landgraf [13] the presence of lipids in food increases the thermal resistance of some microorganisms. This protection is sometimes called lipid protection. It is assumed that the increase in thermal resistance is directly linked to the fact that fat affects the water content in the cell. The resistance of the spores appears due to protoplast dehydration, mineralization and thermal adaptation.

Busatta *et al.* [11] evaluating the destruction of spores in milk, revealed that there is a need for a longer time than 15 minutes at 121°C and the elimination at that time was 99%, leaving some spores probably germinate under favorable. In relation to milk, it was found that the spores did not survive the same conditions of temperature and time, the elimination of spores capable of germination was complete. In this process, should be taken into account the nutritional and organoleptic characteristics of UHT milk remain appropriate.

It can be concluded that 100% of UHT milk analyzed, regardless of the brand, was in accordance with the applicable legal standard for the parameter analyzed

(mesophilic aerobic viable microorganism). This indicates that the BST is a mesophyll adapted exceptionally resistant and can survive UHT treatments.

One possible hypothesis for the presence of BST in UHT milk is due to the higher fat content compared to skim milk. As the presence of BST was only observed in UHT integral milk, certainly fat molecules surrounding the spores giving them protection and increased thermal resistance.

It is worth noting that the method of detection and quantification of *Bacillus sporothermodurans* routinely employed by microbiology laboratories of food requires different culture media, incubators, confirmation of characteristics colonies and a long time to obtain the results. A method that would reduce the disadvantage of the conventional, especially in reducing the time to obtain analytical results should be necessary.

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