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Microbiological quality of refrigerated raw milk in the dairy farm and after transport to the processing dairy plant

Qualidade microbiológica do leite cru refrigerado na propriedade produtora de leite e após o transporte para a indústria beneficiadora

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ABSTRACT: Transport of cooled raw milk in bulk has greatly improved the quality of the raw material collected by dairy plants as it reduces the proliferation of mesophilic microorganisms that cause milk acidity and hinder its processing. However, refrigeration has favored the growth of psychrotrophic microorganisms which are able to grow at low temperatures (below 10ºC) and that produce heat resistant enzymes which degrade some milk components, reducing milk shelf life and causing organoleptic changes. The aim of this paper was to evaluate the microbiological quality of raw milk in dairy farms and after its transport to the processing dairy plant, through plate counting of mesophilic and psychrotrophic microorganisms. Fourteen milk samples were collected from tanks of the dairy-farming properties, and one sample was collected from their milk transport tanker at the entrance of the processing plant. Our results showed that the mean number of mesophilic microorganisms was higher in samples collected straight from the dairy farm tanks than in the samples collected from the transportation tankers at the entrance of the plant. Of the 14 sampled tanks, 64.3% were non-compliant with legislation. The sample collected from the milk transportation tanker containing milk from all properties showed a higher mean number of psychrotrophic microorganisms than the dairy farm samples. We conclude that the milk from dairy properties showed a higher amount of mesophilic microorganisms, and after transportation, at the entrance of the processing plant, there is a higher amount of psychrotrophic microorganisms.

KEYWORDS: quality of milk; mesophilic; psychrotrophic; contamination.

RESUMO: O transporte do leite cru refrigerado a granel melhorou muito a qualidade da matéria-prima recebida pelas indústrias de laticínios, reduzindo a proliferação de micro-organismos mesófilos que causam a acidez do leite e que impossibilitam seu beneficiamento. Porém, a refrigeração favoreceu o crescimento de micro-organismos psicrotróficos que são capazes de crescer em baixas temperaturas (abaixo de 10ºC) e que produzem enzimas termorresistentes que degradam alguns componentes do leite, diminuindo o seu tempo de prateleira e causando alterações organolépticas. Este trabalho teve como objetivo avaliar a qualidade microbiológica do leite cru presente na propriedade leiteira e após o transporte para a indústria beneficiadora, por meio da contagem em placas de micro-organismos mesófilos e psicrotróficos. Foram coletadas 14 amostras de leite nos tanques das propriedades produtoras e uma amostra do caminhão de transporte dessas propriedades, na chegada à indústria beneficiadora. Os resultados mostraram que, nas amostras coletadas diretamente nos tanques das propriedades, a média de micro-organismos mesófilos foi mais alta do que a amostra coletada diretamente do caminhão de transporte ao chegar à indústria. Dos 14 tanques amostrados, 64,3% estão em desacordo com a legislação. A amostra coletada do caminhão de transporte do leite, contendo o leite de todas as propriedades, apresentou média maior de micro-organismos psicrotróficos em relação à média das propriedades. Conclui-se que o leite das propriedades apresentou maior quantidade de mesófilos e que, após o transporte, na chegada à indústria beneficiadora, há maior quantidade de psicrotróficos.

PALAVRAS-CHAVE: qualidade do leite; mesófilos; psicrotróficos; contaminação.

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INTRODUCTION

The dairy production segment is one of the most important industries in Brazil, and it represents a source of income to many municipalities in Rio Grande do Sul, through the generation of taxes and job opportunities (BITENCOURT et al., 2000), hence the importance of producing quality milk that can compete with other markets.

Milk is considered a complete, highly nutritious food, excellent for people's health, and an excellent culture medium for most microorganisms present in nature (ZENI et al., 2013). Milk produced in Brazil generally contains high counts of microorganisms, which indicates flaws in production hygiene. Simple conducts such as discarding the three first milk jets, cleansing of materials used in milking with chlorinated alkaline detergent, submersion in chlorinated solution, and elimination of residual water from milking utensils have proved to be effective practices to improve the microbiological quality of milk (SANTANA et al., 2001a; VALLIN et al., 2009; ANGELIS et al., 2016).

From milking to the entrance at the reception platform in the industrial plant, milk goes through several steps before reaching the table. Great attention to hygiene is required in all processes undergone by milk, so that it is not susceptible to contamination by microorganisms, mostly mesophilic and psychrotrophic bacteria, which cause deterioration and compromise the quality of milk and dairy products.

With the growing dairy sector, the need for prolonged milk storage has arisen. Aiming at preventing these microorganisms from interfering in the quality of milk and dairy products, national milk quality standards were implemented by the Ministry of Agriculture, Livestock, and Food Supply (MAPA) through Normative Instruction no. 51 in 2002 (BRASIL, 2002), which institutes the collection of refrigerated raw milk and transport in bulk (CITADIN et al., 2009).

Normative Instruction no. 62 (BRASIL, 2011) sets forth the conditions under which cooled raw milk must be obtained, conserved, collected, and transported, in order to preserve its quality from milking to its entrance at the dairy plant under official sanitary inspection.

With raw milk transportation in bulk in the rural property, production started to be delivered any time of the day, because expansion tanks ensure milk quality for up to 48 hours, provided it is quality milk when obtained (SANTOS et al., 2009).

Before milk-cooling equipment appeared, the main milk deteriorating microorganisms were lactose-fermenting mesophilic bacteria, which caused rapid acidification in non-refrigerated milk (SANTOS et al., 2009). However, low temperatures, at around 4ºC, favor the growth of psychrotrophic microorganisms when milk is kept under these conditions for too long. These microorganisms derive from environmental sources, *i.e.*, from water, vegetation, teats/udder, and from incorrectly sanitized milking equipment. They produce thermo-resistant enzymes, causing technological and economic problems in dairy farms and decreasing milk shelf time (SANTANA et al., 2001b).

In order to know the microbiological quality of milk from its production in the dairy farm to its transport to the processing plant, the present study compared the amount of mesophilic and psychrotrophic bacteria found in refrigerated raw milk in the dairy farm and after transport, checking potential means of contamination and its possible consequences to the end product.

MATERIAL AND METHODS

Refrigerated raw milk samples were collected at temperatures no higher than 7ºC from 14 cooling tanks in dairy farms, following the transport tanker's route. Samples were collected after milk was homogenized by agitation, and then, 250 mL of milk were collected from each tank in each farm, using a stainless steel collector and sterile flasks. The 14 samples were mixed and transported in one single cooling tanker at a temperature no higher than 10ºC, and, when they reached the processing plant, a sample was also collected from the tanker, which was a mixture of milk from all producers. Samples were packaged in isothermal boxes containing ice and were sent to the Laboratory of Microbiology of Universidade do Vale do Taquari (Univates).

The dairy industry is a Private Limited Society, with active enrollment since October 14, 2014, and it is situated in a municipality in Rio Grande do Sul. It provides approximately 27,000 liters of milk a day. Its main activity is the preparation of milk, and its secondary economic activities are dairy manufacture and bulk commerce of milk and dairy products.

In the laboratory, samples were diluted according to the methodology for mesophilic microorganisms detailed by SALVATORI et al. (2013); pipetting 1 mL of the sample and transferring it to a tube containing 9 mL of 0.1% peptoned water. From this dilution, decimal dilutions were performed up to 10^{-6}. Petri dishes received 1 mL of the 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions with approximately 20 mL of plate count agar (PCA agar), using the pour plate method (depth), with incubation of inverted plates at 36 ± 1ºC for 48 hours.

For psychrotrophic microorganisms, we followed the protocol by Ordinance no. 101 of August 11, 1993, of MAPA (BRASIL, 1993). The agar surface received 0.1 mL of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions, using the spread plate method (surface), with incubation of inverted plates at 7ºC for 10 days. To ensure data reliability, analyses were performed in duplicates. Counts were performed with a colony counter, with a precision interval of 25 to 250 colonies, and results were expressed in UFC mL^{-1} (Colony-Forming Units per mL) of milk.

To analyze the results obtained during the study and to compare the microbiological quality of milk before and after transport, data were tabulated in a Microsoft Excel 2010 spreadsheet and were shown as absolute and relative frequencies. To check the microbiological quality of the milk, standard plate counts were compared to the limit of 3×10^5 UFC mL^{-1} defined by Normative Instruction no. 62, 2011, of MAPA, for mesophilic microorganisms in refrigerated raw milk. For psychrotrophic microorganisms, comparison was performed according to values defined by NÖRNBERG; TONDO; BRANDELLI (2009), 1×10^6 UFC mL^{-1}; as this analysis is not mandatory, the Brazilian legislation has not defined these limits.

RESULTS AND DISCUSSION

Of the milk samples analyzed, only five samples (35.7%) were within the standard value (3×10^5 UFC mL^{-1}) set forth by legislation for mesophilic bacteria (BRASIL, 2011) (Fig. 1). The remaining nine samples (64.3%) analyzed are out of the established standards. When present in the milk, mesophilic bacteria ferment lactose, producing lactic acid, which causes milk acidification thus compromising its use in the industry (SANTANA et al., 2001a). NERO et al. (2005) observed that in the region of Pelotas 56% of the milk samples analyzed were non-compliant with legislation regarding mesophilic microorganisms and that only refrigeration in the farm and transport were not enough to ensure the microbiological quality of the milk; therefore, greater attention to hygiene practices during milking was required.

Although it is not a mandatory analysis for the MAPA, psychrotrophic microorganism count is very important in milk quality control. According to literature, it would be reckless to manufacture dairy products from raw milk with a psychrotrophic microorganism count higher than 5×10^6 UFC mL^{-1} (PINTO et al., 2006). Other authors do not recommend using milk with counts higher than 1×10^6 UFC mL^{-1} (NÖRNBERG et al., 2009). Even if most psychrotrophic bacteria are destroyed in the pasteurization process, these microorganisms are capable of producing lypolitic and proteolytic thermo-resistant enzymes after pasteurization or even after ultra-high temperature (UHT) treatment. These enzymes might cause change in milk flavor and odor, loss of consistency, and gelatinization throughout the commercial life of UHT milk, thus reducing the product's shelf life (ROSSI JUNIOR et al., 2006).

In this study, two samples (14.3%) were out of the limit used as parameter (1×10^6 UFC mL^{-1}). The remaining 12 samples (85.7%) were in accordance with the limit defined by NÖRNBERG et al. (2009). Our results differ from the findings by ZENI et al. (2013), who observed that all silos showed values higher than those ones recommended by literature. They hence assumed that resistant proteolytic enzymes were produced, which caused technological problems in milk and dairy products. According to MENEZES et al. (2015), in the quality raw milk produced in Minas Gerais, milk samples showed high contamination by mesophilic and psychrotrophic microorganisms. These authors diagnosed that contaminations compromised the sanitary hygienic quality of milk, since the majority of the samples analyzed were not in compliance with the limits set forth by legislation either at the time of the study or presently.

There is a weak and non-significant correlation between the number of mesophilic and psychrotrophic microorganisms in the samples analyzed ($r = 0.2660$; $p = 0.3579$). The mean number of mesophilic microorganisms was higher in the samples obtained in dairy farms than in the sample from the milk transport tanker at the entrance of the milk plant (Fig. 2). PINTO et al. (2006) observed that high contamination by

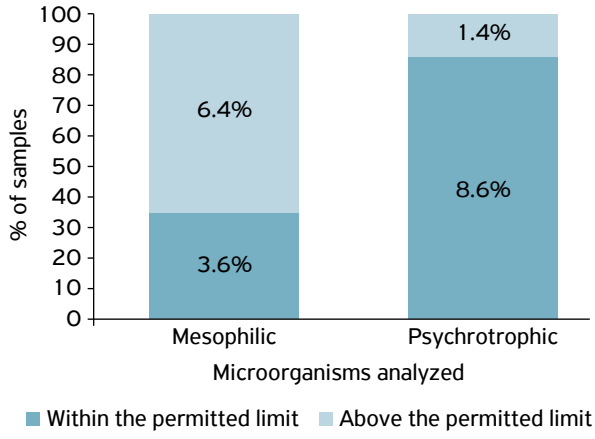

Figure 1. Percentage of compliant and non-compliant milk samples with the limit for mesophilic and psychrotrophic microorganisms permitted by the referred legislation.

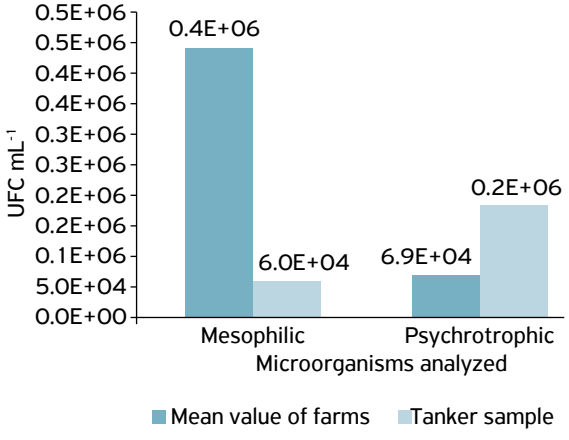

Figure 2. Mean count of mesophilic and psychrotrophic microorganisms found in milk-producing rural farms and in the sample from the milk transport tanker at the entrance of the processing milk plant.

mesophilic microorganisms in cooled raw milk samples might be associated to inadequate hygiene procedures in the production system, to contact of milk with dirty animals, inappropriate environment for production, failure in milk cooling, or milk derived from animals with mastitis, which might have occurred in the farms analyzed, with unsatisfactory results. FRANÇA et al. (2015) observed that poor milk refrigeration at the production source results in a higher total bacterial count than the one allowed by Brazilian legislation. The authors point out that potential causes for this occurrence would be the lack of severity in the monitoring of milk storage temperature, as well as poor maintenance of expansion tanks.

On the other hand, for psychrotrophic microorganisms, the mean value was higher in the milk sample from the transport tanker, due mainly to two samples that had counts higher than 1.6×10^6 UFC mL^{-1}. ROSA et al. (2012), studying somatic cell count (SCC), observed that individual milk samples also had lower percentages than SCC in tankers. The explanation provided by the authors is that milk from healthy cows dilutes the SCC of sick cows, thus indicating that SCC in tankers is not a reliable indicator of milk quality either.

SANTOS et al. (2009) show increased psychrotrophic microorganism count with increased milk storage time, which occurs during transport. According to JAY; TONDO (2005), most psychrotrophic microorganisms are in fact mesophilic microorganisms that have altered their metabolism as a means to adapt to the extraneous conditions of the environment. This could explain the higher mean number of psychrotrophic microorganisms in the milk samples of the transport tanker, since farm samples showed a higher mean number of mesophilic microorganisms.

In the samples from the transport tanker, the milk from the 14 farms evaluated is mixed; some have low microorganism counts and other ones have high microorganism counts. Mesophilic microorganism count in the milk sample from the transport tanker, although lower than the mean count in the milk from the farms (6×10^5 UFC/mL), is still higher than the limits set forth by legislation (3×10^5 UFC mL^{-1}). Therefore, we might infer that there are flaws in milk production hygiene in some farms, thus causing contamination in the milk that shall be processed for commercialization.

In the case of psychrotrophic microorganisms, the sample collected in the milk transport tanker has a high count (1.8×10^6 UFC mL^{-1}), but it is still within the limit defined by PINTO et al. (2006), which is 5×10^6 UFC mL^{-1}. However, it is important to emphasize that psychrotrophic microorganisms multiply rapidly when stored for a long time at low temperatures. Even if psychrotrophic microorganisms are eliminated from milk submitted to UHT treatment, enzymes produced by these organisms will remain and might cause milk gelatinization, as mentioned previously. BÜRGER et al. (2011), studying the microbiological characteristics of UHT processed whole milk and dairy beverage throughout the validity period, observed that the population of psychrotrophic microorganisms in the milk increased by 26.6% in the samples after they were opened and kept under refrigeration for 48 hours.

SANTOS et al. (2009) observed that the number of psychrotrophic microorganisms increased with increased storage time, which thus makes raw milk storage for over 48 hours a cause for concern. Therefore, it is important to control the time this milk will remain in the industry prior to its processing, the hygiene of utensils used in the industry itself, and also the water used in sanitation, which might increase contamination by psychrotrophic microorganisms and, consequently, produce low quality products with shorter shelf life.

CONCLUSIONS

Based on the present study, we might conclude that the sample collected in the milk transport tanker containing milk from all farms had higher mean number of psychrotrophic microorganisms than the samples from dairy farms, and mesophilic microorganism count exceeded the value set forth by legislation, thus indicating low quality milk.

Differential payment to producers who produce milk with good microbiological quality and the training of these farmers in order to produce milk under good hygienic conditions would reduce microorganism count. It is quite important for the company, which will produce better quality products, avoid future technological problems, and increase shelf life of their dairy products.

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