

**Contagem de bactérias probióticas em bebida láctea fermentada sabor morango**

**Probiotic bacteria counting in strawberry-flavored fermented milk beverage**

**Conteo de bacterias probióticas en sabor de fresa de leche fermentada**

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**Laíse Ferreira Lacerda Furtado**

ORCID: <https://orcid.org/0000-0001-7638-4125>

University Center of Technology and Sciences, Brazil

E-mail: [llalaay@gmail.com](mailto:llalaay@gmail.com)

**Ianca Amaral Teixeira Gonçalves**

ORCID: <https://orcid.org/0000-0001-7206-1433>

University Center of Technology and Sciences, Brazil

E-mail: [ianca\\_amaral@outlook.com](mailto:ianca_amaral@outlook.com)

**Clara Mariana Gonçalves Lima**

ORCID: <https://orcid.org/0000-0002-3150-9139>

Federal University of Lavras, Brazil

Email: [claramarianalima@gmail.com](mailto:claramarianalima@gmail.com)

**Jorge Pamplona Pagnossa**

ORCID: <https://orcid.org/0000-0002-7710-2254>

Federal University of Lavras, Brazil

Email: [jorgepampa@gmail.com](mailto:jorgepampa@gmail.com)

**Roseane Mendonça de Figueiredo**

ORCID: <https://orcid.org/0000-0002-5404-7045>

State University of Southwestern Bahia, Brazil

Email: [fraurose@hotmail.com](mailto:fraurose@hotmail.com)

**Ueslei Bruno Cardoso Medeiros**

ORCID: <https://orcid.org/0000-0002-2013-4060>

State University of Southwestern Bahia, Brazil

Email: [ueslei.cmedeiros@hotmail.com](mailto:ueslei.cmedeiros@hotmail.com)

**Ada Azevedo Barbosa**

ORCID: <https://orcid.org/0000-0002-9169-0574>

Federal University of Pernambuco, Brazil

Email: [adabarbosa@hotmail.com](mailto:adabarbosa@hotmail.com)

**Marcelo Silva Brito**

Cândido Tostes Dairy Institute, Brazil

ORCID: <https://orcid.org/0000-0002-6295-0242>

Email: [marcelosbnutricionista@gmail.com](mailto:marcelosbnutricionista@gmail.com)

**Renata Ferreira Santana**

ORCID: <https://orcid.org/0000-0001-6621-6662>

State University of Southwestern Bahia, Brazil

Email: [rena\\_nutri@yahoo.com.br](mailto:rena_nutri@yahoo.com.br)

## Resumo

O objetivo do estudo foi realizar contagem de bactérias lácticas em bebidas lácteas fermentadas de sabor morango, em porções de 90 gramas, comercializados em supermercados de Vitória da Conquista – Bahia. Foram coletadas quatro unidades de amostras de cada uma das quatro marcas escolhidas, de forma arbitrária, em 2 lotes, totalizando 32 amostras. A metodologia adotada foi a descrita pela American Public Health Association (APHA), que envolve as seguintes etapas: diluição seriada da amostra, plaqueamento das diluições pela técnica de *pour plate* com sobrecamada, incubação e contagem de colônias em placa. Das amostras avaliadas, apenas a Amostras A1 se enquadraram no padrão da Normativa Nº 46 de 23 de outubro de 2007, com bactérias viáveis de  $10^6$  UFC/g. Acredita-se que tal resultado possa ser em decorrência de altas temperaturas de armazenamento além da adição de polpas de frutas que também pode influenciar na redução do número de bactérias lácticas, fato evidenciado em iogurtes. Cabe aos estabelecimentos e à Vigilância Sanitária Municipal realizar a fiscalização para que tais produtos tenham suas propriedades funcionais asseguradas.

**Palavras-chave:** Bebida láctea; Fruta; Saúde.

## Abstract

The aim of the study was to count lactic bacteria in strawberry-flavored fermented milk beverages, in portions of 90 grams, marketed in supermarkets in Vitória da Conquista – Bahia, Brazil. Four units of samples were collected from each of the four marks chosen, arbitrarily, in 2 lots, with a total of 32 samples. The methodology adopted was that described by the American Public Health Association (APHA), which involves the following steps:

serial dilution of the sample, plating of dilutions by *pour plate* technique with growth medium overlay, incubation and plate counting of colonies. Of the samples evaluated, only the sample A1 fit the standard of Normative N° 46 of October 23, 2007, with viable population bacteria of  $10^6$  CFU/g. It is believed that this result may be due to high storage temperatures in addition to the inclusion of fruit pulps that can also influence the reduction of the number of lactic bacteria, an evidenced fact in yogurts. It is up to the establishments and the Municipal Sanitary Surveillance to carry out the inspection so that such products have their functional properties ensured.

**Keywords:** Milk beverage; Fruit; Health.

### Resumen

El objetivo del estudio fue contar las bacterias del ácido láctico en las bebidas de leche fermentada con sabor a fresa, en porciones de 90 gramos, que se venden en supermercados en Vitória da Conquista - Bahía. Se recolectaron cuatro unidades de muestras de cada una de las cuatro marcas elegidas, arbitrariamente, en 2 lotes, totalizando 32 muestras. La metodología adoptada fue la descrita por la American Public Health Association (APHA), que implica los siguientes pasos: dilución en serie de la muestra, enchapado de las diluciones mediante la técnica de placa de vertido con recubrimiento, incubación y recuento de colonias en placa. De las muestras evaluadas, solo la Muestra A1 cumplieron con el estándar de la Normativa N° 46 del 23 de octubre de 2007, con bacterias viables de  $10^6$  UFC / g. Se cree que este resultado puede deberse a las altas temperaturas de almacenamiento, además de la adición de pulpas de fruta que también pueden influir en la reducción del número de bacterias lácticas, un hecho evidenciado en el yogur. Depende de los establecimientos y de la Vigilancia Municipal de Salud llevar a cabo la inspección para que dichos productos tengan aseguradas sus propiedades funcionales.

**Palabras clave:** Bebida de leche; Fruta; Salud.

### 1. Introduction

The development of alternatives for the proper use of whey from the production of cheeses is extremely important due to its nutritional quality, volume produced, economic importance and harmful impact to the environment when discarded inadequately (Sousa et al., 2020; Alves, 2014; Debowski, 2014; Paula et al., 2012).

Among the various options for the use of whey, the preparation of dairy beverages is one of the simplest and most attractive alternatives for its use, since it is possible to use equipment previously available in the dairy industries (Melo et al., 2018; Castro et al., 2004).

As reported by Haraguchi et al. (2006) whey proteins (alpha and beta-lactoglobulin, bovine serum albumin and immunoglobulins) exhibit an exceptional profile of essential amino acids, qualifying them as proteins of high biological value. It also has bioactive peptides (immunopeptides, phosphopeptides and exorphins), in which assure distinct functional properties to these proteins.

In this sense, the fermented milk beverages can be defined as the product resulting from the mixture of milk and whey, with or without the addition of products, food substances, vegetable fat, fermented milk, selected dairy starter cultures and other dairy products. Fermentation occurs through the action of cultivation of specific microorganisms and/or added fermented milk(s) and that cannot be subjected to heat treatment after fermentation. The total count of viable lactic bacteria must be at least  $10^6$  CFU/g, in the final product, for the specific lactic cultivation(s) employed, throughout the shelf life (Brasil, 2007).

The objective of this work was to count lactic bacteria which are probiotic in strawberry-flavored fermented dairy beverages marketed in supermarkets in Vitória da Conquista – Bahia, Brazil.

## 2. Material and Methods

Samples of strawberry-flavored milk beverages were obtained in supermarkets in the city of Vitória da Conquista- Bahia. To perform microbiological analyses, four brands commercialized in portions of 90 grams were selected.

Four units of samples from each chosen brand were arbitrarily collected in 2 lots, using 32 samples. The beverages were packed in an isothermal bag of and taken to the laboratory.

Subsequently, the samples were properly identified and homogenized in the packaging itself, according to the established Ministry of Livestock Agriculture and Supply (Brasil, 2003). Before the opening of the packaging, aseptic procedures with 70% ethanol were performed, as recommended by the same institution.

The methodology adopted was that described by the American Public Health Association (2001), which involves the following steps: serial dilution of the sample, plating of dilutions by *pour plate* with overlayer technique, incubation and plate counting of colonies.

After homogenization of the sample, 25 mL of the fermented milk beverage was transferred to dilution tubes using sterilized material. Then, the sample was diluted in 225 mL of buffered and sterilized peptone water at 0.1%, in an Erlenmeyer vial, corresponding to the dilution of  $10^{-1}$ . From the first dilution, the second dilution was performed, in which 1 ml of dilution  $10^{-1}$  was diluted in 9 ml of peptone water ( $10^{-2}$ ). This procedure was performed up to  $10^{-5}$ .

For plating, the *pour plate* technique with overlayer was adopted. For this, 1 mL of each dilution was packed in empty sterile Petri dishes. Then, 20 mL of MRS agar medium (Synth) was added, previously sterilized and fused at 45 °C. The set of samples was carefully homogenized manually. After solidification of the medium, a layer of the same medium was added. The plates were incubated at 35 °C/48h.

The Equation 1 was used to calculate the number of colony forming units (CFU) per mL. Dilution plates were selected in the range between 25 and 250 colonies.

**Equation (1):**  $CFU/mL = n^{\circ} \text{ of colonies on plate} \times \text{inverse of inoculated dilution}$ .

All analyses were performed in triplicate, with 2 repetitions of distinct lots and the results were calculated by the average of the 3 values found and expressed in CFU/mL and presence or absence of lactic bacteria.

### 3. Results and Discussion

Table 1 shows the results of the counting of lactic bacteria in strawberry-flavored fermented milk beverage marketed in Vitória da Conquista – Bahia, Brazil.

Table 1. Lactic bacteria count in fermented milk beverage

Samples	Lactic bacteria count (CFU/mL)	Standard values
A1	$3,3 \times 10^7$	$10^6$
A2	$1,4 \times 10^4$	$10^6$
B1	$4,4 \times 10^4$	$10^6$
B2	$2,1 \times 10^4$	$10^6$
C1	< 25 colonies	$10^6$
C2	$8,8 \times 10^5$	$10^6$
D1	$1,6 \times 10^4$	$10^6$
D2	$1,6 \times 10^5$	$10^6$

Source: Research data

Analyzing the number population of lactic bacteria of the beverage in this study, it was observed that, of the samples evaluated, only one (sample A1) stands within the standard of Normative N<sup>o</sup> 46 of October 23, 2007, with viable bacteria of  $10^6$  CFU/g (Brasil, 2007). It is believed that this result may be due to high storage temperatures, as Silva and Ueno (2013) highlights the storage temperature as a contributing factor to the low value of colony-forming units. In addition to this factor, Rodrigues et al. (2010) reported that the addition of fruit pulps can also influence the reduction of the number of lactic bacteria, a fact evidenced in yogurts.

As in this study, Castilho et al. (2012), analyzing the number of lactic bacteria in fermented milks, marketed in Viçosa - MG, of the 5 brands analyzed in 5 different batches, most were in compliance with the number of viable lactic bacteria.

The lack of these bacteria in their viable form in the product directly impacts the consumer, because people often acquire the product for the benefit of the action of lactic bacteria in the body (Gallina, 2010). Products that have significant counts of lactic acid bacteria have anticholesterolemic, anticarcinogenic, inhibitory effects of pathogenic agents, besides being important in the maintenance of healthy intestinal microbiota (Forsythe, 2013).

Oliveira et al., (2018) evaluated ten samples of fermented milks from different commercial brands in Curitiba - Paraná, all of which had a count above  $10^6$  CFU/mL lactic bacteria during the shelf life of the product. Thus, it is of physiological importance to the health of the consumer, providing therapeutic effect.

Further studies are needed to quantify probiotic bacteria in fermented beverages to determine if there are non-conformities. The limitation of this study is the fact that it evaluated only one type of beverage.

#### 4. Final Considerations

Only one of the analyzed samples of strawberry-flavored milk beverages presented bacteria counting according to the recommendations of current legislation. It is suggested that the decrease in the count may have been caused by the addition of strawberry pulp and failures in the maintenance of temperatures during storage. It is up to the establishments and the Municipal Sanitary Surveillance to carry out the inspection so that such products have their functional properties ensured.

As a suggestion of future works, it is the indication to measure the exposure temperatures of strawberry-flavored dairy beverages at the exhibition counters to secure whether or not they are adequate as recommended by Brazilian legislation.

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**Percentage contribution of each author in the manuscript**

Laíse Ferreira Lacerda Furtado – 10%

Ianca Amaral Teixeira Gonçalves – 10%

Clara Mariana Gonçalves Lima – 10%

Jorge Pamplona Pagnossa – 10%

Roseane Mendonça de Figueiredo – 10%

Ueslei Bruno Cardoso Medeiros – 10%

Ada Azevedo Barbosa – 10%

Marcelo Silva Brito – 10%

Renata Ferreira Santana – 20%