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Physicochemical and microbiological quality of bovine milk from Vale do Taquari in Rio Grande do Sul, Brazil

Qualidade físico-química e microbiológica do leite bovino do Vale do Taquari no Rio Grande do Sul, Brasil

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Abstract
The goal of the present study was to verify the quality of refrigerated raw milk from dairy properties and also of refrigerated raw milk, pasteurized and Ultra High Temperature (UHT) milk from industries in Vale do Taquari in Rio Grande do Sul, Brazil. Physicochemical, microbiological and milk composition analysis were carried out, as established by legislation, in addition to total and thermotolerant coliforms and psychrotrophic counts in the three types of milk and mesophiles count in pasteurized milk and UHT milk from the industries. The collections took place in two industries and 33 dairy properties. Regarding the properties, two (6%) had milk with acidity above that established by legislation and three (9%) had milk with a total bacterial count (TBC) above the established. The milk from industry 1 presented acidity, TBC and density out of the established standards. The two industries and 53.2% of the properties had milk with somatic cell counts (SCC) above that determined by legislation. The milk from the industries showed higher amounts of SCC, TBC, psychrotrophic and total and thermotolerant coliforms than the milk from the dairy properties, and the milk from industry 1 showed higher amounts than the industry 2, in the microbiological parameters.
Keywords: Physicochemical parameters; Microbiological parameters; Milk composition.

Resumo
O objetivo do presente estudo foi verificar a qualidade do leite cru refrigerado das propriedades produtoras de leite e do leite cru refrigerado, pasteurizado e *Ultra High Temperature* (UHT) das indústrias do Vale do Taquari no Rio Grande do Sul, Brasil. Foram realizadas análises de composição do leite, análises físico-químicas e análises microbiológicas, estabelecidas pela legislação, além de coliformes totais e termotolerantes, contagem de psicrotróficos nos três tipos de leite, contagem de mesófilos no leite pasteurizado e no leite UHT das indústrias. As coletas ocorreram em duas indústrias e 33 propriedades produtoras de leite. Em relação às propriedades, duas (6%) apresentaram leite com acidez acima do estabelecido pela legislação e três (9%) apresentaram leite com contagem bacteriana total (CBT) acima do estabelecido. O leite da indústria 1 apresentou acidez, CBT e densidade fora dos padrões estabelecidos. As duas indústrias e 53,2% das propriedades apresentaram leite com contagem de células somáticas (CCS) acima do determinado pela legislação. O leite das indústrias demonstrou maiores quantidades de CCS, CBT, psicrotróficos e coliformes totais e termotolerantes que o leite das propriedades produtoras de leite e o leite da indústria 1 apresentou maiores quantidades que a indústria 2, nos parâmetros microbiológicos.
Palavras-chave: Parâmetros físico-químicos; Parâmetros microbiológicos; Composição do leite.

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Introduction

Milk and dairy products are necessary foods, consumed daily and provide a valuable source of several macro and micronutrients [1]. Milk production in Brazil was 6.2 billion liters in the third quarter of 2021, with an amount of 25.3 billion in the same year. The state of Rio Grande do Sul (RS), with a production of 15.1 billion liters, is considered the second largest national milk producer [2]. The Vale do Taquari in RS 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 compose it.

The quality of raw milk produced in Brazil must be analyzed by the Brazilian Milk Quality Network (RBQL)[3]. The parameters used for the diagnosis of milk quality include microbiological analysis, such as total bacterial count (TBC) for refrigerated raw milk and mesophilic microorganism count for pasteurized and UHT milk. In addition to physicochemical analysis, such as temperature, acidity, density, cryoscopic index, and analysis of milk composition, such as lactose, protein, fat, total dry extract (TDE) or total solids (TS) and defatted dry extract (DDE) or non-fat solids (NFS), for the three types of milk and, additionally, somatic cell count (SCC) and alizarol alcohol test, for refrigerated raw milk. These parameters and their limits are determined by the Ministry of Agriculture, Livestock and Supply (MAPA), through Normative Instructions N. 76/2018[4] and N. 77/2018[3], which determine the guidelines for refrigerated raw milk and for pasteurized milk and Ordinance N. 370/1997[5], which establishes the regulation of sterilized milk by the UHT process.

Considering the public health risks associated with the consumption of raw milk, heat treatment is applied to ensure the microbiological safety of dairy products[6]. The heat treatments used are pasteurization and UHT and aim to reduce the number of microorganisms to levels that are safe for consumer health[1]. In addition to the beneficiation processes, cooling the milk after milking and during transport at a temperature of up to 5 °C[7] is important for maintaining its quality, making the product viable for industrial processing. Low temperature reduces the microbial growth in the milk, but favors the proliferation of psychrotrophic microorganisms. Thus, counting psychrotrophic microorganisms is an important tool for evaluating the quality of produced milk. In addition to psychrotrophs, another important group used worldwide as indicators of hygienic conditions during milk processing are total and thermotolerant coliforms[8].

Thus, the information obtained through physicochemical, microbiological and milk composition analysis, determined by legislation and, additionally, counting of psychrotrophic microorganisms and analysis of total and thermotolerant coliforms, promote an accurate diagnosis of the quality of the produced milk. The goal of the present study was to verify the quality of refrigerated raw milk from dairy properties and of refrigerated, pasteurized and UHT raw milk from industries in Vale do Taquari in Rio Grande do Sul, Brazil, through physicochemical and microbiological analyzes, established by current legislation, in addition to the counting of mesophilic and psychrotrophic microorganisms and analysis of total and thermotolerant coliforms.

Material and methods

Study Area

The study was carried out in the region of Vale do Taquari (VT), Rio Grande do Sul, Brazil. Milk samples were collected from two dairy industries in the region and from 33 dairy properties, located in 33 of the 36 municipalities that compose the region. In the dairy industries, a total of six samples were collected: one of refrigerated raw milk from the tank truck, one of pasteurized milk and one of milk UHT, in each industry. In the properties, samples were collected from the milk cooler, one sample in each property, totaling 33 samples. The dairy industries were named I1 (Industry 1) and I2 (Industry 2) and the types of milk were given different abbreviations, being "Raw" for refrigerated raw milk, "Past." for pasteurized milk and "UHT" for UHT milk. The properties were named by the initial "P" followed by a number, being then determined from "P1" to "P33". The samples were collected in sterile flasks, stored and transported in isothermal boxes with ice. Analyzes took place within six hours after sample collection.

Physicochemical and milk composition analysis

In the refrigerated raw milk from the tank truck of the industries and properties, the milk composition analyzes followed the methods determined in Normative Instructions N. 77/2018, of MAPA[3]. The SCC analysis was performed using ISO 13366-2-IDF148-2:2006[9], and for this analysis a 40 mL bottle with Bronopol preservative was used to collect the samples. The other analyzes of milk composition: protein, lactose, total solids (TS) and non-fat solids (NFS) were carried out using ISO 9622-IDF141:2013[10].

To perform the alizarol test in the raw milk from the properties and the tank truck from the industries, a 100 mL beaker and 75% alizarol-alcohol was used, in which 10 mL of the alizarol-alcohol solution was mixed at 10 mL of milk homogenizing[11]. For processed milk from the industries, pasteurized and UHT, 1L of sample was collected and the norms used for the analysis of milk composition were: non-fat solids (NFS): according to the manual of official methods for the analysis of foods of animal origin of MAPA[12]; Total solids: ISO 6731-IDF

21:2010[13]; Lactose: ISO 22662-IDF 198:2007[14]; Lipids: NMKL 40:2005[15] and Total Protein: ISO 8968-1-IDF 20-1:2014[16].

The acidity and density analyzes, performed on the refrigerated raw milk from the properties and on the three types of milk from the industries (refrigerated raw from the tank truck, pasteurized and UHT), followed the same methodology. 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 diluted until a persistent pink color identical to the standard for approximately 30 s. 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 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[12]. Density analysis was performed using the lactometer equipment, in which 500 mL of the sample was placed into a measuring cylinder, without creating foam, and the equipment was inserted to perform the reading[12]. The temperatures of all samples were measured using an Incoterm thermometer (model 5135) and were performed at the time of collection. All physicochemical and milk composition analyzes were performed in triplicate to ensure the reliability of the results.

Microbiological analyzes

The TBC was carried out on the refrigerated raw milk of the properties and on the refrigerated raw milk from the tank trucks of the industries and was performed according to the methodology recommended by ISO 21187-IDF196:2004[17], which is determined by Normative Instructions N. 77/2018[3]. The analysis of mesophilic microorganisms was carried out in pasteurized milk and in UHT milk from the industries. The analysis of psychrotrophic microorganisms and the analysis of total and thermotolerant coliforms were carried out in the refrigerated raw milk, both from the properties and from the industries, and in the pasteurized and UHT milks from the industries. All microbiological analyzes were performed in triplicate.

The analysis of mesophilic and psychrotrophic microorganisms followed the methodology recommended by the *Standard Methods for the examination of dairy products*[18]. Five decimal dilutions were performed in tubes containing 9 mL of 0.1% peptone. For the determination and quantification of mesophilic aerobic microorganisms, the pour plating method was used, in which the Petri dishes received 1 mL of dilutions 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}, with approximately 20 mL of Plate Count Agar (PCA) (OXOID®).

The Petri dishes were incubated inverted at 36 ± 1 °C for 48 hours. For the determination and quantification of aerobic psychrotrophic microorganisms, the spread plate method was used, in which the surface of PCA agar (OXOID®) received 0.1 mL of the dilutions. The inverted plates were incubated at 7 °C for 10 days. The counting of microorganism colonies was performed using 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 [19], 1mL 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 0.1% 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 were incubated at 30 ºC for 24 or 48 hours in a bacteriological incubator. The tubes with a positive presumptive reaction, evidenced by the production of gas, were then submitted to the confirmatory test in Brilliant Green Bile Lactose (2%) 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.

To check the milk quality, the levels found in the analyzes were compared with the limits defined by Normative Instruction N. 76/2018 of MAPA[4], for refrigerated and pasteurized raw milk and by Ordinance N. 370/1997 of MAPA[5], for UHT milk. The legislation does not establish levels of psychrotrophs and total and thermotolerant coliforms. The results were then compared with the limits established by authors in the area[20-8].

Data analysis

The data were tabulated using an Excel spreadsheet and the descriptive statistics were performed for quantitative data with the Bioestat 5.0 program, multivariate analysis of principal components (PCA) and correlation with the Past program. The results were compared: of the physicochemical and milk composition analyzes and the amounts of microorganisms found in the samples of the three types of milk (refrigerated raw, pasteurized and UHT), from the two industries analyzed in this study, the refrigerated raw milk from the dairy properties and the milk of the industrial tank truck.

Results and discussion

The results of the analysis of the parameters of milk composition (protein, lactose, fat, TS, NFS and SCC) and physicochemical parameters (temperature, acidity and density) show that the storage temperature in 31 of the 33 properties (93.93%) was lower than 5 °C, and only two properties (P9, P4) had a temperature higher than this (Table 1). In property P9, the temperature measured at the time of collection was 7.8 °C. This is due to the fact that in this property the, collection occurred minutes after the milking, not allowing the milk to be sufficiently cooled in the cooler.

Table 1. Levels of physicochemical and milk composition parameters, average, standard deviation and standard error of refrigerated raw milk samples from dairy properties in Vale do Taquari - RS. ex. fat: excess fat - sample with a fat level around 10%; TS: Total solids; NFS: Non-fat solids

Unity	**Temp. °C**	**Acidity g latic acid/100 mL**	**Density g/mL**	**Fat g/100g**	**Protein g/100g**	**Lactose g/100g**	**TS g/100g**	**NFS g/100g**	**SCC SC/mL**
Levels	**up to 5**	**0.14 to 0.18**	**1.028 to1.034**	**min.3.00**	**min. 2.90**	**min.4.30**	**min.11.40**	**min.8.40**	**up to500,000**
P1	3.0	0.18	1.032	4.05	3.45	4.42	13.00	8.95	1,004,000
P2	3.6	0.15	1.033	3.64	2.94	4.45	12.04	8.40	459
P3	2.8	0.18	1.033	4.09	3.25	4.49	12.89	8.80	182
P4	5.1	0.18	1.033	4.09	3.42	4.46	13.17	9.08	1,812,000
P5	4.8	0.16	1.032	3.46	3.27	4.42	12.40	8.94	1,340,000
P6	3.7	0.15	1.031	3.57	3.14	4.49	12.45	8.88	333
P7	3.4	0.19	1.033	4.50	3.45	4.25	13.27	8.77	514
P8	3.7	0.16	1.031	3.67	2.89	4.45	12.12	8.45	159
P9	7.8	0.17	1.032	3.93	3.12	4.66	12.69	8.76	365
P10	3.4	0.15	1.033	3.62	3.07	4.45	12.22	8.60	909
P11	4.2	0.17	1.033	3.94	3.30	4.33	12.66	8.72	658
P12	4.4	0.16	1.031	3.85	3.29	4.59	12.79	8.94	404
P13	4.1	0.16	1.033	3.76	3.10	4.50	12.48	8.72	455
P14	4.2	0.19	1.033	3.65	3.18	4.44	12.40	8.75	1,180,000
P15	4.2	0.16	1.032	4.00	3.37	4.38	12.87	8.87	383
P16	4.1	0.16	1.033	3.86	3.37	4.55	12.78	8.92	365
P17	4.5	0.18	1.032	3.89	3.36	4.36	12.72	8.83	705
P18	4.8	0.15	1.030	4.35	3.11	4.22	12.80	8.45	1,489,000
P19	4.9	0.16	1.033	7.88	3.03	4.29	15.99	8.11	1,218,000
P20	4.2	0.18	1.033	4.48	3.80	4.37	13.76	9.28	1,290,000
P21	3.5	0.15	1.029	3.25	3.01	4.15	11.65	8.40	495
P22	3.4	0.20	1.032	3.70	3.61	4.30	12.81	9.11	894
P23	2.8	0.18	1.031	3.74	3.14	4.57	12.55	8.81	230
P24	3.9	0.15	1.031	3.69	3.10	4.45	12.36	8.67	467
P25	3.7	0.16	1.032	3.77	3.41	4.49	12.81	9.04	507
P26	4.1	0.18	1.030	4.42	3.47	4.25	13.32	8.90	883
P27	4.3	0.16	1.032	3.82	3.28	4.35	12.51	8.69	780
P28	4.2	0.17	1.032	3.66	3.01	4.51	12.22	8.56	133
P29	2.8	0.17	1.029	3.86	3.11	4.35	12.39	8.53	409
P30	1.8	0.18	1.032	4.02	3.31	4.62	12.95	8.93	551
P31	4.1	0.16	1.030	3.69	3.29	4.25	12.19	8.50	706
P32	4.2	0.17	1.030	8.11	2.59	4.30	15.98	7.87	190
P33	4.3	0.17	1.030	ex. fat	ex. fat	ex. fat	ex. fat	ex. fat	exc, fat
Average	4.04	0.17	1.031	4.14	3.23	4.41	12.87	8.73	670,906.25
Standart Deviation	1.03	0.00	1.26	1.12	0.29	0.00	0.80	0.38	425,317.13
Standart Error	0.18	0.00	0.22	0.19	0.05	0.00	0.14	0.06	75,186.15

Proper cooling of milk, on the farm and during transport, decreases the rate of bacterial growth, preventing the increase in bacterial counts, before the milk reaches milk collection centers or processing industries [21]. The physicochemical parameters of milk are very important for its acceptance by the consumer. Industry processes depend directly on these parameters to ensure a long shelf life for the product, adequate characteristics and nutritional benefits for the consumer. Parameters such as pH, oxidative stability and milk composition are extremely important for the processing performed in dairy industries [22].

Regarding the industries, all samples of refrigerated raw milk and pasteurized milk had a temperature below the maximum allowed (Table 2), which is up to 5 °C, for refrigerated raw milk[7] and up to 4 °C, for pasteurized milk[4]. The pasteurized milk produced by industries in Vale do Taquari is originating from several herds, on several properties and transported under refrigeration[3]. The UHT milk from the industries is stored at room temperature, and the samples analyzed in this study had temperatures of 22.1 °C and 28 °C, in industries 1 and 2, respectively (Table 2).

Table 2. Levels of physicochemical, microbiological and milk composition parameters in samples of refrigerated raw milk, pasteurized and UHT milk from industries in Vale do Taquari - RS. *: for refrigerated raw milk; **: for pasteurized milk; ***: for UHT milk; nd: not determined. TS: Total solids; NFS: Non-fat solids.; ----: not performed

Parameters		Industry 1			Industry 2		
		Raw	Past.	UHT	Raw	Past.	UHT
Temperature (°C)	up to 5*; 4**	4.8	3.7	22.1	3.6	4.0	28.0
Acidity (g latic acid/100 mL)	0.14 to 0.18	0.67	0.20	0.24	0.18	0.17	0.18
Density (g/mL)	1.028 to 1.034	1.028	1.037	1.028	1.033	1.033	1.033
Fat (g/100g)	min. 3.00	4.37	3.80	3.00	3.80	3.20	3.00
Protein (g/100g)	min. 2.90	3.30	3.24	3.28	3.27	3.26	3.27
Lactose (g/100g)	min. 4.30	4.33	4.89	4.72	4.48	4.92	4.78
TS (g/100g)	min. 11.40*	13.08	12.42	11.50	12.59	12.02	11.92
NFS (g/100g)	min. 8.40 e 8.20***	8.71	8.60	8.50	8.79	8.80	8.90
SCC (SC/mL)	up to 500,000	1,079,000	------	------	638	------	------
TBC*/Mesophils (CFU/mL)	up to 900,000*up to 100***	4,599,000	9,7	0	466	510	0
Psychrotrophs (CFU/mL)	nd	10,000,000	80	0	10,000,000	40	0
Total coliforms (MPN/mL)	nd	110	0	0	110	0	0
Therm. Coliforms (MPN/mL)	nd	110	0	0	110	0	0

Regarding acidity, two properties, P7 and P22, showed the result above the limit established by current legislation (Table 1), with values of 0.19 and 0.20 g of lactic acid/100 mL, respectively [4]. According to Normative Instructions N. 76/2018 of MAPA[4], the acidity levels of refrigerated raw milk must remain between 0.14 and 0.18g of lactic acid/100 mL. The acidity analysis of the milks from the industries showed that industry 1 presented acidity above the maximum allowed level, in the three types of milk analyzed, raw refrigerated, pasteurized and UHT, with acidity levels of 0.67, 0.20 and 0 .24 g lactic acid/100mL, respectively (Table 2). The recommended acidity levels for pasteurized and UHT milk are also 0.14 to 0.18 g of lactic acid/100 mL, and are determined by Normative Instructions N. 76/2018 [4], if pasteurized milk, and by Ordinance 370/1997[5], if UHT milk. Acidity above the maximum allowed level indicates that failures in Good Agricultural Practices (GAP) may have occurred, or that the milk has been stored for a long time in the cooling tank[24]. Microorganisms present in milk ferment lactose, forming mainly lactic acid, which results in an increase in total acidity[24]. In industry 2, all physicochemical parameters and milk composition are in accordance with the parameters established by legislation.

The alizarol test, performed only on refrigerated raw milk, showed that clumps were formed in seven properties (P13, P15, P17, P27, P28, P29 and P32), which corresponds to 21.21% of the properties analyzed. The Alizarol test is one of the most used test to evaluate the milk quality and aims to verify the stability of milk proteins, when subjected to dehydration caused by alcohol, in order to estimate the stability of milk when subjected to heat treatment. In this way, it is possible to verify if the milk has sufficient thermal stability to support the processing processes of the industry, especially the UHT process.

The alizarol alcohol test, together with the milk acidity test, are used to identify unstable non-acid milk (UNAM) which is characterized as a set of alterations, in which the raw material presents acidity within normal standards, but they react positively to the alcohol test[25]. In this study, none of the properties that presented a positive alizarol test had acidity above the limit allowed in the samples, indicating the occurrence of UNAM.

In the refrigerated raw milk of the industries, the industry 1 showed a positive alizarol test for the refrigerated raw milk, with a yellowish color. The raw milk from the same industry showed acidity above the maximum allowed limit, being 0.67 g of lactic acid/100 mL. Pasteurized and UHT milk from industry 1 also had acidity above the allowed level, which can be explained by the very high acidity in refrigerated raw milk, almost four times higher than the maximum allowed.

Producing good quality raw milk is necessary to produce quality pasteurized and UHT milk. The relationships between practices on dairy properties, the composition, properties of raw milk and the quality of milk and dairy products are closely associated[26].

Regarding density, all samples from the properties were in accordance with the legislation, which should be from 1.028 g/mL to 1.033 g/mL[4]. Industry 1 showed density above the maximum allowed for pasteurized milk, which was 1.037 g/mL. Density is used as one of the parameters that seek to evaluate milk adulteration by adding water or constituents. In the case of fraud involving the removal of cream from milk, the density tends to increase, as the fat has a density of 0.930 g/mL. In the case of frauds by adding water, the density of the milk tends to decrease, and the density below the level can also indicate nutritional problems or health problems in the animal [24].

Regarding the averages of the physicochemical parameters and milk composition, the properties of Vale do Taquari showed higher averages of fat, TS and NFS than the dairy industries in the region and the industries showed a higher average of density than the properties analyzed. Acidity and lactose had equal averages, both in the industries and in the properties (Figure 1a). The temperature of refrigerated raw milk from the industries and properties showed a similar average. The average temperature of refrigerated raw milk from the industries was 4.20 ºC and that of refrigerated raw milk from the properties was 4.04 ºC. The pasteurized milk from the industries had an average temperature of 3.85 °C and UHT milk had an average of 25.05 °C. This is because the UHT milk from the industries is stored at room temperature.

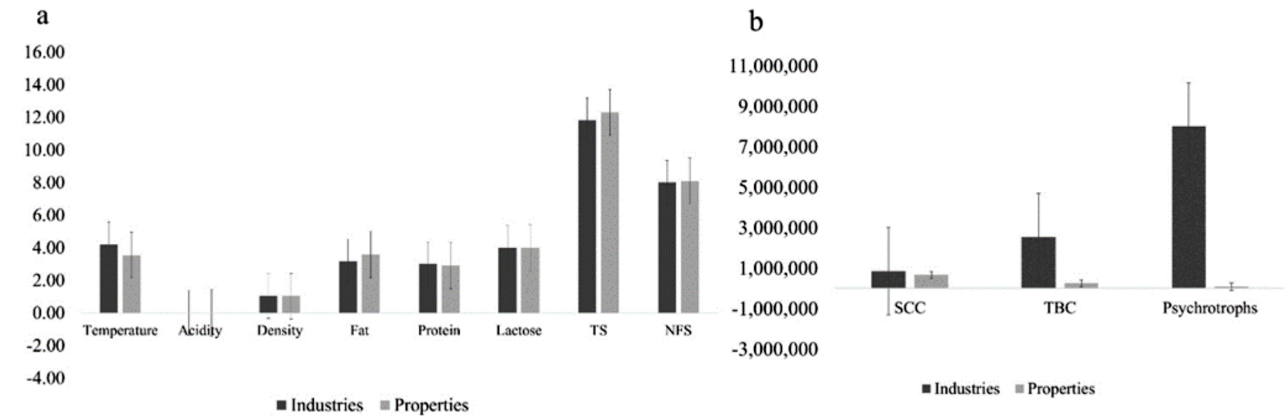

Figure 1. (a) Average of physicochemical parameters and milk composition of refrigerated raw milk samples from milk producing properties and samples of refrigerated, pasteurized and UHT raw milk from industries in Vale do Taquari - RS. For the average temperature of the industries, only refrigerated raw milk was considered. Figure with standard error. (b) Mean of SCC, TBC and psychrotrophic microorganisms found in samples of refrigerated raw milk from farms and dairy industries in Vale do Taquari-RS, with standard error.

The SCC analysis of the properties showed that 17 properties, which corresponds to 53.2% of the samples, had SCC levels above those allowed by the legislation, which is a maximum of 500,000 cells/mL[4]. Among these properties, seven (P1, P4, P5, P14, P8, P19 and P20), which corresponds to 21.2% of the samples, extrapolated the maximum measured by the method, with the SCC level established by estimation. The SCC of the properties ranged from 133,000 cells/mL, in property P28, to 1,812,000 cells/mL, in property P4. The average of the SCC in the properties was 670,906.25 cells/mL (Table 1). One of the samples, P33, had a fat content of around 10%, making it impossible for the method to measure the milk composition parameters (SCC, protein, lactose, ST and

NFS) and the fat itself accurately. This may be due to insufficient homogenization of the milk in the cooler.

According to the legislation, the analysis of SCC must be performed only on refrigerated raw milk[4]. Refrigerated raw milk from the dairy farm must be analyzed monthly and the milk transported in tank trucks must be performed daily[3].

The SCC rate in milk may be related to the immune reaction after infection of the mammary gland, called mastitis. Mastitis can be subclinical, when the number of leukocytes increases in the milk without apparent visual changes, or clinical when there are apparent changes in the milk. At times, this may be combined with signs in the udder or systemic clinical signs in the animal, which can be recognized by the farmer. A large amount of leukocytes presented as SCC and high TBC in milk can result in the production of enzymes that degrade its components, such as fats and proteins, reducing the quality of milk and dairy products. This also affects the shelf life of the product and reduces consumer acceptance[21]. In addition, the mastitic udder releases a large number of pathogenic microorganisms (including *Streptococcus* sp., *Staphylococcus* sp. and *Escherichia* sp.), which can contaminate bulk milk, becoming a public health problem[27].

When analyzing the relationship between the SCC and the other parameters of milk composition (fat, lactose, proteins, TS and NFS) of the properties, through Spearman's correlation analysis, it is possible to verify that the parameters presented a positive correlation, presenting greater amounts in properties with higher levels of SCC: fat (rs= 0.1712), protein (rs= 0.4185), NFS (rs= 0.2512) and TS (rs= 0.2594). The only exception was lactose (rs= -0.4689), which showed a negative correlation, with lower amounts in properties with higher amounts of SCC. This correlation is considered weak between SCC and fat, ST and NFS and moderates between SCC and fat and lactose. The correlation is significant between SCC and proteins and between SCC and lactose (p<0.05). According to Costa et al.[28], SCC above 200,000 SC/mL are indicative of subclinical mastitis, considering influences on milk components. In this study, only four properties (P32, P28, P8, P3), representing 12.12% of the samples, presented SCC below 200,000 SC/mL. Baggio and Montanhini[29], in their study, also reported a negative correlation between lactose and SCC, a fact that can be explained by the fact that inflammation of the mammary gland causes lesions in alveolar cells, leading to a decrease in lactose synthesis.

The SCC analysis performed in refrigerated raw milk from the tank truck of the industries, showed that both had the SCC above the maximum allowed by legislation[4], being 1,079,000 cells/mL in industry 1 and 638,000 cells/mL in industry 2 (Table 2). In industry 1, the amount of cells extrapolated the maximum amount obtained by the method, having its value established by estimate. The average SCC in the industries was 858,500 cells/mL, being higher than the average SCC of the properties, of 670,906.25 cells/mL (Figure 1b). The general average of SCC of milk in the VT region, taking into account the properties and the two industries, was 681,941.18 cells/mL. According to Ndahetuye et al.[21], increasing milk quality and safety worldwide is highly relevant, as regulations protecting consumer health require adherence to milk quality and safety guidelines, including low SCC.

The multivariate analysis of the principal components of the physicochemical parameters and milk composition show that component 1, temperature, explains 32.42% of the results, and component 2, acidity, explains 23.91%. Together, these two components explain 56.33% of the results. It is possible to observe an association between temperature, fat and TS and between acidity, density, protein, NFS and SCC (Figure 2).

The TBC analysis of the properties showed that 23 properties (69.70%) had counts up to 10^4 CFU/mL. Only three properties (P16, P17 and P33), which corresponds to 9% of the analyzed samples, presented TBC above the limit established by legislation (Table 3), which is up to 300,000 CFU/mL[4]. Sample P16 obtained a count of 596,000 CFU/mL. Sample P33, which was the same sample that showed large amount of fat, had a very high count of 1,559,000 CFU/mL. The bacterial count of milk-producing properties ranged from 8,000 CFU/mL (P6, P8, P9 and P30) to 4,534,000 CFU/mL (P17). In property P17, the result extrapolated the maximum measured by the method, is obtained by estimation. The TBC general average of the properties was 239,363 CFU/mL. The collection of milk by the tank truck, in the studied region, occurs every two days, and in 60% of the properties analyzed in this study, the collection of samples took place the following day or hours after the collection by the tank truck, in the case of properties that received him in the early hours of the morning. Thus, the cooler was almost empty, usually with the amount of one or two milkings in storage. The three properties (P16, P17 and P33) in which the TBC was above the allowed limit, are part of the 40% of the properties in which the sample collection took place hours before the tank truck collection (in properties where the collection took place in the early afternoon). Thus, in these properties, the cooler was full, at its maximum capacity, with the amount from several milkings, up to two days before. This suggests that the storage time of milk in the cooler may have an influence on the result of TBC found in dairy properties. Milk production in the properties analyzed in this study varies considerably, from 1,500 liters/month to 150,000 liters/month. However, most of the properties (85%) are made up of small producers, with an average of 12,000 liters/month.

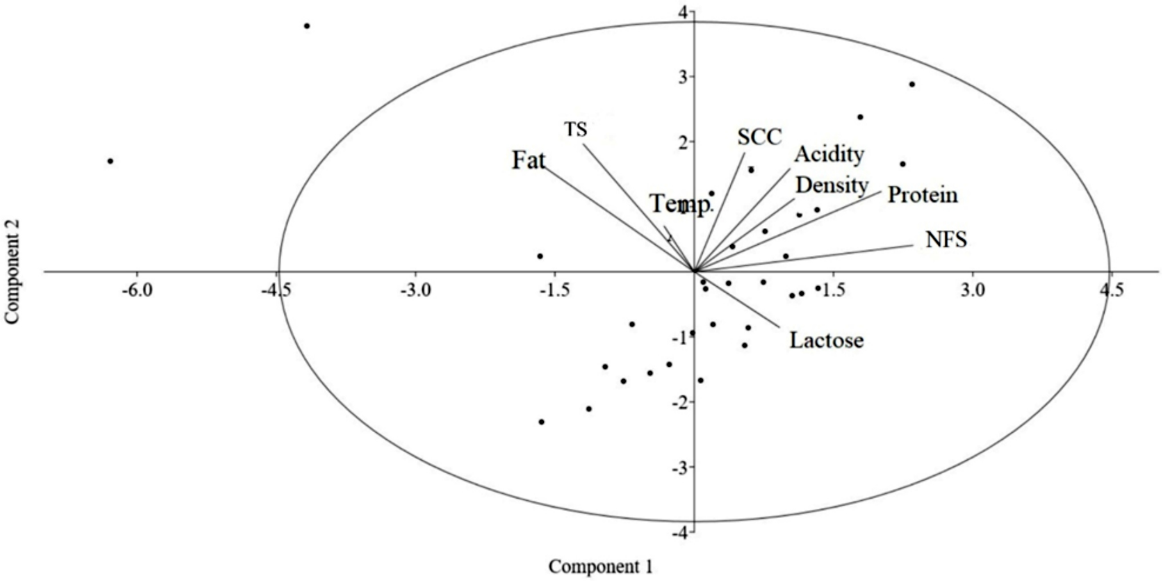

Figure 2. Ordination graphic about the multivariate analysis of the main components of the physicochemical parameters and milk composition of samples of refrigerated raw milk from dairy properties in Vale do Taquari - RS. TS: Total solids; NFS: Non-fat solids; Temp.: Temperature.

Table 3. Count of microorganisms of the analyzed microbiological parameters, average, deviation and standard error of refrigerated raw milk samples from dairy properties in Vale do Taquari - RS. ex. fat: excess fat; nd: not determined

Unity	TBC CFU/mL	Psychrotrophs CFU/mL	Total coliforms MPN/mL	Thermotolerant coliforms MPN/mL
Levels	up to 300,000	nd	nd	nd
P1	13	2	16	1
P2	15	9	21	0
P3	22	4	21	1
P4	113	2	110	21
P5	20	2,3	110	3
P6	8	5	14	1
P7	26000	1	14	3
P8	8	2	21	6
P9	8	1,5	110	110
P10	26	3	6	6
P11	37	4	21	2
P12	86	20	21	16
P13	52	70	21	2
P14	191	90	110	16
P15	50	1,8	3	3
P16	596	100	21	16
P17	4,534,000	2,000,000	110	15
P18	13	10	21	6
P19	15	10	110	6
P20	46	30	110	110
P21	122	2	16	2
P22	91	100	16	2
P23	21	500	21	1
P24	45	10	110	1
P25	15	200	1	1
P26	23	200	110	1
P27	21	900	2	1
P28	31	600	1	1
P29	11	100	6	2
P30	8	10	16	1
P31	24	10	2	1
P32	49	200	110	110
P33	1,559,000	20	16	2
Avarage	239,363.64	91,266.97	42.97	14.24
Standart Deviation	820,663.21	354,012.59	45.34	31.24
Starndart Error	142,589.12	61,625.68	7.89	5.43

Milk has ideal conditions for the growth of several types of microorganisms, including pathogens, due to its high water content, neutral pH and chemical composition[6]. The TBC is used to assess how much the milk production processes have affected its quality and safety. For Ndahuetuye et al.[21], TBC should be interpreted with caution, as different types of bacteria can contaminate milk, coming from different sources. These microorganisms proliferate in milk, as milk contains numerous nutrients for their growth and development.

The difference of TBC in the analyzed samples indicates that each property can be considered as a particular niche containing a dynamic microbiota. Farm management practices, milking systems and housing type have a large effect on TBC and bacterial composition of milk and may explain differences between the properties[27].

In the analyzes of TBC of refrigerated raw milk from the tank truck of the industries, it can be observed that industry 1 has TBC above the maximum amount allowed by current legislation (Table 2), which is up to 900,000 CFU/mL, for milk before industrial processing[4]. The amount of microorganisms in raw milk from industry 1 also extrapolated the maximum value measured by the method, having an estimate at 4,599,000 CFU/mL. Industry 2 presented TBC results within the limits of current legislation, with a TBC of 466,000 CFU/mL. The average TBC of the industries was 2,532,500 CFU/mL, higher than the average of the dairy properties, which was 239,363 CFU/mL (Figure 1b). The general average of refrigerated raw milk samples from Vale do Taquari was 1,387,431 CFU/mL. It should be noted that industry 1 also presented acidity in the samples of the three types of milk, positive alizarol test and SCC above the established limit, for refrigerated raw milk, and density out of the standards established by the legislation, for pasteurized milk.

Microbiological contamination of raw milk occurs mainly on the dairy farm, and the bacteria can be on milking equipment, milk storage places or inside the udder. Another fact is that the milk is not sterile inside the udder, not even in healthy cows, and these microorganisms can enter milk through the entero-mammary route[27]. The samples from the dairy properties in this study show great variation in microbiological parameters and SCC and, due to this, the standard deviation and standard error of the samples were extremely high (Table 1 and Table 3).

Pasteurized milk from industry 1 showed a mesophilic microorganism count of 9,700 CFU/mL. In industry 2, the count of mesophilic microorganisms was much lower, being 510 CFU/mL. The Normative Instructions N. 76/2018[4] does not establish the maximum amounts of mesophilic microorganisms for pasteurized milk. The UHT milk from both industries did not show colony growth, with the count of mesophilic microorganisms equal to zero (Table 2). According to Ordinance 370/1997, the amount of aerobic mesophiles must not exceed 100 CFU/mL in UHT milk[5].

Heat treatment in milk, pasteurization or UHT, is applied to ensure the microbiological safety of dairy products. In addition, it allows the inactivation of spoilage microorganisms and enzymes and, in this way, improves the quality and prolongs the shelf life of the products[6].

Pasteurization aims to reduce the number of any pathogenic microorganisms to a level where there is no significant danger to the health of the consuming public, still resulting in a shelf life of about ten days under refrigerated conditions. The UHT treatment, with temperatures of 135 °C for a few seconds, can guarantee a shelf life of up to six months, at room temperature, when kept in a closed bottle[1].

The analyzes of psychrotrophic microorganisms in refrigerated raw milk from dairy properties showed that most properties obtained counts up to 10^3 CFU/mL (13 properties) and 10^4 CFU/mL (9 properties), followed by 10^2 CFU/mL (6 properties) and 10^5 CFU/mL (3 properties). One property (P17) had a psychrotrophic count up to 10^6 CFU/mL, being 2,000,000 CFU/mL. The amount of psychrotrophic microorganisms ranged from only 10 CFU/mL, in property P30, to up to 2,000,000 CFU/mL, in property P17 (Table 3). The average number of psychrotrophic microorganisms from the properties was 91,266.97 CFU/mL, which is lower than the average TBC found in raw milk from dairy properties, which was 239,363.64 CFU/mL.

Property P17 presented the highest average in the count of psychrotrophic microorganisms, formation of clumps in the alizarol test, in addition to SCC and TBC above the limit established by legislation. Property P17 had its cooler at its maximum capacity, with the milk coming from several milkings, and the temperature was 4.5 °C at the time of collection, higher than the general average of the temperatures of the properties (4. 04 °C).

Psychrotrophic microorganisms are not considered an important component of the bovine udder microbiota and their presence in refrigerated raw milk probably occurs due to contamination during and/or after milking, with milking equipment, in most cases, being responsible for contamination. These microorganisms produce extracellular proteolytic and lipolytic enzymes during their growth in raw milk and these enzymes remain active after heat treatment. Psychrotrophic bacteria can grow considerably during cold storage, and both their metabolism and the production of extracellular enzymes can reduce the quality and shelf life of commercial milk and other dairy products[30].

The amount of psychrotrophic microorganisms in raw milk from dairy farms (with an average of 91,266.97 CFU/mL) represents 38.12% of the TBC (with an average of 239,363.64 CFU/mL). Ribeiro Junior et al.[20], in their study, found a percentage of 78% of psychrotrophs in relation to the total bacterial count. For Mariotto et al.[31], milk with a good quality should not have a psychrotrophic count greater than 10% of the count of mesophilic microorganisms (or TBC), and this proportion gradually increases in contaminated milk.

The count of psychrotrophic microorganisms in the refrigerated raw milk from the industries was 10,000,000 CFU/mL, in industry 1 and 6,000,000 CFU/mL, in industry 2 (Table 2). The average amount of psychrotrophic microorganisms in raw milk from the analyzed industries was 8,000,000 CFU/mL, much higher than the average of psychrotrophic microorganisms in raw milk from dairy properties, which was 91,266.96 CFU/mL (Figure 1b). In relation to TBC, the average count of psychrotrophic microorganisms, of 8,000,000 CFU/mL, was more than three times higher than the average of TBC found in raw milk from the industries, 2,532,500 CFU/mL.

According to Paludetti, Kelly and Gleeson[32], the most common psychrotrophs found in raw milk during cold storage belong to the genus *Pseudomonas* sp. and milk with a psychrotrophic count greater than 5.0 x 10^6 CFU/mL should be rejected for processing, due to the possibility of production of enzymes by these microorganisms. Regarding the amounts of psychrotrophs found in the properties of this study, none exceeded this limit, and only one property (P17), representing 3% of the samples, had psychrotroph counts up to 10^6 CFU/mL. According to Ribeiro Junior et al.[20], who also obtained counts below 10^5 CFU/mL, in their study with raw milk, this evidences a high quality of milk produced on the properties. However, in relation to the industries, both presented counts between 10^6 CFU/mL (industry 2) and 10^7 CFU/mL (industry 1), exceeding the limit considered acceptable. According to the same author, the deterioration of milk by psychrotrophs is noticeable when the count reaches 10^6 CFU/mL.

The count of psychrotrophic microorganisms for pasteurized milk from the industries showed amounts above 10^3 CFU/mL in both industries, being 80,000 CFU/mL, in Industry 1, and 40,000 CFU/mL, in industry 2. The UHT milk from the industries did not show a count of psychrotrophic microorganisms (Table 2). The amount of psychrotrophic microorganisms in pasteurized milk from the industries (80,000 CFU/mL, in industry 1 and 40,000 CFU/mL, in industry 2) was much higher than the amount of mesophilic microorganisms of the same type of milk (9,700 CFU/mL, in the industry 1 and 510 CFU/mL, in industry 2). In industry 1, the amount of psychrotrophs is almost eight times greater than the amount of mesophiles and in industry 2, the amount of psychrotrophs is almost 80 times greater than the amount of mesophiles.

Although the quality of fluid milk and dairy products can be degraded through different mechanisms, chemical and/or microbial, microbial growth is predominant in spoilage. For pasteurized milk, one of the main causes of microbial spoilage of milk is post-pasteurization contamination (PPC) with gram-negative bacteria during processing[33]. A large number of bacteria can cause problems to the processed milk quality. These microorganisms are psychrotrophic, thermoduric, heat resistant and pathogenic. Spore-forming microorganisms and thermoduric enzymes produced by psychrotrophs are not destroyed by pasteurization and are common quality problems in milk and dairy products. Therefore, greater control of the initial microbial composition of raw milk is very important for the dairy industry in order to reduce or limit the economic losses caused by quality problems[27].

The amount of total coliforms in the properties ranged from 1 MPN/mL (two properties) to 110 MPN/mL (ten properties) and the amount of thermotolerant coliforms ranged from 1 MPN/mL (eleven properties) to 110 MPN/mL (three properties). The average amount of total coliforms in samples from dairy properties in Vale do Taquari was 42.97 MPN/mL and the average of thermotolerant coliforms was 14.24 MPN/mL. The ten properties (P4, P5, P9, P14, P17, P19, P20, P24, P26, P32) that presented total coliform counts at 110 MPN/mL, correspond to 33.03% of the analyzed samples. Of those, only three (P9, P20 and P32), 10% of the samples, presented the same amount for thermotolerant coliforms. Among these ten properties, seven (P4, P5, P14, P17, P19, P20, P26) had SCC above the limit established by legislation (Table 1). Of the three exceptions (P9, 924 and P32), two had SCC above 300,000 cells/mL, 365,000 (P9) and 467,000 (P24). Property P32, which also presented the amount of thermotolerant coliforms at 110 NMP/mL, showed low SCC, with a value of 190,000 cells/mL. Property P17, which showed positive SCC, TBC, alizarol and the highest count of psychrotrophs, as already mentioned, also showed higher amounts of total coliforms, showing failures in the hygiene of the property.

In industries, the amount of coliforms and thermotolerants in refrigerated raw milk was 110 NMP/mL for both, showing an average higher than the amount found in dairy properties (Figure 3a). Pasteurized milk and UHT milk from industries did not show the presence of coliform microorganisms.

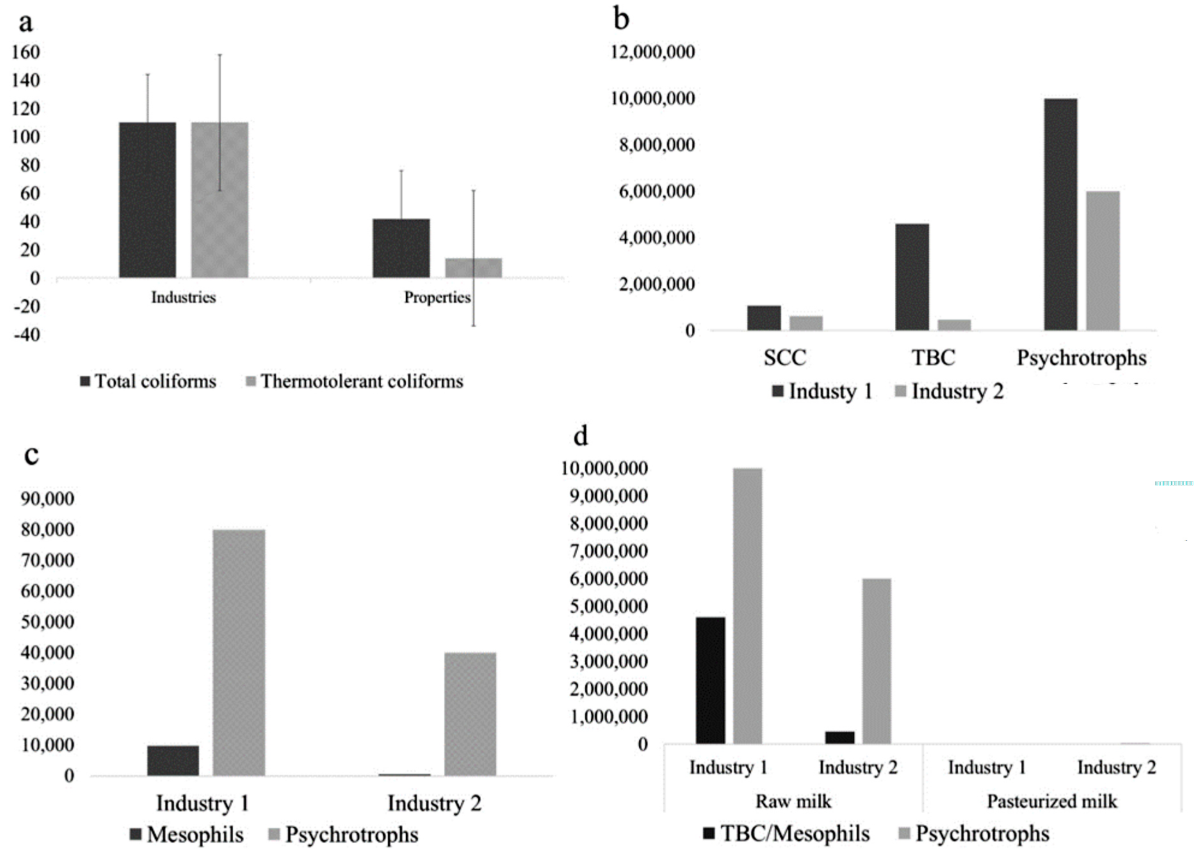

Figure 3. (a) Average amount of total coliforms and thermotolerant coliforms found in samples of refrigerated raw milk from industries and dairy properties in Vale do Taquari -RS and standard error; (b) Amount of SCC, TBC and psychrotrophic microorganisms found in refrigerated raw milk from industries 1 and 2 of Vale do Taquari – RS; (c) Quantity of mesophilic microorganisms and psychrotrophic microorganisms found in samples of pasteurized milk from industries 1 and 2 of Vale do Taquari – RS; (d) Amount of mesophilic microorganisms (or TBC) and psychrotrophic microorganisms found in refrigerated raw milk and pasteurized milk from industries in Vale do Taquari – RS.

The use of indicator bacteria, such as the group of total and thermotolerant coliforms, had as its initial concept the investigation of fecal contamination, later serving as an indirect indicator of pathogens. However, several studies over the years have shown a lack of correlation between the presence of indicators and pathogens in the samples. Thus, these indicators are currently used as sanitation indicators. Coliform bacteria can be found in low amounts, up to 100 CFU/mL, in refrigerated raw milk with good quality. However, the presence of large quantities has been mainly attributed to unsanitary processing conditions. Total and thermotolerant coliforms are not inherent microflora in raw milk and are introduced from the environment, udder and milking equipment[8]. In cases of *Escherichia. coli* in high numbers, foodborne outbreaks may occur[21].

The fact that refrigerated raw milk from industries has higher amounts of TBC, psychrotrophs and total and thermotolerant coliforms than refrigerated raw milk from dairy properties in Vale do Taquari, can be explained by the milk storage time, which can cause the proliferation of microorganisms, mainly psychrotrophic, which multiply at low temperatures. About the microorganisms of the coliform group, the contamination can come from the environment, at the time of collection of milk by the tank truck. The higher amount of SCC can be explained through the fact that the milk from the tank truck comes from several properties, which results in the mixing of milk with high SCC and milk with acceptable levels, causing a general increase in SCC in the milk of the tank truck.

When comparing the amount of SCC, TBC, mesophiles, psychrotrophs and total and thermotolerant coliforms of the two analyzed industries, it can be seen that industry 1 had the highest amount of SCC, TBC and psychrotrophs (Figure 3b), in refrigerated raw milk and higher quantity of mesophiles and psychrotrophs in pasteurized milk (Figure 3c), in relation to industry 2. Thus, the milk from industry 2 has a higher microbiological quality than the milk from industry 1. Industry 1 needs greater attention in the storage and processing of milk, in addition to checking for possible post-processing contamination. The counts of psychrotrophs and SCC are 40% and 70%, respectively, higher compared to industry 2. The TBC of industry 1 is 10 times higher than the TBC of industry 2. Relative to pasteurized milk, the mesophilic count is 19 times higher in Industry 1 than in Industry 2, and the psychrotrophic count is exactly double the count found in Industry 2.

Despite this, it is possible to verify the reduction of TBC and the number of mesophilic and psychrotrophic microorganisms from refrigerated raw milk from the industries related to pasteurized and UHT milks. This indicates that, in general, the beneficiation processes of the industries have been effective (Figure 3d) in the reduction of microorganisms. This reduction reaches 100% of refrigerated raw milk compared to UHT milk. as it did not show the growth of mesophiles, psychrotrophs or the presence of total and thermotolerant coliforms. The pasteurized milk from the industries also did not present microorganisms of the coliform group, reinforcing the efficiency of this thermal process in the elimination of this group of microorganisms.

Conclusion

The physicochemical analyzes showed that two properties presented acidity above that established by the legislation for refrigerated raw milk. The milk composition analyzes showed that all properties and industries were in accordance with the established for lactose, protein, fat, ST and NFS, with the exception of property 17, which showed excess fat. The SCC was above the established limit for 53.2% of the properties and for the two analyzed industries. Microbiological analyzes showed that three properties and industry 1 had TBC above the established limit. Industry 1 also presented acidity above the allowed in the three types of milk, raw refrigerated, pasteurized and UHT, and density above the established for pasteurized milk. The industries showed higher amounts of SCC, TBC, psychrotrophs and total and thermotolerant coliforms than the dairy properties of Vale do Taquari. The raw milk from the properties showed higher quality compared to the refrigerated raw milk found in industrial tank trucks, a fact that can be caused by failures in cooling or prolonged storage periods. The refrigerated and pasteurized raw milk from industry 2 showed a higher microbiological and physicochemical quality than the refrigerated and pasteurized raw milk from industry 1. The evaluation of the quality of the milk produced is extremely important for the identification of possible failures in the production process, being essential for the implementation of improvements in all stages of the production chain.

Conflict of interests

The authors declare no conflict of interest

Author contributions

Conceptualization: T. Müller, M.J.Maciel and C. Rempel. *Data curation*: T. Müller. *Formal analysis*: T. Müller. *Investigation*: T. Müller. *Methodology*: M.J.Maciel. *Resources*: M.J.Maciel and C. Rempel. *Project management*: C. Rempel. *Validation*: T. Müller. *Visualization:* T. Müller. *Supervision*: M.J.Maciel and C. Rempel. *Writing (original draft):* T. Müller. Writing (review and editing): M.J.Maciel and C. Rempel.

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