

**Microbiological aspects of type C pasteurized milk marketed in the cariri region of the
State of Ceará**

**Aspectos microbiológicos do leite pasteurizado tipo C comercializado na região do cariri
do Estado do Ceará**

**Aspectos microbiológicos de la leche pasteurizada tipo C comercializada en la región
cariri del estado de Ceará**

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Abstract

The quality of milk is directly linked to a reduced number of deteriorating microorganisms, low somatic cell count and absence of pathogens. Therefore, the objective of this study was to evaluate the microbiological quality of type C pasteurized milk, marketed in the Cariri region, in the state of Ceará. The Most Probable Number (MPN) of Coliforms at 45°C and the presence of *Salmonella* spp were determined, according to the limits established by the Resolution of the Collegiate Board (RCD) number 12, 02/2001. 33 samples of pasteurized type C milk sold in commercial establishments, including supermarkets and bakeries, were collected in different cities in the state of Ceará. The results showed that 39.40%, equivalent to 13 of the samples, were outside the standards established by the current legislation regarding the coliform count at 45°C. Regarding the analysis of *Salmonella* spp. all samples showed absence of the microorganism.

Keywords: *Coliforms*; Food legislation; Food microbiology; *Salmonella* Phages.

Resumo

A qualidade do leite está diretamente ligada a um número reduzido de microrganismos em deterioração, baixa contagem de células somáticas e ausência de patógenos. Portanto, o

objetivo deste estudo foi avaliar a qualidade microbiológica do leite pasteurizado tipo C, comercializado na região do Cariri, estado do Ceará. Foram determinados o Número Mais Provável (NMP) de Coliformes a 45 ° C e a presença de *Salmonella* spp, de acordo com os limites estabelecidos pela Resolução da Diretoria Colegiada (RCD) nº 12, de 02/2001. 33 amostras de leite pasteurizado tipo C vendidas em estabelecimentos comerciais, incluindo supermercados e padarias, foram coletadas em diferentes cidades do estado do Ceará. Os resultados mostraram que 39,40%, o equivalente a 13 das amostras, estavam fora dos padrões estabelecidos pela legislação em vigor para a contagem de coliformes a 45 ° C. Em relação à análise de *Salmonella* spp. todas as amostras apresentaram ausência do microrganismo.

Palavras-chave: *Coliformes*; Legislação alimentar; Microbiologia de alimentos; *Salmonella* Phages.

Resumen

La calidad de la leche está directamente relacionada con un número reducido de microorganismos en deterioro, bajo recuento de células somáticas y ausencia de patógenos. Por tanto, el objetivo de este estudio fue evaluar la calidad microbiológica de la leche pasteurizada tipo C, comercializada en la región de Cariri, en el estado de Ceará. Se determinó el Número Más Probable (NMP) de Coliformes a 45 ° C y la presencia de *Salmonella* spp, de acuerdo con los límites establecidos por la Resolución de la Junta Colegiada (RCD) número 12, 02/2001. Se recolectaron 33 muestras de leche pasteurizada tipo C que se venden en establecimientos comerciales, incluidos supermercados y panaderías, en diferentes ciudades del estado de Ceará. Los resultados arrojaron que el 39,40%, equivalente a 13 de las muestras, se encontraba fuera de los estándares establecidos por la legislación vigente en cuanto al recuento de coliformes a 45 ° C. En cuanto al análisis de *Salmonella* spp. todas las muestras mostraron ausencia del microorganismo.

Palabras clave: *Coliformes*; Derecho alimentario; Microbiología de los alimentos; *Salmonella* Phages.

1. Introduction

Milk is a food product obtained by complete and interrupted milking of medium and large ruminant animals, rested, healthy and well fed (Brasil, 2011). Presents essential nutrients such as vitamins, minerals and proteins of high biological value (Magan et al. 2020; Mandrioli et al. 2020; Poulsen et al. 2015).

According to Fusco and Quero (2014), milk presents ideal conditions for the development of desirable and undesirable microorganisms, due to its constituents, being an excellent substrate for bacterial proliferation. For this reason, the microbiological quality of milk must be constantly monitored, in order to provide a quality product, reducing losses and free of toxicity (Afriat et al. 2020).

According to Berhe et al. (2020), sensory changes and microbiological growth in milk can be minimized by carrying out practical hygiene and sanitation measures at the milking site and during milk handling, using clean containers and utensils, as well as temperature suitable for transport and storage. Atasever et al. (2020) certified that the control of the food chain must be monitored to ensure that the food does not present any complications to public health, therefore, monitoring the quality of milk hygiene is necessary in order to avoid the growth of undesirable microorganisms.

When preserving pasteurized milk, the maximum temperature limits must be met, since milk after pasteurization must be stored in a cold room and shipped at up to 4,0°C, and for delivery to consumption, the temperature limit must be respected. maximum 7,0°C (Brasil, 2018). Pasteurized milk can be classified according to its type, as: type A, B and C milk.

Type C milk is obtained mechanically or manually, in different herds, does not undergo refrigeration on the farm and is sent in appropriate tanks, direct to the processing industry until 10 am on the day of obtaining it (Brasil, 2002).

The chemical composition, physical-chemical and hygienic characteristics of milk are important parameters for its quality, being recommended by the Ministry of Agriculture, Livestock and Supply, of Normative Instruction 76/2018. Brazilian law establishes minimum and maximum values for bacterial counting, which is allowed in pasteurized milk, thus, the standards that advocate the hygienic-sanitary quality of milk evaluate its microbiological aspect, with the objective of improving its sensory characteristics, since the it can be consumed in its fresh form or added as an ingredient for the production of other products (Brasil, 2018).

Several studies available in the literature have confirmed the presence of microorganisms in quantities above the levels of acceptability of Brazilian legislation, since type C milk has a high degree of contamination, due to some factors such as inadequate processing and / or post-processing recontamination, handling inadequate, poor hygiene of the manipulator and / or process line and failure to implement good manufacturing practices (Santiago et al. 2011; Moura, 2012; Fonseca et al. 2016; Rodrigues and Ferreira, 2016; Santos et al. 2017; Lopes et al. 2018; Pereira et al. 2019)

Therefore, it is assumed that pasteurized milk is a food suitable for consumption, without any risk of contamination by pathogenic bacteria and with its sensory characteristics preserved. The objective of this study was to evaluate the microbiological quality of type C pasteurized milk, sold in the Cariri region, in the state of Ceará.

2. Material and Methods

Samples of type C pasteurized milk sold in commercial establishments, including supermarkets and bakeries, were collected in different cities in the state of Ceará. At the time of collection, the storage temperature of the samples was checked. Then, the samples were transported in isothermal containers with ice to the Food Microbiology Laboratory of the Faculty of Technology Centec - Cariri, where the temperature was subsequently verified, with the aid of an infrared thermometer. Coliforms were counted at 45°C and the presence of *Salmonella* spp. in triplicate, according to the methodology described by the American Public Health Association (Apha, 2005). The results were compared with the microbiological criteria established in the Brazilian legislation, governed by the Resolution of the Collegiate Board (RCB) n°12, 02/2001 (Brasil, 2001).

For analysis of coliforms at 45 ° C, successive dilutions of the samples were prepared in lactated broth that were incubated in a bacteriological oven at 35 ° C for 48 hours, to verify the gas production. From the tubes with gas production, a handle was transferred to tubes containing bright green broth 2% lactose bile, being incubated at 35 ° C for 48 hours. After that time, gas was produced in the tubes and an elevation of the positive tubes was transferred to the Escherichia coli broth, incubated in a water bath at 44.5 ° C for 24 hours. The result was considered as positive if the tubes had gas in the Durhan tube. Next, together with the Most Probable Number (MPN) table, the MPN of total coliforms per mL of sample was determined.

In the research of *Salmonella* spp. a 25g portion of the sample was added to 225 mL of the lactated broth and incubated at 35°C for 24 hours. After the incubation time, the sample was placed in tubes containing tetrathionate broth, selenite-cystine broth and Rappaport-Vassiliadis broth and incubated at 42-43°C for 24 hours. After the incubation period, differential plating was performed by streaking with a nickel loop in the selective media: Brilliant Green Agar, Xylose Lysine, Hektoen Enteric Agar and *Salmonella*-Shigella Agar, incubated at 35-37 ° C for 24 hours. After the incubation period of the differential plating, the biochemical test was carried out, transferring the colonies with the aid of a platinum needle

and inoculation by pricking and streaking in the inclined tubes with the following means: A Lysine Iron agar and Triple Iron Sugar Agar. Finally, incubated at 35-37 °C for 24 hours, and later, it was observed whether there was a typical *Salmonella* reaction.

3. Results and Discussion

The results obtained in the microbiological analyzes of the 33 milk samples are shown in Table I.

Table I. Results of microbiological analysis of type C pasteurized milk.

SAMPLE	Coliforms at 45°C (MPN/mL)	<i>Salmonella</i> spp (25 mL)	Temperature °C (collect point)
1	2x10	Absence	13
2	2,8x10	Absence	10
3	<3	Absence	10
4	<3	Absence	10
5	<3	Absence	19
6	<3	Absence	15
7	<3	Absence	11
8	9	Absence	10
9	2,8x10	Absence	9
10	<3	Absence	16
11	<3	Absence	13
12	1,1x10	Absence	11
13	≥2,4x10 ³	Absence	12
14	2,8x10	Absence	11
15	<3	Absence	10
16	<3	Absence	10
17	<3	Absence	11
18	<3	Absence	10
19	4	Absence	16
20	3	Absence	5

21	7	Absence	8
22	<3	Absence	9
23	<3	Absence	11
24	<3	Absence	10
25	4	Absence	10
26	2,8x10	Absence	10
27	1,1x10	Absence	9
28	1,1x10	Absence	5
29	<3	Absence	10
30	<3	Absence	10
31	1,5x10	Absence	10
32	<3	Absence	12
33	9	Absence	16
Standard allowed (BRASIL, 2001)	Maximum of 4 MPN/mL	Absence	Maximum of 7°C (Normative Instruction n° 76/2018 (BRASIL, 2018))

^a MPN - Most Probable Number. Source: Authors.

In the microbiological analysis for coliform count at 45 °C, the results obtained were 39.40% (n = 13) unsatisfactory, considering that the current legislation requires a standard of up to 4 MPN/ mL. Considering that the bacterial genera belonging to the coliform group are destroyed under pasteurization temperature, the permanence of this would indicate the inefficiency of the heat treatment.

Lower results than the present research were obtained by Santiago et al. (2011), when evaluating 18 samples from three brands and six batches of pasteurized milk marketed in the municipality of Diamantina - MG, under the microbiological aspects in the count of thermotolerant coliforms, found that 22.0% of the samples (n = 4) were at odds with the legislation.

It should be noted that thermotolerant coliforms are hygienic-sanitary indicators, therefore, it is essential to observe and respect good manufacturing practices in order to guarantee product safety. They are microorganisms that live in symbiosis with many warm-blooded animals such as oxen, cats, pigs as well as humans (Menezes et al. 2014).

Research carried out by Lopes et al. (2018) where they analyzed the count of thermotolerant coliforms in pasteurized milk, it was found that there was no contamination by these microorganisms in any sample of pasteurized milk. Conversely, Rodrigues and Ferreira (2016) found that 80% of the milk samples evaluated were contaminated by coliforms at 45°C.

Pereira et al. (2019) regarding the group of thermotolerant coliforms in none of the evaluated milk samples presented contamination by this microorganism. On the other hand, Santos et al. (2017) analyzed samples of pasteurized milk and showed contamination by thermotolerant coliforms in 33% of the samples, at their lowest dilution, which was 10^{-4} , thus showing a high level of contamination.

In a study by Fonseca et al. (2016) the determination of the MPN for thermotolerant coliforms showed a lower count than the values established by legislation, where all samples of pasteurized milk were acceptable. Moura (2012) observed that of the 90 samples analyzed, 31.4% are unfit for consumption, with coliforms at 45°C.

These results may indicate inadequate processing and / or post-processing recontamination, especially with regard to the handling of these foods. The most frequent causes are those resulting from raw material, dirty equipment or handling without proper hygiene care. Thus, the good manufacturing practices (GMP's), required by RCB number 216 of 2004, are fundamental principles to guarantee the proper hygienic-sanitary conditions of the prepared food (Brasil, 2004).

In view of the impact that these contaminations can generate on health, it is of fundamental importance to adopt public policies aimed at training producers and distributors, with regard to care with milk handling. Actions aimed at hygienic-sanitary care throughout the process are essential to guarantee product quality (Santos et al. 2019).

Guido et al. (2010) highlighted the importance of GMP's when developing a study with producers in the municipality of Barbosa Ferraz - PR, where they verified through microbiological analysis a reduction in the level of contamination after the insertion of training on good production practices. However, these same authors stressed that it is essential to provide assistance after training, considering that there is a period of adaptation, until all recommendations are followed in a certain and safe way (Lopes et al. 2018).

The research for *Salmonella* spp showed that all samples analyzed did not contain the presence of this bacterium, where the current legislation establishes a standard of absence

in 25 g, being classified, in this parameter, as “products acceptable for consumption as for microbiological analysis” (Brasil, 2001).

Discordant results to those of the present study were obtained in the research for *Salmonella* spp., In which 25% of the pasteurized milk samples analyzed obtained a positive result, which was confirmed through biochemical tests (Santos et al. 2019). Moura (2012) analyzing 90 milk samples provided by the Ceará state government program, found that 14.1% of the samples were contaminated by *Salmonella* spp. Teixeira et al. (2014) found that 100% (n = 20) of the samples evaluated in the study did not present the presence of *Salmonella* spp.

The shipment of pasteurized milk must be carried out at a maximum temperature of 4°C, by means of its adequate packaging, and taken to the retail trade through vehicles equipped with thermal insulation and equipped with a refrigeration unit, to reach points of sale with a temperature not exceeding 7°C (Brasil, 2018).

Fonseca et al. (2016) obtained mean temperature of type C pasteurized milk samples, 14.4° C. Thus, according to Freire (2006), any change in the temperature of collection or storage can increase the possibility of multiplication of microorganisms present and due to the metabolism of these pathogens there is an increase in the final acidity, which can make the product unfit for consumption.

The result identified through the temperature averages of this study, showed that only 9% (n = 3) samples were below the value allowed by Normative Instruction number 76 of 2018, where a maximum value of 7°C is recommended. Thus, most samples were at inadequate temperatures (91%) at the collection site, which represents a risk to consumers' health.

4. Conclusion

Type C pasteurized milk has great economic and nutritional value for the Brazilian market, but through the results of the present study, it was found that there are still irregularities in the techniques performed in the processing performed with these products. The results suggest the need for actions aimed at improving microbiological quality and the adequate processing of milk, stricter hygiene practices, in addition to compliance with good manufacturing practices in order to ensure greater quality and safety of the final product. In view of this, it is verified the importance of an efficient heat treatment in order to eliminate most of the pathogenic and deteriorating microorganisms.

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