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# Effect of pasteurization on the decay of *Mycobacterium bovis* in milk cream

# Efeito da pasteurização no decaimento de *Mycobacterium bovis* em creme de leite

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## Abstract

Milk cream must be pasteurized in order to be sold in Brazil. However, there are no specific legal requirements for this product, and producers set their own pasteurization parameters using the ones approved for milk as a reference. Considering that fat protects bacteria from heat, that no thermal inactivation studies have been performed on *Mycobacterium bovis* present in cream, and that bovine tuberculosis is endemic in Brazil, the aim of this study was to evaluate the inactivation of *M. bovis* in milk cream subjected to commercial parameters of pasteurization. Milk cream samples were contaminated and pasteurized in a water bath at 75, 80, 85, and 90°C for 5 and 15 s. *M. bovis* cells were plated onto Stonebrink-Leslie medium, incubated at 36°C for 45 days, and quantified; the result was expressed in log CFU $mL^{-1}$. The fat content of the samples ranged from 34% to 37% and the average initial load of *M. bovis* was 8.0 Log CFU $mL^{-1}$. The average decay of the *M. bovis* populations was 4.0, 4.3, 4.9 and 6.7 log CFU $mL^{-1}$ when the cream was treated for 15 sec at 75, 80, 85 and 90°C, respectively, showing that the efficiency of the heat treatment was improved by increasing the temperature of the process. Given the lipophilic nature of *M. bovis*, the cream should be subjected to more intense parameters of pasteurization than those applied to milk.
**Key words**: Fat. Mycobacteria. Thermal death. Thermal treatment.

## Resumo

A pasteurização do creme de leite é obrigatória no Brasil, mas não há parâmetros legais específicos para esse produto, de forma que as empresas estabelecem seus próprios parâmetros tendo como referência mínima os aprovados para a pasteurização do leite. Assim, considerando que a gordura tem efeito termo protetor para as bactérias, que não há estudos de inativação térmica do *Mycobacterium bovis* em creme de leite e que a tuberculose bovina é endêmica no Brasil, o objetivo deste estudo foi avaliar a inativação de *M. bovis* em creme de leite, submetido aos parâmetros comerciais de pasteurização. Amostras de creme de leite foram contaminadas e pasteurizadas em Banho-Maria a 75°C, 80°C, 85°C e 90°C, por

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até 15 s. O *M. bovis* foi quantificado por semeadura em meio Stonebrink-Leslie, incubado a 36ºC por 45 dias, e o resultado expresso em Log UFC $mL^{-1}$. O teor de gordura das amostras variou de 34 a 37% e a carga inicial média de *M. bovis* foi de 8,0 Log UFC $mL^{-1}$. O decaimento médio da população de *M. bovis* foi de 4,0, 4,3, 4,9 e 6,7 Log UFC $mL^{-1}$, quando expostos respectivamente às temperaturas estudadas por 15 s, demonstrando que a eficácia do tratamento térmico melhorou com o aumento da temperatura do processo. Devido ao caráter lipofílico do *M. bovis*, o creme de leite deveria ser submetido a parâmetros de pasteurização mais intensos do que aqueles aplicados ao leite.
**Palavras-chave:** Gordura. Morte térmica. Micobacteria. Tratamento térmico.

## Introduction

Milk cream is a dairy product in the form of a fat emulsion in water and can be classified as low-fat (fat content of 10.0%-19.9%), medium-fat (fat content of 20.0-49.9%), and high-fat (fat content > 50%) (BRASIL, 1996).

The Brazilian law imposes the pasteurization of milk cream before marketing; however, it does not define specific treatment parameters (BRASIL, 1996); therefore, dairy product companies are free to establish their thermal processing procedures. Some temperature/time treatment combinations employed by Brazilian companies are, for example: 65°C for 30 min, 75°C for 15 s, 80°C for 15 s, and 85°C for 3 to 5 s (personal communication)[5].

Considering the protective effect of fat on various microorganisms (MOLIN; SNYGG, 1967; DONNELLY, 1986; FRANCO, LANDGRAF; 1996; MACDONALD; SUTHERLAND, 1993; CHABRA, 1999; BUSSATA, 2005). Ordóñes Pereda et al. (2005) recommended the use of rapid pasteurization (72°C for 15 s) for low-fat milk cream and high-temperature treatments for the other two classes (85 to 100°C for 10 to 15 s).

*Mycobacterium bovis* causes bovine tuberculosis and is one of the non-spore-forming bacteria most resistant to heat. Despite its transmission in milk and its lipophilic nature (SINHA, 1994), no previous studies have assessed the thermal resistance of this pathogen in milk cream. The objective of this study is to evaluate the decay of *Mycobacterium bovis* in milk cream subjected to different thermal treatments because bovine tuberculosis is endemic in the country and recent studies carried out in 13 States showed prevalence of tuberculosis infected herds among 0.36% in the Federal District, and 9.0%, in São Paulo (BAHIENSE et al., 2016; BARBIERI et al., 2016; DIAS et al., 2016; GALVIS et al., 2016; GUEDES et al., 2016; LIMA et al., 2016; NÉSPOLI et al., 2016; QUEIROZ et al., 2016; RIBEIRO et al., 2016b; ROCHA et al., 2016; SILVA et al., 2016; VELOSO et al., 2016; VENDRAME et al., 2016).

## Materials and Methods

An isolate of *M. bovis* (spoligotype SB1141) was obtained from a bovine slaughterhouse in the state of São Paulo (RODRIGUEZ at al., 2004). The isolate was grown at 36°C for 10 days in Stonebrink-Leslie medium (CENTRO PANAMERICANO DE ZOONOSIS, 1985). The inoculum (with a total volume of 12 mL) was prepared suspending 0.3 g of the culture in 0.85% saline solution with 0.05% Tween 80; 6 mL of this inoculum were used to infect 100 mL of commercial pasteurized milk cream previously incubated at 90°C for 15 min in a water bath (WB) to inactivate contaminants, and an aliquot (5 mL) was taken to determine the initial load (IL). Before the contamination, milk cream was analyzed for fat content using the Gerber method (BRASIL, 2006).

The contaminated milk cream was distributed (5 mL) into three test tubes (16 x 160 mm) for each heat treatment investigated. To heat the samples we used a WB at 95°C until the target temperatures (75°C, 80°C, 85°C, and 90°C) were reached. One

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of the test tubes was collected for quantitation of the bacterial load (time 0), and the other two test tubes were incubated at each temperature for 5 and 15 s. Five repetitions of the test were performed at each temperature. The temperature of cream was monitored using a mercury thermometer in another test tube containing 5 mL of not contaminated sample.

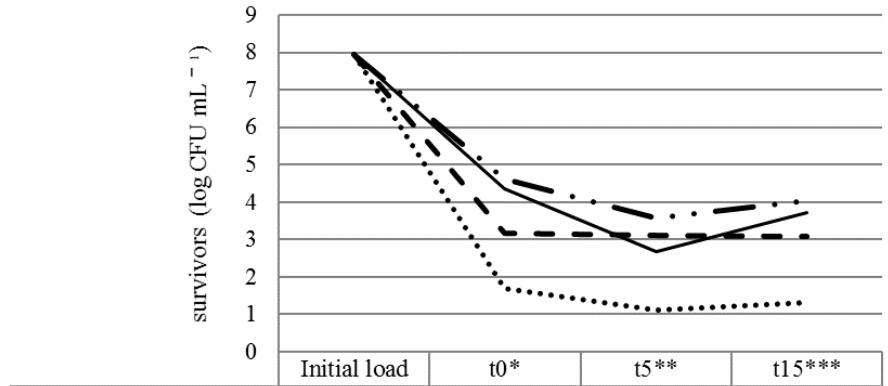
The heating curve of the milk cream was determined separately, and the length of the heating process was recorded seven times at all temperatures tested.

Subsequently, samples were subjected to decimal and serial dilutions in 0.1% peptone water with 0.05% Tween 80 and plated in duplicate onto Stonebrink-Leslie medium (CENTRO PANAMERICANO DE ZOONOSIS, 1985). The readings were performed after 45 days of incubation at 36°C. The dilution chosen for the bacterial count contained between 10 and 150 colonies, and the result was expressed in log CFU $mL^{-1}$.

## Results and Discussion

The fat content of the milk cream ranged between 34% and 37%, and this product was classified as medium-fat milk cream (BRASIL, 1996). The average heating time was 1 min and 41 s for the product to reach a temperature of 75°C, 2 min and 13 s to reach 80°C, 2 min and 46 s to reach 85°C, and 3 min and 51 s to reach 90°C. The IL was 8.0 log CFU $mL^{-1}$ and the bacterial load ranged between 7.8 and 8.3 log CFU $mL^{-1}$. The load of *M. bovis* decreased as a function of length and temperature of the treatment. The most effective temperature for the reduction of the bacterial count was 90°C, which led to a complete inactivation of the inoculum in some replicates; more specifically, counts were lower than 100 CFU $mL^{-1}$. Figure 1 shows the decay of *M. bovis* for each thermal treatment.

**Figure 1.** Decay of the population of *Mycobacterium bovis* (spoligotype SB1141, log CFU $mL^{-1}$) in milk cream as a function of time and temperature of the treatment in a water bath.




| | Initial load | t0* | t5** | t15*** |
|---|---|---|---|---|
| average 75 degrees | 8,0 | 4,6 | 3,6 | 4,0 |
| average 80 degrees | 8,0 | 4,3 | 2,7 | 3,7 |
| average 85 degrees | 8,0 | 3,2 | 3,1 | 3,1 |
| average 90 degrees | 8,0 | 1,7 | 1,1 | 1,3 |

*: when samples reached the target temperature
**: after 5 seconds on target temperature
**: after 15 seconds on target temperature.

Ribeiro et al. (2016a) evaluated the survival of the same spoligotype (SB1141) at 72°C for 20 s in UHT whole milk and the results obtained were similar to the ones we obtained for the treatment combination of 75°C and 15 s. This finding is of particular interest because milk cream contains approximately 10 times the fat content that is usually found in UHT whole milk. In addition, the heating time needed for the sample to reach the target temperature was similar in both studies [1 min 45 sec (WB at 85°C), Ribeiro et al. (2016a); 1 min and 41 sec (WB at 95°C), present study]. This result indicates that the penetration of heat in milk cream is similar to that in whole milk and suggests that the effect of this process is similar in both substrates.

The heating step was the most efficient in inactivating the microorganism (Figure 1), which is consistent with the result obtained by Ribeiro et al. (2016a). Grant et al. (1996) evaluated the resistance of *Mycobacterium paratuberculosis* under conditions of slow pasteurization and observed a significant decrease in bacterial load in the first third of the process followed by a slight reduction in the bacterial load. The results obtained by Sung and Collins (1998) suggest that mycobacteria can adjust to different temperatures by producing heat shock proteins or other molecules able to protect them against the deleterious effects of heat during the later phase of temperature maintenance. The authors also found that, when *M. paratuberculosis* was inoculated into an unheated substrate, a reduction of 1 log CFU $mL^{-1}$ was observed after 90 s of incubation at 71°C; however, when the same substrate previously heated to 71°C was inoculated with *M. paratuberculosis*, a reduction of 6 log CFU $mL^{-1}$ was observed after the same time.

We found that the treatment at 90°C was the most effective and resulted in a decrease in the number of *M. bovis* cells of up to 6.9 log CFU $mL^{-1}$ in milk cream; in addition, although not as effective, the treatment at 75°C significantly decreased the viability of *M. bovis* in milk cream (4 log CFU $mL^{-1}$).

The similarity between the results of bacterial survival in whole milk and milk cream, together with the comparable heating times used for the two products, suggests that the fat content does not influence the lethal effect of the pasteurization process in the presence of the fat percentages tested.

It can be inferred that pasteurization at 75°C for 15 s has the same level of safety for milk cream and whole milk, with respect to contamination with *M. bovis*. However, considering the lipophilic character of *M. bovis* and the higher concentration of fat in milk cream, approximately 10-fold in this case, it is reasonable to assume that, in the natural pasteurization process of milk cream, the initial bacterial load would be 10 times higher than the one found in whole milk. After pasteurization, the final bacterial load in milk cream would also be 10 times higher than the one in pasteurized milk. In this scenario, assuming an initial load of $10^4$ CFU $mL^{-1}$ in whole milk, which constitutes the maximum natural contamination of milk (BALL, 1943), it can be assumed that the initial load in milk cream would be approximately $10^5$ CFU $mL^{-1}$. For milk cream subjected to pasteurization at 75°C for 15 s, the final load would be 10 CFU $mL^{-1}$. However, it is also reasonable to assume that the initial load of *M. bovis* in milk cream is lower than the one obtained in the simulation, considering its likely dilution with uncontaminated milk.

It is difficult to determine the actual significance of these findings for public health, because the initial bacterial load in the milk mixture and the oral infectious dose of *M. bovis*, among other factors, remain unknown (SINHA, 1994); however, we suggest that pasteurization temperatures higher than those approved for the pasteurization of whole milk should be used for milk cream, unless thermal inactivation is performed before the separation of the cream.

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