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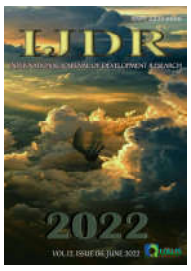

RESEARCH ARTICLE OPEN ACCESS

QUALITY OF REFRIGERATED, PASTEURIZED AND STERILIZED RAW BOVINE MILK FROM INDUSTRIES IN VALE DO TAQUARI – RS

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ABSTRACT

Milk is a food of animal origin, rich in nutrients and which, due to its nutritional properties, is a matrix for the growth of microorganisms. Physicochemical, microbiological and microbiome analysis in milk allow an accurate diagnosis of its quality. The aim of the present study was to evaluate the quality of refrigerated, pasteurized and sterilized raw milk from dairy industries in Vale do Taquari, Rio Grande do Sul, Brazil. Physicochemical and microbiological analyzes were performed, established by Brazilian legislation and the analysis of psychrotrophic microorganisms and total and thermotolerant coliforms. In addition, the microbiome was analyzed through high-throughput sequencing of the 16S rRNA gene. Both industries had somatic cell counts (SCC) above the limit established for refrigerated raw milk and psychrotrophic levels higher than those of mesophiles. Industry 1 presented acidity above the limit in the three types of milk, total bacterial count (TBC) and density for refrigerated raw milk and for pasteurized milk, respectively. The samples presented a wide diversity of genera, composed of psychrotolerant (*Kurthia*, *Acinetobacter*, *Viridibacillus*), biofilm formers (*Pseudomonas*), mastogenic (*Streptococcus*) and lactic acid (*Lactococcus*), in addition to genera considered harmful (*Escherichia*, *Citrobacter*, *Aeromonas* and *Enterobacter*).

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INTRODUCTION

Milk is an example of a nutrient-rich food of animal origin, which contains lipids, proteins (casein), carbohydrates (lactose), amino acids, vitamins and minerals (calcium), and which has several dietary benefits for humans. Due to all these nutritional properties, milk is also a growth matrix for a wide variety of spoilage and/or potentially pathogenic microorganisms (Lindsay *et al*., 2021). Bacterial contaminants in milk are usually animal skin, feed, air, soil, and milking equipment (Elegbeleye & Buys, 2022). The production of this drink represents an important contribution to the economy and social development, as around 150 million families work in milk production worldwide. Most dairy farmers use this activity for their subsistence and are small farmers residing in developing countries (Fao, 2021). Milk production in Brazil was 25.3 billion liters during the year 2021, and in the third quarter this production reached 6.2 billion liters. Rio Grande do Sul is the second largest national milk producer, with a production of 15.1 billion (IBGE, 2021).

Vale do Taquari, a region located in the central part of the state, is responsible for a large part of the state's milk production, and this activity is the basis of the economy of the small municipalities that comprise it. The quality of milk is influenced by several factors ranging from production on milk-producing properties to transport and processing carried out by the industry. The parameters used for the diagnosis of milk quality include analysis of the composition (lactose, protein, fat, total dry extract or total solids and defatted dry extract or non-fat solids), physical-chemical analysis (temperature, acidity, density and cryoscopic) and microbiological analyses, such as total bacterial count (TBC) or mesophilic microorganism count, for the three types of milk (refrigerated raw, pasteurized and sterilized milk) and additionally, alizarol test and somatic cell count (SCC) for refrigerated raw milk.These parameters and their limits are determined by current legislation, Normative Instruction (NI) No. 76/2018 (Brazil, 2018a), NI No. 77/2018 (Brazil, 2018b) and Ordinance No. 370/1997 (Brazil, 1997), from the Ministry of Agriculture, Livestock and Supply (MAPA). NI No. 76/2018 provides information on the identity and quality characteristics that refrigerated

raw milk, pasteurized milk and type A pasteurized milk must present (Brazil, 2018a). IN No. 77/2018 establishes the criteria and procedures for the production, packaging, conservation, transport, selection and reception of raw milk in establishments registered with the official inspection service (Brazil, 2018b), and Ordinance No. 370/1997 regulates the identity and quality of milk sterilized by the Ultra High Temperature (UHT) process (Brazil, 1997).One of the ways used to reduce the microbial growth of milk, causing its degradation, is the cooling right after milking and during transport. This cooling must occur at a temperature of up to 5 °C (Brazil, 2020) and remain below this temperature until reaching the dairy industry, where it will undergo processing. The decrease in temperature reduces bacterial proliferation, however, favors the proliferation of psychrotrophic microorganisms. Psychrotrophic microorganisms can produce heat-resistant proteolytic and lipolytic enzymes that remain active after heat treatment, potentially affecting the quality and shelf life of milk and dairy products. Thus, it is necessary to investigate the psychrotrophic bacteria existing in milk in order to control contamination and proliferation from its source (Yang *et al*., 2020).

In addition to the analysis of psychrotrophic microorganisms, the analysis of total and thermotolerant coliforms in milk plays an important role in the dairy industry, as these microorganisms are often used as indicators of hygiene in the milk production process (Masiello *et al*., 2016). Another current tool that has been used to identify the quality of milk produced is high-throughput sequencing, which provides detailed and valuable information about the microbial community in milk and dairy products in general.High-throughput sequencing enriches the understanding of the role of microorganisms in milk and dairy products (You *et al*., 2022). In this way, the information obtained through conventional physical-chemical and microbiological analyses, together with genetic sequencing, promote an accurate diagnosis of the quality of the milk produced, being also a tool for the improvement of the processing processes used in the dairy industry. Current Brazilian legislation does not specify acceptable levels of psychrotrophic microorganisms and total and thermotolerant coliforms and does not regulate the analysis of data obtained by sequencing. The objective of the present study was to evaluate the quality of refrigerated raw milk from tank trucks, pasteurized milk and milk sterilized by the UHT process, from dairy industries in Vale do Taquari - RS, through physical-chemical and microbiological analyzes established by current legislation, in addition to counting psychrotrophic microorganisms, analysis of total and thermotolerant coliforms and the microbiome, through high-throughput sequencing of the 16S rRNA gene.

MATERIALS AND METHODS

Six samples were collected in two industries, in two cities in Vale do Taquari - RS, one of refrigerated raw milk from the tank truck, one of pasteurized milk and one of milk sterilized by the UHT process in each of the industries. The industries were named "I1" for Industry 1 and "I2" for Industry 2, and the types of milk had their abbreviated names, being "Raw" for refrigerated raw milk, "Past.", for pasteurized milk and "Ster. ", for sterilized milk.Samples were collected using sterilized plastic bottles, and all hygiene precautions were followed. The samples were placed in a styrofoam box with ice, kept at a temperature below 7 °C. Physicochemical, microbiological and milk composition analyzes were performed up to 10 hours after sample collection and sequencing up to 24 hours after collection.

Molecular Analysis: The identification of microorganism genera was performed using high-performance sequencing of the V3/V4 regions of the 16S rRNA gene. The primers for the V3-V4 region of the 16S rRNA gene were: 341F (CCTACGGGRSGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). PCR reactions were performed in triplicates, with the conditions: 95 °C for 5 min, 25 cycles of 95 °C for 45 s, 55 °C for 30 s and 72 °C for 45 s and a final extension of 72 °C for 2 min. The MiSeq Sequencing System equipment (Illumina Inc., USA) was used to sequence the genomic libraries. For single-end sequencing, the V2 kit with 300 cycles was used. The sequences were analyzed using the Sentinel pipeline. In the Sentinel pipeline, fastq files are evaluated for Phred quality (QP) using the FastQC v.0.11.8 program (Andrews, 2010). Therefore, the fastq files were submitted to low quality primers and sequence trimming (Phred< 20). The software used for this purpose was built in Python v.3.6, which is inspired by the features of the BioPython project (Cock *et al*. 2009). For paired-end data, before the trimming step, two pairs of files (R1 and R2) were merged into one file using pandaseq v.2.11. Clusters with abundances less than two were removed from the analysis, as such structures are usually related to chimera sequences (Smyth *et al*., 2010). Taxonomic identifications were performed with BLASTn v.2.6.0 (Altschul *et al*., 1990), using a proprietary or public database as a reference.

Physicochemical and milk composition analysis: Sample temperatures were measured using an Incoterm thermometer (model 5135) at the time of collection. In refrigerated raw milk, the analyzes of milk composition: protein, lactose, total dry extract (TDE) and defatted dry extract (DDE) were carried out using ISO 9622-IDF141:2013 (ISO, 2013). The SCC analysis was performed using ISO 13366-2-IDF148-2:2006 (ISO, 2006), and for this analysis a 40 mL bottle with Bronopol preservative was used to collect the samples. To perform the alizarol test, a 10 mL beaker and 75% alizarol-alcohol was used. 10 mL of the alcohol-alizarol solution was mixed with 10 mL of milk and homogenized (Gasparotto *et al*., 2020). The rules used for the composition of milk in processed milk (pasteurized and sterilized) were: defatted dry extract, according to the manual of official methods for analyzing foods of animal origin by MAPA (Brazil, 2019); Total dry extract: ISO 6731-IDF 21:2010 (ISO, 2010); Lactose: ISO 22662-IDF 198:2007 (ISO,2007); Lipids: NMKL 40:2005 (NMKL,2005) and Total Protein: ISO 8968-1-IDF 20-1:2014 (ISO,2014). 1 liter of sample was collected to perform these analyses. The acidity and density analyzes followed the same methodology in the three types of milk. The acidity analysis was performed by titration, in which 10 mL of milk was pipetted into a 100 mL beaker, and 5 drops of 1% phenolphthalein were added. Sodium hydroxide (NaOH) 0.1N was then diluted until a persistent pink color identical to the standard for approximately 30s. The acidity was calculated as follows: Titratable acidity, % lactic acid = V. 0.09. N x 100/v, in which: V: corresponds to the volume of 0.1N NaOH solution spent in the solution in mL; v: is the sample volume in mL; 0.09: refers to the lactic acid conversion factor and N: is the normality of the 0.1N NaOH solution (Brazil, 2019). Density analysis was performed using a thermolacton densimeter equipment, in which 500 mL of the sample was poured into a beaker, without creating foam, and the equipment was inserted to perform the reading (Brazil, 2019). All analyzes were performed in triplicate.

Microbiological analyzes: The TBC analyzes were carried out according to the methodology recommended by ISO 21187-IDF196:2004 (ISO, 2004). The analyzes of mesophilic and psychrotrophic microorganisms were carried out using the methodology described in the Standard Methods for the examination of dairy products (Apha, 2004). For the determination and quantification of aerobic mesophilic microorganisms, the decimal dilution methodology was used, in which 1 mL of the sample was pipetted, transferring it to a tube containing 9 mL of 0.1% peptone. From this dilution, decimal dilutions 100, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made. Petri dishes received 1 mL of the dilutions, with approximately 20 mL of Plate Count Agar (PCA) agar (OXOID®), using the depth plating method, with inverted plates incubated at 36 ± 1 ºC for 48 hours. For the determination and quantification of aerobic psychrotrophic microorganisms, the surface of PCA agar (OXOID®) received 0.1 mL of dilutions 100, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}, using the method of plate dispersion (surface), with incubation of inverted plates at 7 ºC for 10 days. Counts were performed with a colony counter and the results were expressed in CFU/mL (Colony Forming Units per mL). The analysis of total and thermotolerant coliforms was performed using the Multiple Tube Technique, a method recommended by ISO 4831:2006 (ISO, 2006). 1 mL of the sample was inoculated in a series of 3 tubes in Lauryl Sulfate Tryptose Broth (OXOID®) in test tubes containing inverted Durham tubes. A dilution

was performed using saline peptone solution, concentration 10^{-1} and 1 mL was added in a series of 3 tubes Lauryl Sulfate Tryptose Broth (OXOID®). The inoculated tubes will be incubated at 30 ºC for 24 or 48 hours in a bacteriological oven. The tubes with a positive presumptive reaction, evidenced by the production of gas, were then submitted to the confirmatory test in 2% Brilliant Green Lactose Bile Broth (OXOID®). The tubes that showed gas formation in the Brilliant Green Bile 2% test were transferred to Escherichia coli broth (EC) and remained in a water bath for 48 hours at a temperature of 45 ± 0.2 ºC. All microbiological analyzes were performed in triplicates. To verify the quality of the milk, the results found in the analyzes were compared with the limits defined by IN No. 76/2018 of MAPA (Brazil, 2018a), for refrigerated and pasteurized raw milk and by Ordinance No. 370/1997 of MAPA (Brazil, 1997), for sterilized milk. The legislation does not establish amounts of psychrotrophic microorganisms and total and thermotolerant coliforms, however, the results were confronted with such material or with recent scientific publications.

Data Analysis: The data were tabulated using the Excel spreadsheet and statistical tests of Q-square (χ^2) and Shannon's biodiversity index were performed using the Bioestat 5.0 program and principal component analysis (PCA) using the Past program. The genders and the number of microorganisms found in the samples of the three types of milk, raw refrigerated, pasteurized and sterilized, and the two industries analyzed in this study were compared.

RESULTS

The physical-chemical analyzes show that the milk composition parameters: proteins, lactose, fat, EST and ESD and the physical-chemical parameters: temperature, acidity and density are in accordance with the parameters established by legislation, NI No. 76/ 2018 of MAPA (Brazil, 2018a), in industry 2 (Table 1). Industry 1 presented acidity results above the maximum parameter allowed for the three types of milk analyzed (refrigerated, pasteurized and sterilized raw). According to NI No. 76/2018 and Ordinance No. 370/1997 (MAPA), milk acidity levels must remain between 0.14 and 0.18g of lactic acid/100 mL (Brazil, 1997; Brazil, 2018a). In addition, pasteurized milk from industry 1 had a density above the permitted level, with a value of 1.037.

sample having a yellowish color. According to legislation, the SCC analysis and the alizarol test must be performed only on refrigerated raw milk. The average of TBC, mesophilic and psychrotrophic results in refrigerated and pasteurized raw milk are shown in Figure 1. Industry 1 has TBC above the maximum amount allowed by current legislation for refrigerated raw milk, which is up to 900,000 CFU/mL (Brazil, 2018a) in the tanker truck (Figure 1a). The amount of microorganisms in raw milk from industry 1 also extrapolated the maximum value measured by the method, having an estimate of 4,599,000 CFU/mL (4.59 x 10^6 CFU/mL). Industry 2 presented TBC results within the limits of current legislation, and the total bacterial count of refrigerated raw milk from the tanker was 466,000 CFU/mL (4.66 x 10^5 CFU/mL).

Pasteurized milk from industry 1 (Figure 1b) had a mesophilic microorganism count of 9,700 CFU/mL (9.7 x 10^3 CFU/mL). In industry 2, the count of mesophilic microorganisms was 510 CFU/mL (5.1 x 10^2 CFU/mL). NI No. 76/2018 (Brazil, 2018a) does not establish the maximum amounts of mesophilic microorganisms for pasteurized milk. The sterilized milk from both industries did not show colony growth, with the count of mesophilic organisms equal to zero. When evaluating the amount of psychrotrophic microorganisms, industry 1 had a count of 10,000,000 CFU/mL (1.0 x 10^7 CFU/mL) and industry 2 of 6,000,000 CFU/mL (6.0 x 10^6 CFU/mL), for refrigerated raw milk. Pasteurized milk showed counts above 10^3 CFU/mLin both industries, being 80,000 CFU/mL (8.0 x 10^4 CFU/mL) in Industry 1, and 40,000 CFU/mL (4.0 x 10^4 CFU/mL) in industry 2. The count of psychrotrophic microorganisms was much higher than the count of mesophilic microorganisms in the two industries analyzed (Figure 1). The sterilized milk from the industries showed a count of psychrotrophic microorganisms equal to zero. The analysis of total and thermotolerant coliforms showed that the two samples of refrigerated raw milk from the industries of Vale do Taquari - RS presented values equal to 110 MPN/mL, both for total coliforms and for thermotolerant coliforms. Pasteurized milk and sterilized milk from the industries did not present microorganisms of the coliform group in the analyzed samples. Genetic sequencing analyzes showed a total of 51,401 sequences of microorganisms distributed in 41 genera of individuals of the Bacteria Domain. The eight main genera found in the total samples from the industries were: *Bacillus* (14,146), *Kurthia* (9,569), *Streptococcus* (9,222), *Enterobacter* (5,747), *Lysinibacillus* (3,530), *Aeromonas* (1,776),

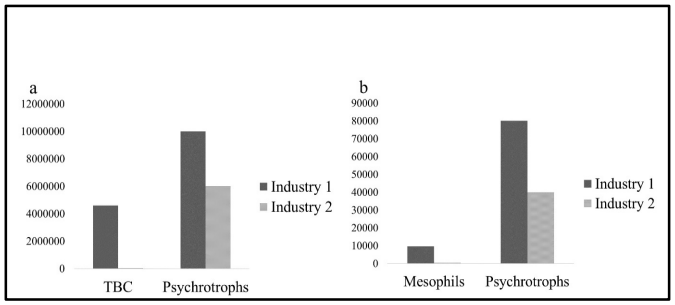
Table 1. Results of physicochemical and compositional analyzes found in milk samples from industries in Vale do Taquari-RS

Parameter		Industry 1				Industry 2			
	Limits	Raw	Past.	Ster.	Average	Raw	Past.	Ster.	Average
Temperature(ºC)	upto 5°C* e 4 °C**	4.8	3.7	22.1	10.2	3.6	4.0	28	11.86
Acidity (g latic acid/100 mL)	0.14 to 0.18	0.67	0.21	0.24	0.37	0.18	0.17	0.18	0.18
Density (g/mL)	1.028 to 1.034	1.028	1.037	1.028	1.031	1.033	1.033	1.033	1.033
Fat (g/100g)	min. 3.00	4.37	3.80	3.00	3.72	3.80	3.20	3.00	3.33
Protein (g/100g)	min. 2.90	3.30	3.24	3.28	3.27	3.27	3.26	3.27	3.27
Lactose (g/100g)	min. 4.30	4.33	4.89	4.72	4.64	4.48	4.92	4.78	4.72
TDE (g/100g)	min. 11.40	13.08	12.42	11.50	12.33	12.59	12.02	11.92	12.17
DDE (g/100g)	min. 8.40	8.71	8.60	8.50	8.60	8.79	8.80	8.90	8.83

Limits and and average of the physical-chemical and composition parameters of raw, pasteurized and sterilized milk from samples from the industries of Vale do Taquari -RS. TDE: Total Dry Extract; DDE: Defatted Dry Extract; *: for refrigerated raw milk; **: for pasteurized milk.

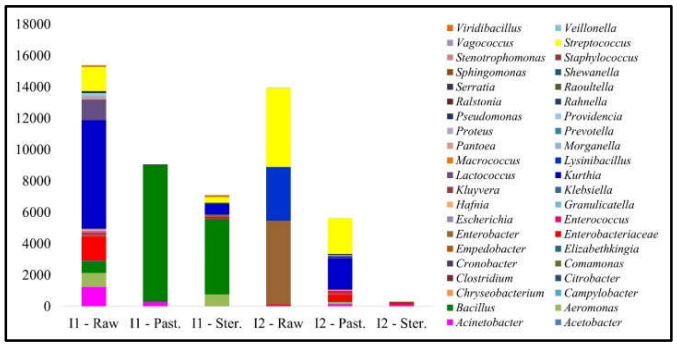
The legislation establishes that the density of milk must be between 1.028 to 1.034 g/mL (Brazil, 2018a). All samples of refrigerated and pasteurized raw milk had a temperature below the maximum allowed at the time of collection, which is up to 5 °C for refrigerated raw milk (Brazil, 2020) and up to 4 °C for pasteurized milk (Brazil, 2020; Brazil, 2018a). The sterilized milk is stored at room temperature and, at the time of collection, it presented a temperature of 22.1 °C in the sample from industry 1 and 28 °C in the sample from industry 2. The SCC analysis showed that the two industries presented values above the maximum allowed by the legislation, which is up to 500,000 SC/mL (Brazil, 2018a), with a value of 1,079,000 SC/mL being obtained in industry 1 and 638,000 SC /mL in industry 2. In industry 1, the amount of SC extrapolated the maximum level obtained by the method, having its value established by estimate. Industry 1 also showed positive alizarol test for refrigerated raw milk, with the

Acinetobacter (1,759) and *Lactococcus* (1,358). Another 33 genera appear with sequences ranging from 263 (*Enterococcus*) to only two (*Clostridium*) and 2,201 sequences were not identified at the genus level, being classified as bacteria of the *Enterobacteriaceae* family. The refrigerated raw milk sample from industry 1 (I1-Raw) presented 15,384 sequences distributed in 32 genera. The 11 main genera were: *Kurthia* (6,907 sequences), *Streptococcus* (1,516), *Lactococcus* (1,307), *Acinetobacter* (1,233), *Aeromonas* (892), *Bacillus* (701), *Providencia* (181), *Enterobacter* (139), *Escherichia* (121), *Enterococcus* (111) and *Hafnia* (101). Another 21 genera appear in the sample with sequences between 1 and 93 (Figure 2). In pasteurized milk (I1-Past.), industry 1 presented a total of 9,052 sequences of microorganisms distributed in 13 genera, the main two being: *Bacillus* (8,699) and *Acinetobacter* (290).



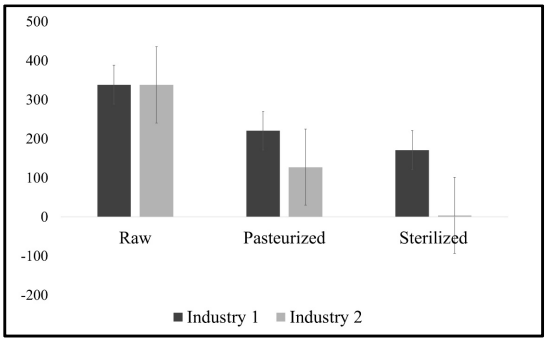
Total bacterial count (TBC) and mesophilic and psychrotrophic microorganisms in CFU/mL, in samples of raw and pasteurized milk from industries in Vale do Taquari. (a) Total bacterial count and amount of psychrotrophic microorganisms in samples of refrigerated raw milk from industries 1 and 2. (b) Count of mesophilic microorganisms and psychrotrophic microorganisms in samples of pasteurized milk from industries 1 and 2.

Figure 1. Average of total bacterial counts, mesophiles and psychrotrophs in samples milk collected at industries in Vale do Taquari- RS



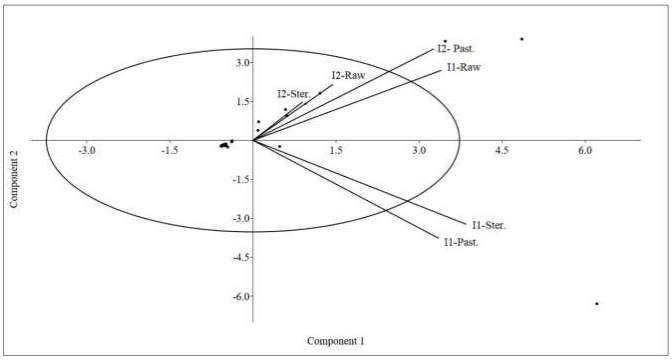
Genus and *Enterobacteriaceae* family found in the milk samples from the industries of Vale do Taquari – RS. I1-Raw: refrigerated raw milk from industry 1; I1-Past.: Pasteurized milk from industry 1; I1-Ster.: Sterilized milk from industry 1; I2-Raw: refrigerated raw milk from industry 2; I2-Past.: Pasteurized milk from industry 2; I2-Ster.: Sterilized milk from industry 2.

Figure 2. Genus and family of microorganisms identified in milk samples from industries in Vale do Taquari - RS



Average amount of microorganisms found in samples of raw, pasteurized and sterilized milk from industries in Vale do Taquari -RS and standard error.

Figure 3. Arithmetic average of the genus found in the milk samples from the industries of Vale do Taquari – RS



Sorting chart using multivariate analysis in the Past program. I1-Raw: refrigerated raw milk from industry 1; I1-Past.: Pasteurized milk from industry 1; I1-Ster.: Sterilized milk from industry 1; I2-Raw: refrigerated raw milk from industry 2; I2-Past.: Pasteurized milk from industry 2; I2-Ster.: Sterilized milk from industry 2.

Figure 4. Perceptual map of multivariate analysis of milk samples collected in industries in Vale do Taquari – RS

In third place, the genus *Pseudomonas* was observed, with only 26 sequences. Another ten genera appear in the sample with less than 10 sequences. The sterilized milk from industry 1 (I1-Ster.) presented a total of 7,090 sequences distributed in 22 genera, nine of which were the main ones: *Bacillus* (4,743), *Aeromonas* (754), *Kurthia* (661), *Streptococcus* (369), *Enterobacter* (145), *Viridibacillus* (113), *Citrobacter* (59) and *Pseudomonas* and *Comamonas* (47). Another 13 genera appear in the sample with less than 21 sequences. The refrigerated raw milk sample from industry 2 (I2-Raw) presented a total of 13,950 sequences, distributed in 11 genera, three of which were the most abundant: *Enterobacter* with 5,351 sequences, *Streptococcus* with 5,053 and *Lysinibacillus*, with 3,402. In fourth place appears the genus *Acinetobacter*, with only 30 sequences. Another seven genres have an insignificant amount of sequences, ranging from 14 to a single sequence. Pasteurized milk from industry 2 (I2-Past.) presented a total of 5,622 sequences distributed in 34 genera, seven of which were more abundant: *Streptococcus* (2,276), *Kurthia (*1,992), *Enterococcus* (150), *Aeromonas* (127), *Acinetobacter* (113), *Enterobacter* (109) and *Lysinibacillus* (103). Another 11 genera appear with sequences between 10 and 50 and 16 genera had a number of sequences less than 10. The sterilized milk from industry 2 (I2-Ster.) showed 303 sequences distributed in 15 genera, three of which were more expressive: *Acinetobacter* (91), *Lactococcus* (17) and *Pseudomonas* (17) and 140 sequences identified at the family level, *Enterobacteriaceae*. Twelve other genera appear in the sample with less than 10 sequences. Using χ^2 to compare the genera found in the milk samples from the industries of Vale do Taquari, it can be seen that there is a statistically significant difference ($p = 0.0001$).

This difference occurs between samples of refrigerated raw milk, pasteurized milk and sterilized milk from the same industry and between the same types of milk from different industries. When calculating the average of genera of microorganisms found in the milk samples from the industries of Vale do Taquari (Figure 3), it is possible to observe that the two samples of refrigerated raw milk have the same and higher average of microorganisms, these being 338.54 and 338 .12 sequences (Industry 1 and Industry 2, respectively). Then comes the sample of pasteurized milk (220.78) and the sample of sterilized milk (171.02) from industry 1. Pasteurized milk from industry 2 showed an average of 127.56 microorganisms and finally, the sterilized milk from industry 2, showed the lowest average of all samples, 3.98 microorganisms. The standard error of the samples ranged from 212.07 in the sample of pasteurized milk from industry 1 and 2.26 in the sterilized milk from industry 2. The sample with the greatest diversity of genera was pasteurized milk from industry 2 with 34 genera, followed by the raw milk sample from industry 1, with 32 genera, and the sample of sterilized milk also from industry 1, with 22 genera. The raw milk sample from industry 2 had the lowest number of genera with only 11. The sterilized milk sample from industry 2 had 15 genera and the pasteurized milk sample from industry 1 had 13 genera. The Shannon diversity index of the milk samples analyzed in this study ranged from 0.1961 in the pasteurized milk sample from industry 1 to 1.845 in the refrigerated raw milk sample also from industry 1. The sterilized milk sample from industry 2 had an index of 1.649, followed by the pasteurized milk sample from industry 2 (1.54), the sterilized milk sample from industry 1 (1.206) and the refrigerated raw milk sample from industry 2 (1.108). The Shannon-Weaver diversity index considers equal weight between rare and abundant species and the lower the index value, the lower the degree of uncertainty and, therefore, the sample diversity is low (Furtado and Vieira, 2020). The analysis of the principal components (PCA) of the genera found in the samples from the analyzed industries (Figure 4) demonstrates that there is an association between the refrigerated raw milk sample from industry 1 and the three samples from industry 2 (refrigerated raw, pasteurized and sterilized). The sample of pasteurized milk from industry 1 is associated with the sample of sterilized milk from industry 1. Component 1, sample of refrigerated raw milk from industry 1, explains 35.07% of the results and component 2, sample of pasteurized milk from industry 1 explains 31.05% of the results, together these components explain 66.12% of the results.

DISCUSSION

Physico-chemical parameters and milk composition of industries: Industry 1 showed a positive alizarol test for raw milk and acidity above the maximum level allowed for refrigerated, pasteurized and sterilized raw milk. Pasteurized milk from the same industry presented density above the maximum allowed. The alizarol test aims to verify the stability of the milk, confirmed by the formation of a brick color in the analyzed sample. When the milk is unstable, clumps form and its color may be violet or yellow, being rejected by the industries. The yellow color represents acidification of the sample, usually caused by microbial activity and the violet color indicates fraud by addition of constituents, such as acidity reducers, with sodium bicarbonate being the most used (Ulisses *et al*., 2022). The sample from industry 1 showed a yellowish color, indicating acidification of the sample by microbial metabolism, and this same sample showed acidity above the allowed level. According to Sandoval and Ribeiro (2021), milk acidity is characterized by the presence of microorganisms that metabolize lactose, forming lactic acid. The fact that pasteurized milk and sterilized milk from industry 1 (0.21 and 0.24 g lactic acid/100 mL, respectively) present milk acidification may be a result of acidity much higher than that allowed in refrigerated raw milk (0 .67 g lactic acid/100 mL), which was almost four times higher than the maximum level allowed by legislation, which is up to 0.18 g lactic acid/100 mL. In addition, pasteurized and sterilized milk showed a higher number of sequences of microorganisms than those observed in industry 2, with this difference being more expressive in sterilized milk (7,090 sequences: industry 1 and 303 sequences: industry 2). The density of milk is variable, depending on its composition and is used to control fraud, up to a certain limit, the main ones being previous skimming and the addition of water. Samples with densities below or above that determined by NI No. 76 (Brazil, 2018a) cause the rejection of milk by industries (Ulisses *et al*., 2022). The pasteurized milk sample from industry 1 showed higher density than recommended. According to Souza *et al*. (2018), density above the established levels may indicate that the milk was skimmed or that some corrective product was added.

Microbiological parameters of industrial milk: Microbiological analyzes show that industry 1 has SCC and TBC above the levels allowed by current legislation for refrigerated raw milk. Industry 2 presented TBC within the established, but had SCC levels above the limits established by legislation. The number of somatic cells is 69% higher in Industry 1 compared to Industry 2 (441,000 SC/mL more) and the TBC is 10 times higher in Industry 1 compared to Industry 2. Chemical composition and microbiological quality of milk are extremely important in the production of milk and dairy products. In this context, SCC and TBC have a great influence on the organoleptic characteristics, as well as the durability and shelf life of the milk (Martins Junior *et al*., 2021). There is a direct relationship between SCC and milk quality. This parameter is a well-established and commonly used milk quality criterion for evaluating the intramammary health status of both individual animals and bulk milk tanks. Udder infection is considered the most frequent cause of increased SCC in bovine milk and is mainly caused by pathogenic microorganisms. In the milk of uninfected animals, epithelial cells form about 50% of somatic cells, with the remainder derived from blood and leukocytes. Polymorphonuclear leukocytes, macrophages and lymphocytes represent approximately 25%, 15% and 10%, respectively, of SCC (Moradi *et al*., 2020). High amounts of SCC, in addition to reflecting on the quality of milk, closely interfere with the industrial yield of dairy products (Martins Junior *et al*., 2021). TBC is another important tool in monitoring the quality of raw milk, being an indicator of hygienic-sanitary conditions in obtaining milk. This count is often directly correlated with the count of psychrotrophic bacteria (PBC) in the product (Lampugnani *et al*., 2018). Milk collected shortly after milking often has a low TBC and this may be due to the milk not yet having come into contact with biological contaminants. A very high bacterial count can be caused by the absence or poor hygiene at the time of milking (Melo *et al*., 2021). For Hahne *et al*. (2019), the microbiota of bulk tank milk with high bacterial counts is predominated by cold-adapted species, such as psychrotrophic microorganisms, which have high rates of microbial growth at low temperatures. Industry 1 high TBC is in line with high sample acidity and positive alizarol test. For Fagnani *et al*. (2016), the relationship between TBC and acidity has been widely studied in milk and the main cause of acidity comes from the metabolism of mesophilic aerobic microorganisms. Thus, both acidity, high TBC and the alizarol test indicate microbial activity in refrigerated raw milk from industry 1, extending to processed milks. The counting of mesophilic microorganisms can be performed in pasteurized and sterilized milk. According to MAPA Ordinance No. 370/1997, the number of mesophiles must not exceed 100 CFU/mL in UHT milk (Brazil, 1997). Industry 1 showed a greater number of colonies of mesophilic microorganisms (9,700 CFU/mL) compared to Industry 2, which was only 510 CFU/mL, for pasteurized milk. Industry 1 had a number of mesophiles 19 times greater than industry 2, which is in agreement with the TBC in refrigerated raw milk, which was also much higher in industry 1.

When evaluating the amount of psychrotrophic microorganisms in refrigerated raw milk, industry 1 had a higher count (10,000,000 CFU/mL) than industry 2 (6,000,000 CFU/mL). The same occurs for pasteurized milk, with the count being 80,000 CFU/mL, in industry 1, and 40,000 CFU/mL, in industry 2. Psychrotrophic microorganism is a general term for a class of microorganisms that are able to grow at low temperatures. These microorganisms have the ability to produce enzymes, which can impair the quality of milk and dairy products (Wei *et al*., 2019). The proteolytic and lipolytic enzymes produced by these microorganisms are associated with technological and sensory changes in the product, even after processing, due to their heat resistance capacity (Lampugnani *et al*., 2018). The count of psychrotrophic bacteria (PBC) is considered an important indicator that determines the quality of raw milk and final dairy products. The CBP generally required to initiate spoilage in milk is about 10^6 CFU/mL (Yang *et al*., 2020). In most countries, raw milk is not processed immediately after milking and is therefore kept refrigerated until processing. The entire process can take up to 5 days, depending on milk collection intervals and transport distances, which results in increased numbers of psychrotrophic microorganisms. Furthermore, prolonged cold storage of raw milk can influence the microbial diversity of that milk (Zhang *et al*., 2020). The samples of refrigerated raw milk from the analyzed industries showed psychrotrophic counts up to 10^7 CFU/mL (industry 1) and 10^6 CFU/mL (industry 2), evidencing levels of microorganisms prone to milk deterioration and alteration of its characteristics organoleptic.

The two industries evaluated showed a higher number of psychrotrophic microorganisms than the number of mesophilic microorganisms (pasteurized milk) and the total bacterial count (refrigerated raw milk). In refrigerated raw milk, the psychrotrophic count was twice as high as the TBC for industry 1 (10,000,000 CFU/mL and 4,599,000 CFU/mL, respectively) and more than 10 times higher for industry 2 (6,000 .000 CFU/mL and 466,000 CFU/mL, respectively). The fact that industry 1 has a TBC above that established by legislation explains the smaller difference between mesophiles and psychrotrophs in relation to industry 2. In pasteurized milk, the count of psychrotrophic microorganisms was 8 times higher than the count of mesophilic microorganisms in industry 1 (80,000 CFU/mL and 9,700 CFU/mL, respectively) and 80 times higher in industry 2 (40,000 CFU/mL and 510 CFU/mL, respectively). The average TBC of refrigerated raw milk samples was 2,532,500 CFU/mL, that of mesophilic samples from pasteurized milk was 5,105 CFU/mL and that of psychrotrophs was 8,000,000 CFU/mL for raw milk and 60,000 CFU/mL for pasteurized milk. Although there is no established maximum level, in good quality milk, the psychrotrophic count should be no more than 10% of the total mesophilic aerobic or TBC count. In heavily contaminated milk, the count of psychrotrophs increases proportionally, and can be much higher than the number of mesophiles (Mariotto *et al*., 2020). Prolonged refrigeration may have been responsible for such a large difference between the amount of mesophilic and psychrotrophic

microorganisms in the samples from the industries analyzed in this study. The analysis of total and thermotolerant coliforms showed the presence of the two groups of microorganisms in the refrigerated raw milk of both analyzed industries, in an amount of 100 MPN/mL. Coliforms are defined as aerobic or facultative anaerobic, gram-negative, non-spore forming rods capable of fermenting lactose, resulting in gas and acid production at 35 °C in 48 h (Godziszewska *et al*., 2018). Coliforms have been used in the dairy industry since the early 20th century to identify milk processed under unsanitary conditions or where contamination has occurred after pasteurization. This group is formed by members of the Enterobacteriaceae family and, historically, has shown an important role in the deterioration of fluid milk. Despite the advantages of their use as indicators, current research indicates that coliforms are decreasing considerably in fluid milk (Alles *et al*., 2018). Coliform microorganisms are represented by four main genera: Escherichia, Klebsiella, Citrobacter and Enterobacter (Godziszewska *et al*., 2018). Genetic sequencing demonstrated the presence of the four genera of coliforms in the refrigerated raw milk samples analyzed in this study.

Although the samples of pasteurized milk and sterilized milk did not show total and thermotolerant coliforms in the microbiological analyses, the sequencing showed the presence of the genera Enterobacter, Citrobacter and Escherichia in the pasteurized milk of industry 2 and Enterobacter, Citrobacter, Klebsiella in the sterilized milk of the industry 1. Godziszewska *et al*. (2018), in their study, detected microorganisms from the coliform group in 95% of bulk tank milk samples. For Masiello *et al*. (2019), coliforms are often isolated from raw milk and pasteurized milk, and their presence in processed milk can be explained by contamination after processing. For Odenthal *et al*. (2016), heat treatment of milk by UHT is efficient in inactivating members of the Enterobacteriaceae family, but in this study, genera of this family were found in sterilized milk. Genetic sequencing is a more efficient tool that performs a thorough analysis of the sample, which would explain why the coliform group was not detected by the traditional microbiological method. This is also demonstrated in the analysis of mesophilic and psychrotrophic microorganisms in sterilized milk, which did not show the growth of colonies in the microbiological method, however, in the sequencing, sequences of microorganisms were found in both industries, being very expressive in the case of industry 1 (7,090) and less expressive in industry 2 (303). Industry 1 showed higher amounts for most of the microbiological parameters evaluated in this study. Compared to industry 2, industry 1 has a higher TBC, a greater number of mesophiles, psychrotrophs, somatic cells, in addition to a greater amount of microorganism sequences in the three types of milk analyzed, raw refrigerated, pasteurized and sterilized. Despite this, both industries show a reduction in the amount of mesophilic and psychrotrophic microorganisms and in the amount of sequence of microorganisms from refrigerated raw milk to processed milk (pasteurized and sterilized). In industry 1, the number of mesophiles reduced from 4,599,000 CFU/mL in refrigerated raw milk to 9,700 CFU/mL in pasteurized milk and zero in sterilized milk. The number of psychrotrophs reduced from 10,000,000 CFU/mL in refrigerated raw milk to 80,000 CFU/mL in pasteurized milk and zero in sterilized milk. The number of microorganism sequences reduced from 15,384 in refrigerated raw milk to 9,052 in pasteurized milk (41.15%) and 7,090 in sterilized milk (53.91%). In industry 2, the number of mesophiles reduced from 466,000 CFU/mL in refrigerated raw milk to 510 CFU/mL in pasteurized milk and zero for sterilized milk. The number of psychrotrophs reduced from 6,000,000 CFU/mL in refrigerated raw milk to 40,000 CFU/mL in pasteurized milk and zero in sterilized milk. The number of microorganism sequences reduced from 13,950 in refrigerated raw milk to 5,622 in pasteurized milk (59.69%) and 303 in sterilized milk (2.17%). This indicates that the beneficiation processes of industries have been efficient in reducing the amount of microorganisms.

Industry milk microbiome: When observing the total of the genera found in the six analyzed samples, eight genera are considered more abundant. The genus Bacillus (14,146) represents 27.52% of the total sample, the other 7 (*Kurthia, Streptococcus, Enterobacter, Lysinibacillus, Aeromonas*, *Acinetobacter* and *Lactococcus*) represent 64.12% of the total. The refrigerated raw milk sample from industry 1 (I1-Raw) presented as the most abundant genera: *Kurthia, Streptococcus, Lactococcus, Acinetobacter, Aeromonas, Bacillus, Providencia, Enterobacter, Escherichia, Enterococcus* and *Hafnia*. The genus *Kurthia*alone represents 44.89% of the amount of sequences present in the sample, and the other 10 together represent 40.96%. *Kurthia* is a genus of proteolytic and lipolytic psychrotrophic microorganisms from Brazilian refrigerated raw milk. This genus also has some mesophilic microorganisms (Ribeiro Junior *et al*., 2019). Hahne *et al*. (2019), in their study, found species of the genus Streptococcus as dominant in the microbiota of raw milk samples from bulk tanks. Recent research on the prevalence of mastitis pathogens has reported that Streptococcus is the most common type of pathogen associated with clinical mastitis (Smith *et al*., 2020). Lactococcus is the most extensively studied genus of lactic acid bacteria (LAB) as these bacteria are extensively used in the food industry (Guo *et al*., 2019). Species of the genus Lactococcus were recently associated with the occurrence of clinical mastitis. This may be related to changes in the environment, which facilitate their growth and introduction into the udder, or to biochemical improvements and advanced molecular techniques that have allowed the accurate identification of Lactococcus spp. instead of misclassification in Streptococcus spp. (Smith *et al*., 2020). For Mallappa *et al*. (2020), *Lactococcus*, together with *Lactobacillus, Leuconostoc,Streptococcus* and *Enterococcus* are the most common LAB genera in milk. In addition to these, psychrotrophic microorganisms, yeasts and molds, which establish themselves particularly during cold storage, are the main components of dairy products. Acinetobacter is a genus of psychrotrophic species also very common in raw milk. This genus is ubiquitous in the environment and milk contamination can result from the place where the animals are, such as the stable, hay, air or from ineffective cleaning processes (Hahne *et al*., 2019).

The fourth most abundant genus in the sample was Aeromonas, composed of emerging pathogens capable of colonizing and infecting several hosts. This genus can be isolated from foods such as vegetables, beef and pork. In humans, these microorganisms are capable of causing infections of the gastrointestinal system (Pessoa *et al*., 2019). Aeromonas is one of the main lipase-producing genera, along with Pseudomonas, Moraxella, Acinetobacter, Achromabacter, Aeromonas, Serratia and Alcaligenes. Lipases produced by psychrotrophic microorganisms in raw milk can withstand the heat treatment used in the dairy industry, causing flavor defects in manufactured products that have a long shelf life, such as UHT milk (Deeth, 2021). Bacillus is also one of the genera commonly found in milk and dairy products. Some species of this genus have the potential for biofilm formation, persisting in the industrial environment (Lindsay *et al*., 2022). Spores of some species of *Paenibacillus* (formerly classified as Bacillus) can survive heat treatment in raw milk and can withstand temperatures up to 130 °C. In addition, enzymes produced within biofilms degrade the protein and lipid components of milk, altering its sensory and nutritional properties (Elegbeleye & Buys, 2022). *Providencia* are urease-producing gram-negative microorganisms belonging to the Enterobacteriaceae family. Although these species are present as normal flora of the human intestinal tract, they are opportunistic pathogens, especially in immunocompromised people. Animals such as cattle, sheep, insects, worms, cats, birds, dogs and reptiles are reservoirs of Providencia, in addition to being present in water. Raw milk is a potential source of microorganisms of this genus, and contamination can occur during the milking process, through animal feces or can be related to subclinical mastitis (Al-Gburi, 2020). Like *Providencia*, the *Enterobacter* and *Escherichia* genera are members of the *Enterobacteriaceae* family. The *Enterobacter* genus is ubiquitous in nature and is widely dispersed in various ecosystems and niches such as water, soil, plants, faeces and skin, as well as the alimentary tract of humans, but it has also been isolated in milk (Khalifa, 2020). According to Ionnaou (2019), in recent decades several members of Enterobacteriaceae have been reclassified as unique species within the genus *Escherichia: Escherichia vulneris, Escherichia blattae, Escherichia fegusonii* and *Escherichia*

hermannii. Some of these species can cause infections in humans, as is the case with *E. hermannii*and *Escherichia coli. Enterococcus* are part of the microbiota of many raw and pasteurized foods. These microorganisms have a dual nature, with useful and harmful microorganisms. Scientific evidence confirms the discovery of strains with probiotic and functional potential. Species such as *Enterococcus faecalis* and *Enterococcus faecium* are used as probiotics for humans and also as veterinary food supplements (Giraffa, 2022). *Hafnia* are gram-negative, rod-shaped microorganisms belonging to the *Enterobacteriaceae* family, which has opportunistic pathogenic species of humans and animals. This genus is common in foods and has often been isolated from spoiled food products, especially in raw protein foods stored under refrigeration, such as fish, meat, and milk. This genus can form biofilms, which adhere to the solid surface, which is a potentially important factor that causes food contamination and spoilage (Zhu *et al*., 2019).

According to Olajide and LaPointe (2022), the diversity of microorganisms in raw milk can come from the animal, milking equipment, transport, storage or the environment. Microorganisms in milk can be harmful (pathogenic, spoilage) or beneficial. Some microorganisms, such as LAB, can be used to produce fermented dairy foods when grown under controlled conditions. Milk is a good medium for microorganisms to grow, so controls over storage temperature and hygiene during production and processing are essential to maintain an acceptable product. According to Yang *et al*. (2020), the most abundant psychrotrophic genera in raw milk are *Pseudomonas, Acinetobater, Flavobacterium, Sphingobacterium* and *Serratia,* for gram-negatives, and *Lactococcus, Aerococcus, Bacillus, Kurtha* and *Staphylococcus* for gram-positives. In their study, three genera were reported with high frequency: *Pseudomonas,Lactococcus* and *Acinetobacter.* Zhang *et al*. (2019), reports as the main genera of psychrotrophs found in raw milk with gram-negative properties: *Pseudomonas, Aeromonas, Serratia, Acinetobacter, Alcaligenes, Achromobacter, Enterobacter* and *Flavobacterium* and gram-positive: *Bacillus, Clostridium, Corynebacterium, Microbacterium, Micrococcus, Arthbacter, Staphylococcus* and *Carnobacterium.* In this study, the genera *Aeromonas, Bacillus, Providencia, Acinetobacter, Enterobacter, Pseudomonas* and *Kurthia* were found, as well as *Viridibacillus.* Pasteurized milk from industry 1 (I1-Past.) presented only three abundant genera: *Bacillus, Acinetobacter* and *Pseudomonas*, and the genus *Bacillus*, with 8,699 sequences, represents 96.10% of the total sample (9,052). It is well understood that pasteurization of milk and dairy products keeps consumers safe from foodborne illness, while failure to heat treatment can result in foodborne illness outbreaks (Lindsay *et al*., 2021). For Wei *et al*. (2019), the quality of raw milk is important to determine the quality of processed milk and industrialized dairy products. The genus *Pseudomonas*, with only 26 sequences, represents 0.28% of the total sample and is well known for its ability to produce biofilms. Biofilm formation of microorganisms in the storage tank leads to increased contamination of milk because biofilm-associated organisms exhibit high levels of resistance to cleaning and disinfection (Hanhe *et al*, 2019).

Species of nine main genera were found in the sterilized milk from industry 1 (I1-Ster.): *Bacillus, Aeromonas, Kurthia, Streptococcus, Enterobacter, Viridibacillus*, *Citrobacter*, *Pseudomonas* and *Comamonas*.The genus *Bacillus* (4,743) is the most abundant and represents 66.89% of the sequences present in the sample (7,090). *Viridibacillus*represents 1.59% (113 sequences) of the total microorganisms present in the sample. This genus is composed of microorganisms that are ubiquitous in nature and have already been isolated throughout the dairy chain. In addition, members of this genus are capable of producing spores that survive in adverse conditions. Thus, the ability to reduce the presence or control the growth of psychrotolerant spore formers in the dairy system has the potential to considerably improve the quality of fluid milk (Buehler *et al*., 2018). For Alles *et al*. (2018), spore-forming gram-positive bacteria represented the majority of milk bacteria and their main groups are the *Bacillales*family, with the genera *Bacillus* and *Viridibacillus*being the most frequent. The genus *Citrobacter* (with 59 sequences) represents 0.83% of the total sample. This genus is composed of gram-negative, aerobic and facultative anaerobic bacteria belonging to the *Enterobacteriaceae* family, commonly disseminated in nature. Recent studies have shown Citrobacter infections in fish and the indiscriminate use of antibiotics has given rise to resistant species (Royam; Nachimuthu, 2020). This genus has been found in fruits and vegetables, due to its proximity to the soil and inadequate handling (Adegun *et al*., 2019). Despite the low incidence in the sample, its presence in UHT milk is a cause for concern, and may be an indication of post-processing contamination. The genus *Comamonas*, as well as *Pseudomonas*, is not very expressive in the sample. This genus is formed by gram-negative, non-fermentative and rod-shaped bacteria, most of which are aerobic chemoheterotrophs considered non-pathogenic to humans (Wu *et al*., 2018). The refrigerated raw milk sample from industry 2 (I2-Raw) has only three main genera: *Enterobacter, Streptococcus* and *Lysinibacillus,* representing 38.35%, 36.22% and 24.38% of the total sample (13,950 sequences), respectively.

Together, these three genres add up to 13,806 sequences, that is, 98.95% of the total sample. Members of the genus *Lysinibacillus*have been isolated from diverse environments and are reported to be potential symbionts of animals and plants, as well as free-living soil microorganisms. These microorganisms have long been known as insect biocontrol agents and are also important plant growth-promoting bacteria (Hashmi *et al*., 2020). In the pasteurized milk from industry 2 (I2-Past.) seven main genera were found: *Streptococcus, Kurthia, Enterococcus, Aeromonas, Acinetobacter, Enterobacter* and *Lysinibacillus.* The two main genera, *Streptococcus* and *Kurthia* together represent 75.91% of the total sample (5,622 sequences). Ding *et al*. (2020) found as main genera in pasteurized milk: *Pseudomonas, Corynebacterium, Streptococcus, Cyanobacteria.* Pasteurization can eliminate some pathogenic microorganisms, but there are species capable of withstanding heat treatment. The clear identification of the microorganisms present in the samples is important for the rigorous control of pasteurization in industries. The genera *Streptococcus* and *Pseudomonas* were found in pasteurized milk from industries 1 and 2, in agreement with the study by Ding *et al*. (2020). An important situation is post-pasteurization contamination (PPC) that can occur due to poor hygiene practices in the industry or from existing biofilms on processing equipment (Elegbeleye; Buys, 2022). Post pasteurization contamination is still an obstacle for some industries.

Studies suggest that 40 to 50% of conventional pasteurized fluid milk shows evidence of post-process contamination. CPP is associated with rapid bacterial growth producing unacceptable sensory characteristics, which often lead to spoilage before the product's shelf life (Alles *et al*., 2018). In the sterilized milk from industry 2 (I2-Ster.) three main genera were found, *Acinetobacter, Lactococcus* and *Pseudomonas.* The genus *Acinetobacter* represents 30.03% of the total sample. *Lactococcus* and *Pseudomonas* represent 5.61% each. In this sample, 140 sequences of microorganisms of the *Enterobacteriaceae* family, not identified at the genus level, were identified, which represents 46.20% of the sample. As they belong to the *Enterobacteriaceae* family, they should not be present in UHT milk, indicating possible post-processing contamination. In the present study, the genus *Bacillus* was the most abundant in the total of samples. This genus is commonly found in milk and dairy products and has the potential to form biofilms and spores resistant to heat treatment, which explains its presence in processed milk samples. The refrigerated raw milk samples showed to have in common psychrotrophic, mastitogenic and intestinal origin genera, *Acinetobacter, Enterobacter* and *Streptococcus*. Pasteurized milk samples showed the Acinetobacter and Pseudomonas genera in common, both of which are formed by psychrotrophic microorganisms. The sterilized milk samples showed *Streptococcus, Enterobacter, Kurthia* and *Pseudomonas*. *Kurthia* and *Pseudomonas* are psychrotrophic genera and *Enterobacter* and *Escherichia* are associated with contamination of fecal origin. Improved access to genome-based culture-independent methods has generated great interest in defining the bovine milk microbiome. Several bacterial

genera are routinely identified from milk samples, but the origin and function of these organisms are uncertain, and environmental factors have been shown to strongly influence the composition of these bacterial populations, as sources of microbial DNA may include bacteria introduced from the skin or environment. Understanding the bovine milk microbiome has been hampered by the lack of standardized methods used to collect, process and evaluate bovine milk samples. Furthermore, contamination of samples with bacterial DNA from laboratory reagents is a well-known problem that has affected the results of studies with bovine milk samples (Ruegg, 2022).

CONCLUSION

The analysis of samples of refrigerated, pasteurized and sterilized raw milk from the dairy industries of Vale do Taquari showed that industry 1 presented acidity above the maximum allowed for the three types of milk, TBC and alizarol positive for refrigerated raw milk and density outside the level set for pasteurized milk. The two industries presented SCC above the limit established by the current legislation and obtained the same number of microorganisms from the group of total and thermotolerant coliforms for refrigerated raw milk. The count of psychrotrophic microorganisms was above the recommended and was superior to the TBC and the count of mesophilic microorganisms, in refrigerated raw milk and in pasteurized milk from both industries. The number of SCC, mesophiles and psychrotrophs was higher in industry 1. The samples showed great diversity of genera. The main microorganisms found were psychrotolerant, such as *Kurthia, Acinetobacter, Viridibacillus*, biofilm formers, such as *Pseudomonas, Bacillus,*mastogenic such as *Streptococcus* and lactic acid such as *Lactococcus,* in the three types of milk analyzed. In addition, genera of microorganisms considered harmful such as *Escherichia, Citrobacter, Aeromonas* and *Enterobacter* were found even in processed milk. The physical-chemical, compositional and microbiological analyzes of milk produce an efficient assessment of its quality. However, these analyzes can be used in conjunction with high-throughput sequencing, providing a complete diagnosis, precisely identifying the microorganisms present in the samples, thus enabling the tracking of possible failures or the improvement of the production process throughout the dairy chain.

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