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Quality of ovine raw milk stored under refrigeration

Qualidade do leite cru ovino armazenado sob refrigeração

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ABSTRACT

The dairy sheep industry is recent in Brazil and the production is concentrated in mountainous regions, such as Serra Gaúcha. Ovine milk has a higher solids content compared to bovine milk, thus with higher yield in cheese production. Sheep milk production undergoes seasonal variations and it could be stored until a sufficient quantity is available for processing. The objective of this study was to evaluate microbiological and physicochemical parameters in ovine milk from six different producers under refrigerated storage. Milk was analyzed during seven days, including standard plate count, psychrotrophic bacteria, total and fecal coliforms, Staphylococcus sp. and S. aureus, titratable acidity, pH, water activity, and protein content. The results showed high microbial counts and standard plate counts above those established by legislation for most samples. Psychrotrophic, total and fecal coliforms counts were increased during this period. Titratable acidity increased over the days, while protein content decreased. Samples of milk producer B presented the lowest microbial counts among all tested. Cold storage becomes a short-term alternative when there is a decrease in sheep milk production for dairy processing. Storage period of refrigerated milk must not exceed two days due to deterioration.

KEYWORDS: Milk; Sheep; Microbiological Analysis; Physicochemical Analysis; Shelf Life

RESUMO

A industrialização de lácteos ovinos é recente no Brasil e a produção é concentrada em regiões de serra, como a Gaúcha. O leite ovino possui alto conteúdo de sólidos comparado ao bovino, com maior rendimento na produção de queijo. A produção deste leite sofre variações sazonais e poderia ser estocado até obter uma quantidade suficiente para processamento. O objetivo deste trabalho foi avaliar parâmetros microbiológicos e físico-químicos no leite proveniente de seis produtores sob armazenamento refrigerado. O leite foi analisado durante sete dias, incluindo contagem padrão em placas (CPP), psicrotróficos, coliformes totais e termotolerantes, *Staphylococcus* sp. e *Staphylococcus aureus*, acidez titulável, pH, atividade de água e proteína. Os resultados demonstraram altas contagens microbianas e CPP acima dos limites estabelecidos pela legislação para a maioria das amostras. Contagens de psicrotróficos, coliformes totais e fecais foram elevadas durante este período. A acidez titulável aumentou ao longo dos dias e o conteúdo de proteína diminuiu. As amostras do produtor B apresentaram menores contagens microbianas dentre todas testadas. A estocagem a frio torna-se uma alternativa, a curto prazo, quando há diminuição na produção de leite ovino para beneficiamento a derivados. No entanto, o período de armazenamento refrigerado não deve ultrapassar dois dias, devido à deterioração.

PALAVRAS-CHAVE: Leite; Ovelha; Análise Microbiológica; Análise Físico-química; Vida de Prateleira

INTRODUCTION

Milk production represents an important part of the global economy; however, the American continent generates only about 0.4% of world ovine milk production. The continent contributes 7.2% of the number of sheep, thus a very small portion is destined for milk production[1]. In Brazil, milk production generates numerous jobs in several areas, with social and economic importance[2,3].

Milk is considered a complete food, highly nutritive, contributing to vital functions as an important source of carbohydrates, proteins and lipids[4]. Milk composition varies according to the animal species. When compared to cow's milk, sheep's milk shows great variations in its components. The largest differences relate to the protein levels, in particular casein, and lipids. Cow's milk shows values of 2.6% and 4.2%, while ovine milk has values corresponding to 3.6% and 7.9%, respectively[5].

In natura, ovine milk is currently used mainly for cheese production and, to a lesser extent, to produce other dairy products[6,7]. Among its characteristics, the high protein value[8], high total solids and fat contents stand out, making it ideal for the production of cheeses with high commercial values[6,7].

Milk, regardless of the producing animal species, may contain a high microbial load due to animal contamination by several pathogens, with mastitis as its main source. Inflammation increases the percentages of somatic cells in milk, interfering with their quality[4]. Among the main bacteria related to mastitis are *Staphylococcus aureus*, *Streptococcus*, coliform groups[9] and *Pseudomonas*[10]. This source of contamination can be controlled through proper herd management, as well as hygiene when milking, which reduces the microbial load in the animals' teats[3,4]. Other sources of milk contamination are inadequate practices during storage and transport[8].

Normative Instruction No. 62, of December 29, 2011, establishes the Technical Regulation of Identity and Quality of refrigerated raw milk, with reference values for the milk to be processed and marketed. According to this legislation, raw milk must be cooled to a temperature equal to or lower than 4°C, in the maximum time of three hours after the end of the milking[11]. The purpose of refrigeration is to avoid microbial growth and undesirable milk changes[12].

The quality of the raw material is an extremely important and limiting factor for the presence of pathogenic and deteriorating microorganisms in the process of dairy industrialization[8]. Therefore, the dairy industry must seek ways to obtain milk and dairy with the lowest contamination possible, to ensure product conservation and consumer safety. Thus, this study aimed to evaluate the microbiological and physicochemical quality of sheep's milk obtained from different producers in the Serra Gaúcha region.

METHOD

Raw milk production from the Lacaune sheep was destined for a dairy industry, which has a Federal Inspection Seal (S.I.F.), located in the Serra Gaúcha region. The samples were obtained from six different dairy farms that supply their product to this dairy, in August 2015; the producers were identified with letters from A to F. The milk was obtained by mechanized milking and collected from the refrigerated tank of each property.

The sheep's milk was collected in 80 mL sterile bottles, and we obtained at least two bottles of milk per producer for each day of analysis. The bottles were immediately packed in isothermal boxes, with hard reusable ice to maintain the refrigeration temperature, and sent to the biology laboratory at the Federal University of Pampa (Unipampa), Itaqui-Rio Grande do Sul. The samples were received and kept under refrigeration with controlled temperatures (5°C ± 2.5°C) and monitored during the evaluation period. The analyses started 24 hours after milking, for seven consecutive storage days, and were done at least in duplicate.

The physicochemical parameters analyzed in the samples of refrigerated raw milk were those recommended by the Ministry of Agriculture Livestock and Food Supply Normative Instruction (IN) No 62, of December 29, 2011[11], including total protein, acidity, cryoscopic index and fat. Besides these, pH, water activity and 72% ethanol stability were determined. The physicochemical analyses followed the methodology described by IN No. 68, of December 12, 2006[13]. Water activity was measured according to the manufacturer's manual, AquaLab 4TE[14]. Other evaluations followed official methodologies for pH[15] and cryoscopic index[16].

The methodologies followed the IN No. 62/2003 of August 26, 2003, for the SPC and total and fecal coliform groups; the coliform group was determined by the multiple tube technique and the most probable number analyzed against the Hoskins' table[17]. Besides these analyses, we also performed counts of psychrotrophic microorganisms of *Staphylococcus* sp. and *S. aureus*[18]. The serial dilutions ranged from 10^{-1} to 10^{-10}.

The results were organized in Microsoft Excel 2010 to obtain means and standard deviations of the mean, and the microbiological count values were converted into logarithms. Data were evaluated by the ASSISTAT program 7.7 beta[19], by which analysis of variance was applied, followed by the Tukey Test at the 5% level of significance.

RESULTS AND DISCUSSION

Figure 1 shows the SPC and psychrotrophic counts in raw ovine milk kept under refrigeration.

Figure 1A shows that although refrigeration slows down the microbiological growth process, this process does not cause microbial destruction and, with time, increases the microbiological load of the products[12,21]. The peaks in the graph, observed on the sixth day for producers D, E and F, correspond to the uncountable result in the highest dilution (10^{-10}). This may have occurred due to additional contamination at the time of collection at the properties; or these bottles may have been kept open longer than necessary. The contamination may have occurred at the time, as all the milk was collected at the same moment, and the count for the same producers was lower on the seventh day of refrigeration.

Considering the SPC in the storage period (Figure 1A), the milk collected from milk producer B showed counts close to the maximum limit recommended by IN No. 62/2011[11] and without great variations. Only the average value for day 1 (5.60 log colony-forming units [CFU]/mL) of this milk producer exceeded the maximum limit established for the south region (5.48 logCFU/mL)[11]. The mean counts in other studies were 4.30 and 4.70 logCFU/mL for raw ovine milk[22,23] and 5.36 logCFU/mL for sheep's milk in a refrigerated silo[24]. The latter study evaluated the milk during storage at 4°C for one to four days and the pattern of mesophilic growth over the days was similar to our study. At the end of four days, the mean quantification was 6.33 logCFU/mL[24], while the mean value of these Serra Gaúcha producers on the fourth day was 6.18 logCFU/mL.

The mean SPC for the different milk producers on day 1 was 6.06 logCFU/mL (n = 12), higher than the cited studies and also the European Union limit for ovine and goat milk in refrigerated tanks, which is 5.70 logCFU/mL[24,25]. This is a worrying result, since the SPC includes bacteria that may be pathogenic[26], as well as indicating that animal hygiene practices were deficient, especially at the moment of milking, and through insufficient refrigeration[27]. A high SPC is a limiting factor for the product validity, compromising the dairy quality of this milk[21].

Psychrotrophic counts (Figure 1B) show that milk producer C showed statistically higher values on most days of analysis. On the other hand, the decline shown on the last day for milk producer B represents the non-detection of colonies at the lowest dilution (10^{-1}) evaluated. This behavior may be due to lower substrate availability for microbial growth, considering the advanced storage time; however, the lack of homogenization in the collection of the aliquot for analysis should not be disregarded. Although there is no legal limit to the psychrotrophic count in milk, which is a typical bacterial group in foods kept at low temperatures, high counts were observed[24,26]. In the psychrotrophic group, there are potentially proteolytic bacteria, such as *Pseudomonas*, which can live in the transport tanks, producers' refrigerators or even in the dairy[24,28,29]. Data obtained evaluating raw cow's milk refrigerated in Rio Grande do Sul, indicated

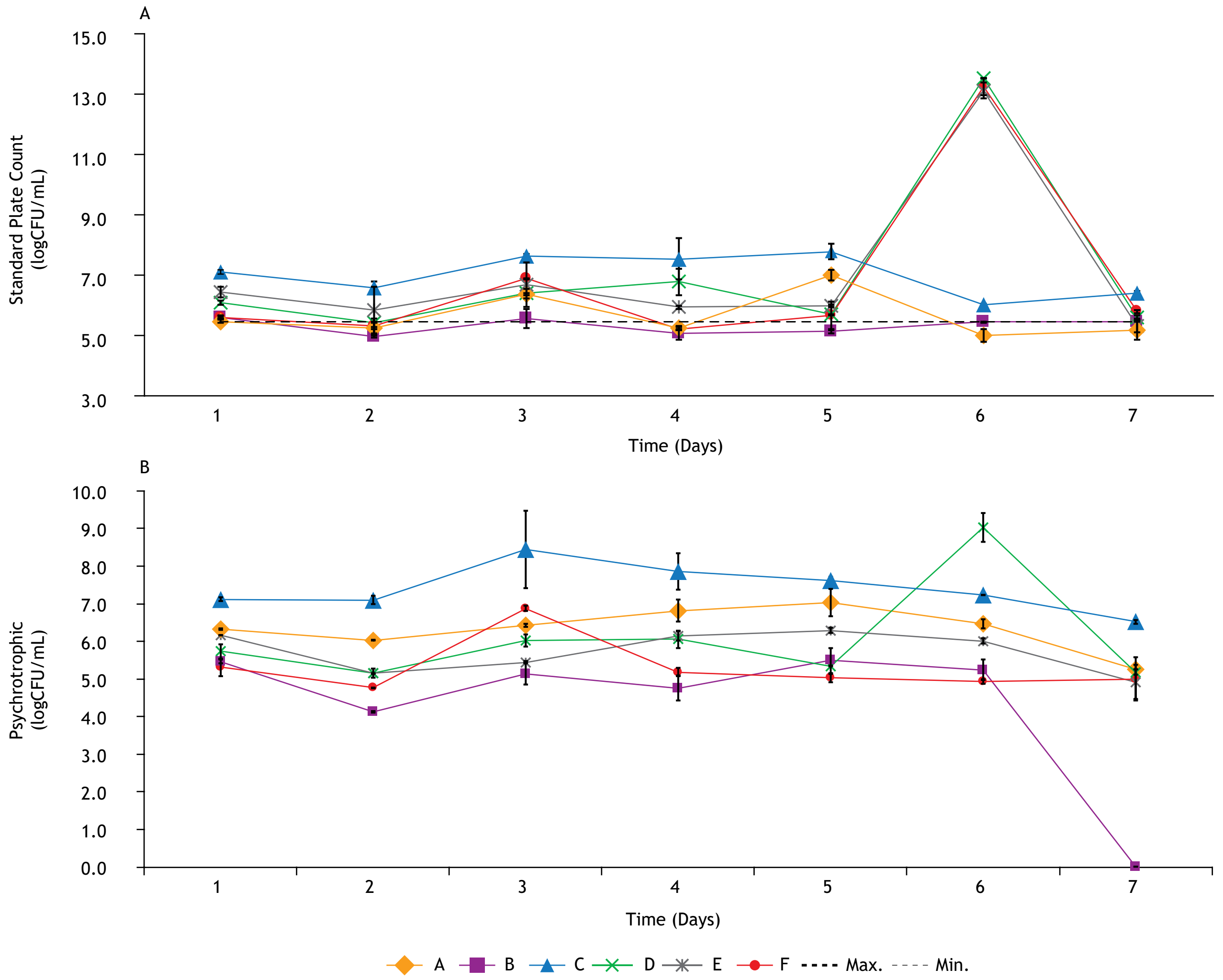

Figure 1. Mean values of (A) Standard Plate Count (SPC) and (B) psychrotrophic bacteria in refrigerated raw ovine milk. The colored lines indicate the means obtained by the milk producer and the bars are the standard deviation of the mean; the dashed line indicates the maximum value allowed by the legislation for standard plate count[11].

psychrotrophic counts from 5.00 to 7.62 logCFU/mL (n = 70)[28]. These values were similar to those obtained on the first day of this study, which ranged from 5.31 to 7.11 logCFU/mL, regardless of the producer. Psychrotrophic counts in samples collected from a dairy silo from dairy sheep in León, Spain, showed mean values of 6.40 logCFU/mL (n = 130) and 7.77 logCFU/mL at the end of four days[24]. In this study, the mean psychrotrophic value among the milk producers was 6.02 logCFU/mL (n = 12) on the first day, 6.09 logCFU/mL (n = 12) on the fourth day and 5.86 logCFU/mL (n = 84) in total samples, indicating values compatible with those obtained for ovine herds.

Raw milk kept at refrigerated temperatures may present with several bacteria of the genera *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Microbacterium*, *Oerskovia*, *Propionibacterium*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Bacillus* and *Listeria*, as well as those belonging to the coliform group[5,26,28]. However, high psychrotrophic counts, not only in ovine milk, but also in bovine, indicate the need to develop control mechanisms to prevent a producer from contaminating the storage silo and creating processing problems[24].

Table 1 shows the rates of total and fecal coliforms and of *Staphylococcus* sp. in refrigerated ovine milk. On the first day, only milk producers B and F showed reduced total coliform values in relation to the others, with values equal to or close to the maximum quantified by the technique. Although the legislation does not have a parameter of coliforms for raw milk, the total coliform group is a good indicator of hygienic conditions in obtaining the milk and the thermotolerant coliforms of the sanitary conditions of *in natura* milk[21].

However, data of thermotolerant coliforms (Table 1) were lower in all milk producers when compared to total coliforms. However, milk producer C stood out with a high contamination number. This group of coliforms represents an imminent danger when the milk is not pasteurized correctly because of the risk of disease to the final consumer[21,26]. The mean value of all samples was 2.51 logCFU/mL (n = 36) for total coliforms and 0.85 logCFU/mL (n = 36) for thermotolerants. The values in other studies with milk from Lacaune sheep in Rio Grande do Sul was found to be 2.04 to 2.34 logCFU/mL for total coliforms and 2.04 logCFU/mL for thermotolerants[22,23]. The values in another study with ovine milk obtained in a refrigerated storage silo were 3.45 logCFU/mL for total coliforms and 2.38 logCFU/mL for *Escherichia coli*[24].

The data are worrisome because they demonstrate that the hygiene of milking places, equipment or during the milking process was inadequate, which impairs the quality of the milk, reducing its shelf life and compromising the dairy production[21,27]. Thus, measures such as milking in a clean place, using potable water to wash the animals' teats and equipment, and submerging the animal's teats in disinfectant solution before and after milking can minimize contamination[22,27], providing a product with improved quality and converting it to a higher payment value per liter for the producer[27].

The presence of *Staphylococcus* sp. (Table 1) was indicated by high scores, with mean values being 3.01 logCFU/mL on the first day and 3.98 logCFU/mL on the seventh day of refrigeration, higher than those observed in another study with *Staphylococcus aureus* in ovine milk from refrigerated silos, with values between 2.62 and 2.92 logCFU/mL[26]. No count was observed on day 1 for the milk producer F, unlike the others, which may indicate better sanitary management in this herd, since *Staphylococcus* is commonly involved in mastitis and may contaminate the milk produced[9]. The investigation of the presence of *S. aureus* in Lacaune raw sheep's milk in other studies showed counts of 3.52 and 4.28 logCFU/mL[22,23]. A study of the *Staphylococcus* prevalence as a cause of mastitis in dairy herds on farms in Egypt found the presence of *S. aureus* in 50% (n = 40) of the isolates, 20% of *Staphylococcus cohnii* and 10% of *Staphylococcus lugdunensis* as the most prevalent in cases of mastitis in sheep[9]. Another similar study was carried out with goats, in Brazil, and the prevalence of *S. aureus* was 26.7% (n = 86), *Staphylococcus intermedius* was 16.3%, with coagulase-negative staphylococci as the most prevalent (51.2%)[30].

The high counts of *Staphylococcus* sp. indicate that either the hygiene conditions at the moment of milking were deficient or that the animal's health was impaired, since they could have had mastitis[5,29,31]. Such species can cause substantial economic losses, resulting in decreased milk production, affecting industrial production, and possible health risk to consumers[9]. The high number of *Staphylococcus* sp. may be associated with the presence of *S. aureus* in milk, which was verified in only two of our samples. The counts were 2.51 ± 0.33 logCFU/mL for milk producer E, on day 1, and 3.46 ± 0.01 logCFU/mL for milk producer C, on day 7. This microorganism can produce staphylococcal toxin and cause food poisoning[26,31], therefore, following-up these producers' herds and establishing rigid control of milk refrigeration is necessary to avoid proliferation of the microorganism and toxin production.

Table 1. Evaluation of total coliforms, thermotolerant and *Staphylococcus* sp. on the first and last days of sheep milk storage under refrigeration.

Producer (day of refrigeration)	Total coliforms (logCFU/mL)	Thermotolerant coliforms (logCFU/mL)	Staphylococcus sp. (logCFU/mL)
A (1)	Higher than 3.04[a]	Lower than 0.48[b]	4.45 ± 0.45[a]
A (7)	Higher than 3.04[a]	Lower than 0.48[b]	4.35 ± 0.07[a]
B (1)	2.20 ± 0.84[ab]	1.30 ± 0.33[ab]	1.81 ± 1.80[a]
B (7)	0.96 ± 0.40[ab]	0.48 ± 0.00[b]	4.26 ± 0.18[a]
C (1)	Higher than 3.04[a]	2.75 ± 0.29[a]	2.66 ± 2.65[a]
C (7)	Higher than 3.04[a]	2.71 ± 0.33[a]	3.90 ± 0.01[a]
D (1)	Higher than 3.04[a]	Lower than 0.48[b]	4.46 ± 0.23[a]
D (7)	2.61 ± 0.43[ab]	Lower than 0.48[b]	4.17 ± 0.26[a]
E (1)	Higher than 3.04[a]	0.72 ± 0.24[b]	3.66 ± 0.18[a]
E (7)	Higher than 3.04[a]	0.52 ± 0.04[b]	2.01 ± 2.00[a]
F (1)	0.96 ± 0.40 [b]	0.52 ± 0.04[b]	n.d.[*a]
F (7)	0.56 ± 0.00[b]	Lower than 0.48[b]	5.19 ± 0.19[a]

Mean values ± standard deviation of the mean (n = 2); Different letters in the same column indicate a statistically significant difference ($p < 0.05$) Define a and b; * n.d. not detected at the lowest dilution 10^{-1}

Figure 2 shows the analysis results of titratable acidity and protein content in refrigerated raw ovine milk. On day 1 (Figure 2A) only milk producers A, B and E showed milk with titratable acidity values within the limits established by Brazilian legislation, which are from 0.14 to 0.18 grams of lactic acid per 100 mL. Over the course of days, there was an increase in the amount of lactic acid in the samples for all milk producers. Milk from producer A kept the acidity lower throughout the period and there was a statistically significant increase between days 1 and 7. Generally, milk producers C, D and F had the worst acidity values from the beginning to the end of the analyses, thus not meeting the values defined by the legislation at any time. According to studies carried out with ovine milk in Serra Gaúcha[32] and with breeds from Europe[33], acidity ranged from 0.22 to 0.25 g lactic acid per 100 mL milk, while in the metropolitan region of Porto Alegre[23] the mean value was 0.15 g lactic acid per 100 mL milk. These previous studies developed in Brazil also used the Lacaune breed. The average value obtained with all samples was 0.19 g lactic acid per 100 mL milk (n = 84), close to that reported in other studies.

The results confirm the tendency of the refrigerated milk to acidify (Figure 2A), which can be caused by microbiological contamination that causes the acidity of the raw material to increase due to the lactic acid production by microorganisms. Compared with a study on the curdled milk yield obtained from fresh, cold and frozen milk of Lacaune sheep, the serum obtained from the milk cooled to 5°C for seven days showed higher acidity levels compared with the others, probably due to possible microbiological contamination during the refrigeration process[34].

Figure 2B shows that in the first two days, all the evaluated milk showed average values above the minimum of 2.9% protein[11]. From this period onward, the values were statistically lower compared to days 1 and 2. In addition, there was a sharp fall on day 3 for milk producers B and C, although an increased count was not observed for psychrotrophic and mesophilic bacteria at these same points, and this decreased protein content may be attributed to a lack of homogenization in the collection of sample or aliquot for analysis. On days 6 and 7, low values that compromise the quality and industrial yield were observed. The mean protein content for all producers on the first refrigeration day was 5.99% (n = 12), remaining this way on the second day and decreasing from the third day. If we consider this mean value, it is higher than that reported in studies on fresh Lacaune ovine milk produced in Brazil, with levels reported from 5.70%[22], 5.52%[23], 5.27%[34], 4.93%[35] to 4.46%[31], but below that observed in European ovine milk, with 6.56% in Italy[36] and 6.20% in different European breeds[32]. However, the mean value after seven days was 3.71% (n = 84), much lower than that obtained in another study with sheep's milk from the same region, kept under refrigeration for seven days, with an average of 5.26% (n = 12)[34].

Protein deterioration and consumption were accentuated in the last two days of analysis, indicating the excessive consumption of this substrate. This may have occurred due to bacteriological contamination, caused by proteolytic microorganisms. Proteolysis can be attributed to psychrotrophic bacteria and *Pseudomonas* is best known for this effect[29]. As noted earlier, high psychrotrophic counts occurred throughout the entire refrigerated storage period (Figure 1B).

Other microorganisms are known to use lactose as a substrate and produce lactic acid, thus acidifying the medium and precipitating nutrients, such as casein[3,12]. In the evaluation of the stability of ovine milk to ethanol, 72% showed that on days 6 and 7 there were lumps in the milk from all producers, from medium to large amounts, indicating that, in addition to protein content, the stability was also compromised. As well as those already mentioned, other proteolytic bacteria that may be involved in this process are *Lactobacilli* and *Enterococci*[28,37]. Although psychrotrophic bacteria have a correlation with proteolytic activity, this was not observed in previous studies with bovine milk[28], and the milk mycobiota itself may be a degrading factor of proteins[37].

Table 2 shows data on pH and water activity in sheep's milk, from the first to the last day of storage. The pH values ranged from 7.04 to 7.35, with a mean value of 7.13 (n = 84). The pH values cited in previous studies were 6.27[22] and 6.53[32] for the Lacaune breed, 6.70 for Corriedale[7] and ranged from 6.51 to 6.85 for European breeds[33]. The increase in pH, above 6.7 and even 7.0, may be associated with changes caused by mastitis, with negative economic impact and changes in the physicochemical composition of milk[38]. Changes in the acidity and pH values of ovine milk may affect the coagulation process and the mass water retention capacity, influencing the desorption process, the size of the clot granules, the texture and the microbial growth in the cheese[33].

Milk water activity remained high throughout the period, with an average value of 0.9822 (n = 84), and small variations among the milk producers were observed, but none were statistically significant. A high concentration of milk moisture decreases its yield by lowering solids content and increasing water activity, favoring contamination and degradation reactions[32].

Cryoscopic index and fat content were obtained from dairy control data, with the exception of milk producer E. The following values were observed for each other producer: -0.578°H (A); -0.565°H (B); -0.563°H (C); -0.564°H (D); and -0.569°H (F). These results are above the limits stipulated by the legislation, which determines values from -0.530°H[1] to -0.550°H[11]. High values of the order of -0.55°C and -0.59°C were observed in Lacaune sheep milk samples in Cascavel in the state of Paraná[39]. The expected freezing point for sheep milk is around -0.535°H, values below this may be evidence of fraud by adding water to milk[5].

Fat contents per milk producer were 7.30% (A); 6.26% (B); 6.81% (C); 9.42% (D); and 6.58% (F), resulting in a mean value of 7.27 ± 0.87% among all producers. The values obtained in samples of Lacaune sheep's milk in other studies were lower, 6.47%[23], 6.70%[21] and 6.84%[38]. Fat content is directly related to cheese yield and texture, and milk with higher fat concentrations is indicated for the production of soft cheese[39]. This high fat value observed in the milk samples represents a gain in the production chain, since

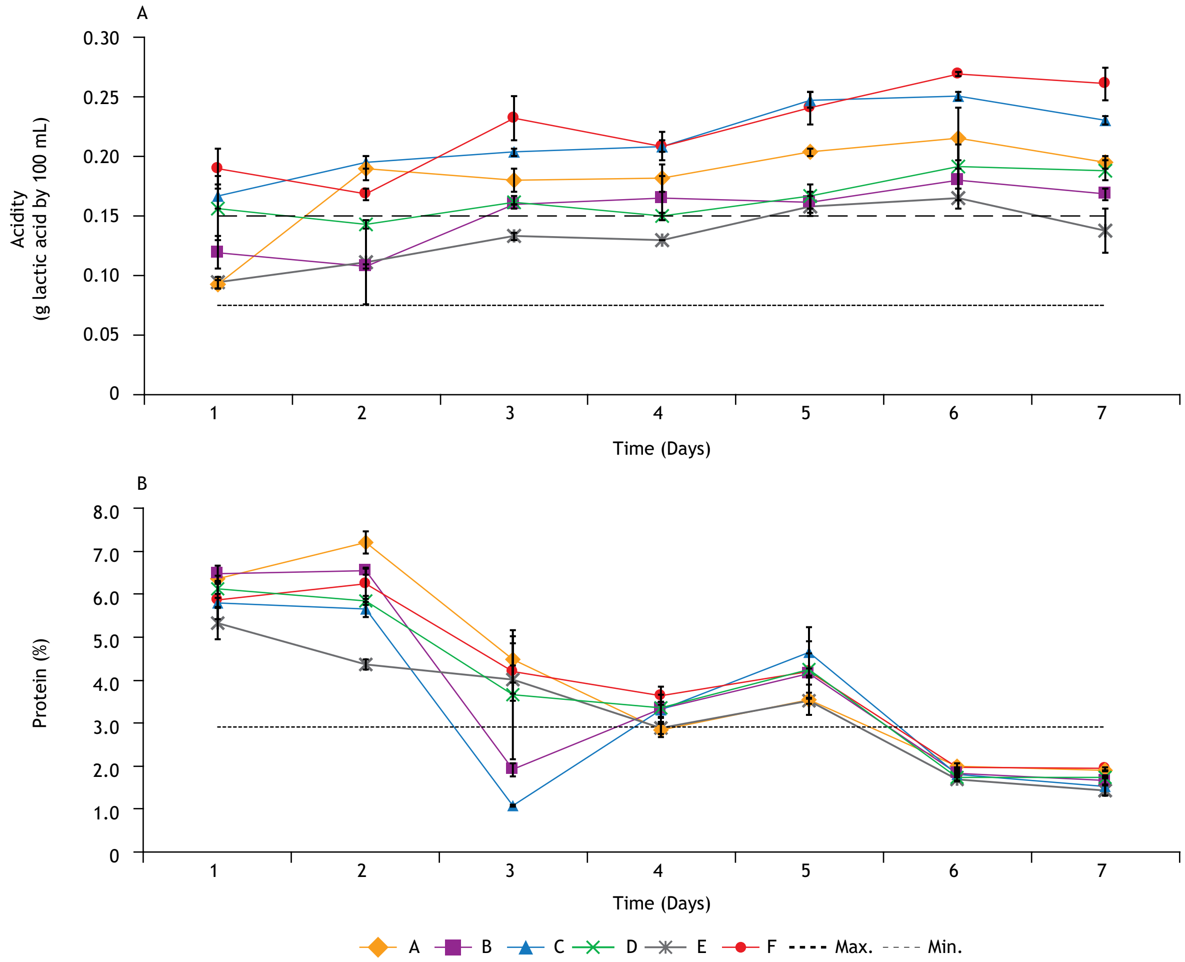

Figure 2. Mean values of (A) titratable acidity and (B) protein in raw ovine milk kept under refrigeration. The colored lines indicate the averages obtained by the producer and the bars are the standard error of the mean; the dotted line indicates the minimum value and the dashed line indicates the maximum value, defined in legislation[11].

the producer receives greater remuneration for the milk and the industry is able to produce more dairy, including taking advantage of the surplus after the milk has been skimmed.

FINAL REMARKS

Most of the samples showed SPC above the maximum allowed by current legislation, as well as high counts for psychrotrophic, total coliforms and *Staphylococcus* sp., which highlights problems related to herd health, sanitary milking conditions and refrigerated storage. There was no producer with better results for all parameters evaluated, with large variations between them occurring during the refrigeration days. However, milk producer B had a larger number of samples below the limit established for SPC, smaller counts for psychrotrophic and total coliforms, and lower acidity at the end of the period. In addition, acidity increased and protein decreased during refrigeration days, demonstrating that this storage cannot be prolonged. If there is a need for storage in seasonal periods, the industry should use refrigerated ovine milk within two days of milking. Thus, economic losses and industrial yield would be avoided and higher quality and safety to dairy provided.

Table 2. Evaluation of pH and water activity of milk from different producers kept under refrigeration.

Producer (day of refrigeration)	pH	Water activity
A (1)	7.15 ± 0.02[de]	0.9916 ± 0.0023[a]
A (7)	7.15 ± 0.02[de]	0.9900 ± 0.0034[a]
B (1)	7.22 ± 0.01[bcd]	0.9900 ± 0.0002[a]
B (7)	7.30 ± 0.01[ab]	0.9900 ± 0.0004[a]
C (1)	7.13 ± 0.01[def]	0.9902 ± 0.0006[a]
C (7)	7.26 ± 0.01[abc]	0.9873 ± 0.0010[a]
D (1)	7.10 ± 0.02[ef]	0.9904 ± 0.0014[a]
D (7)	7.35 ± 0.04[a]	0.9866 ± 0.0018[a]
E (1)	7.26 ± 0.01[abc]	0.9897 ± 0.0011[a]
E (7)	7.28 ± 0.01[abc]	0.9930 ± 0.0061[a]
F (1)	7.04 ± 0.01[f]	0.9887 ± 0.0018[a]
F (7)	7.19 ± 0.01[cde]	0.9857 ± 0.0004[a]

Mean values ± standard deviation of the mean (n = 2); Different letters in the same column indicate statistically significant difference ($p < 0.05$) Define a,b,c,d,e,f

REFERENCES

1. Food and Agriculture Organization. FAOSTAT. 2016[acesso 8 jun 2016]. Disponível em: http://faostat3.fao.org/browse/Q/QA/E
2. Langoni H, Penachio DDS, Citadella JCC, Laurino F, Faccioli-Martins PY, Luncheis SB et al. Aspectos microbiológicos e de qualidade do leite bovino. Pesq Vet Bras. 2011;31(12):1059-65. https://doi.org/10.1590/S0100-736X2011001200004
3. Rohenkohl JE, Corrêa GF, Azambuja DFD, Ferreira FR. O agronegócio de leite de ovinos e caprinos. Ind Econ. 2011;39(2):97-114.
4. Callefe JLR, Langoni H. Qualidade do leite: uma meta a ser atingida. Vet Zootec. 2015;22(2):151-61.
5. Gomes SF. Qualidade do leite cru de ovinos da área geográfica de produção do Queijo Terrincho DOP (Trás-os-Montes): dos fatores de produção animal à qualidade do queijo [dissertação]. Bragança: Instituto Politécnico de Bragança; 2012.
6. Rodrigues ARF. Otimização do processo de fabrico de um queijo de ovelha amanteigado [dissertação]. Porto: Faculdade de Ciências; 2014.
7. Souza ACKOD, Osório MTM, Osório JCDS, Oliveira NMD, Vaz CMS, Souza M et al. Produção, composição química e características físicas do leite de ovinos da raça Corriedale. Rev Bras Agrociência. 2005;11(1):73-7. https://doi.org/10.18539/CAST.V11I1.1173
8. Rossi EM, Zilli D, Scapin D, Roza-Gome MF, Gelinski JMLN. Avaliação da qualidade microbiológica de queijos Minas Frescal comercializados em supermercados da região extremo-oeste de Santa Catarina, Brasil. Evidência. 2010;10(1-2):105-14.
9. El-Jakee JK, Aref NE, Gomaa A, El-Hariri M, Galal HM, Sherif AO et al. Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: an environmental hazard. Int J Vet Sci Med. 2013;1(2):74-8. https://doi.org/10.1016/j.ijvsm.2013.05.006
10. Mallet A. Quantificação e identificação de *Escherichia coli, Pseudomonas aeruginosa e Aeromonas hydrophila* em água de propriedades leiteiras [dissertação]. Lavras: Universidade Federal de Lavras; 2007.
11. Ministério da Agricultura, Pecuária e Abastecimento (BR). Instrução Normativa nº 62, de 29 de dezembro de 2011. Aprova o regulamento técnico de produção, identidade e qualidade do leite tipo a, o regulamento técnico de identidade e qualidade de leite cru refrigerado, o regulamento técnico de identidade e qualidade de leite pasteurizado e o regulamento técnico da coleta de leite cru refrigerado e seu transporte a granel. Diário Oficial União. 29 dez 2011.
12. Chaves ACSD. Leite. In: Koblitz MGB. Matérias primas alimentícias: composição e controle de qualidade. Rio de Janeiro: Guanabara Koogan; 2011. p. 148-84.
13. Ministério da Agricultura, Pecuária e Abastecimento (BR). Instrução Normativa nº 68, 12 de dezembro de 2006. Oficializar os métodos analíticos oficiais físico-químicos, para controle de leite e produtos lácteos. Diário Oficial União. 14 dez 2006.
14. AquaLab. Aqualab series 4TE: atividade de água por ponto de orvalho.São José dos Camps: AquaLab; 2016[acesso 19 jul 2016]. Disponível em: http://aqualab.decagon.com.br/produtos/analisadores-de-atividade-de-agua/aqualab-series-4te-atividade-de-agua-por-ponto-de-orvalho/
15. Instituto Adolfo Lutz. Normas analíticas do Instituto Adolfo Lutz: Métodos químicos e físicos para análise de alimentos. São Paulo: IMESP; 2006.
16. International Dairy Federation. ISO 5764:2002. Milk - Determination of freezing point - Thermistor cryoscope method. Brussels: IDF, 2002.
17. Silva DN, Junqueira VCA, Silveira NFDA, Taniwaki MH, Santos RFSD, Gomes RAR. Manual de métodos de análise microbiológica de alimentos e água. São Paulo: Varela; 2007.
18. Silva FAZ, Azevedo CAV. Principal components analysis in the software assistat-statistical attendance. Proceedings of the 7th World Congress on Computers in Agriculture; 2009 Jun 22-24; Reno, Nevada. St. Joseph: American Society of Agricultural and Biological Engieers; 2009.
19. Ministério da Agricultura e do Abastecimento. Instrução Normativa nº 37, de 31 de outubro de 2000. Aprovar o regulamento técnico de identidade e qualidade de leite de cabra. Diário Oficial União. 31 out 2000.
20. Gonzaga N, Daniel GC, Marezel J, Rodrigues L, Marioto M, Tamanini R et al. Evolução da qualidade microbiológica e físico-química do leite pasteurizado. Semina: Ciên Biol Saúde. 2015;36(1):47-54. https://doi.org/10.5433/1679-0367.2015v36n1p47
21. Nespolo CR, Brandelli A. Characterization of cheeses produced with ovine and caprine milk and microbiological evaluation of processing areas in the dairy plant in Brazil. Int Food Res J. 2012;19(4):1713-21.
22. Nespolo CR, Taffarel LAS, Brandelli A. Parâmetros microbiológicos e físico-químicos durante a produção e maturação do queijo Fascal. Acta Scientiae Veterinariae. 2009;37(4):323-8.
23. Garnica MLD, Santos JA, Gonzalo AC. Influence of storage and preservation on microbiological quality of silo ovine milk. J Dairy Sci. 2011;94(4):1922-7. https://doi.org/10.3168/jds.2010-3787.
24. European Union. Council Directive 92/46/ECC of 16 june 1992. Laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. Diario Official Comunidades Europeas. 16 jun 1992.
25. Jay JM. Microbiologia dos alimentos. Porto Alegre: Artmed; 2005.
26. Dürr JW. Como produzir leite de qualidade. Brasília: Senar; 2012.
27. Nörnberg MDFBL, Tondo EC, Brandelli A. Bactérias psicrotróficas e atividade proteolítica no leite cru refrigerado. Acta Scientiae Veterinariae. 2009;37(2):157-63.
28. Raynal-Ljutovac K, Pirisi A, Crémoux R, Gonzalo C. Somatic cells of goat and sheep milk: Analytical, sanitary, productive and technological aspects. Small Rumin Res. 2007;68(1-2):126-44. https://doi.org/10.1016/j.smallrumres.2006.09.012

29. Salina A, Machado GP, Guimarães FDF, Langoni H. Sensibilidade microbiana de *Staphylococcus* spp. Isolados de leite de cabras com mastite subclínica. Vet Zootec. 2015;22(2):288-94.

30. Brandão MLL, Rosas CDO, Bricio SML, Costa JDCBD, Medeiros VDM, Warnken MB. Produção de materiais de referência para avaliação de métodos microbiológicos em alimentos: estafilococos coagulase positiva e Listeria spp. em leite em pó. Analytica. 2013;63(3):60-70.

31. Brito MA, Gonzalez FD, Ribeiro LA, Campos R, Lacerda L, Barbosa PR, et al. Composição do sangue e do leite em ovinos leiteiros do sul do Brasil: variações na gestação e na lactação. Ciênc Rural. 2006;36(3):942-8. https://doi.org/10.1590/S0103-84782006000300033

32. Park YW, Juárez M, Ramos M, Haenlein GFW. Physico-chemical characteristics of goat and sheep milk. Small Rum Res. 2007;68(1-2):88-113. https://doi.org/10.1016/j.smallrumres.2006.09.013

33. Fava LW, Külkamp-Guerreiro IC, Pinto AT. Rendimento de coalhada obtida a partir de leite fresco, refrigerado e congelado de ovelha da raça Lacaune e característica física do soro obtido. Ciênc Rural. 2014;44(5):937-42. https://doi.org/10.1590/S0103-84782014000500028

34. Ticiani E, Sandri, EC, Souza JD, Batistel F, Oliveira DED. Persistência da lactação e composição do leite em ovelhas leiteiras das raças Lacaune e East Friesian. Ciênc Rural. 2013;43(9):1650-3. doi:10.1590/S0103-84782013000900018

35. Cosentino C, Paolino R, Freschi P, Calusso AM. Jenny milk as an inhibitor of late blowing in cheese: a preliminary report. J Dairy Sci. 2013;96(6):3547-50. https://doi.org/10.3168/jds.2012-6225

36. Ramos CL. Caracterização de *Lactobacillus* spp. e desenvolvimento de um sistema de simulação de sobrevivência bacteriana no trato gastrointestinal [tese]. Lavras: Universidade Federal de Lavras; 2013.

37. Pinheiro KG. Características físico-químicas do leite caprino na época seca e chuvosa na microrregião de Mossoró-RN [dissertação]. Mossoró: Universidade Federal Rural do Semiárido; 2012.

38. Malho EL. Análise físico química comparativa entre leite de ovelha Lacaune e leite de vaca Jersey no oeste do Paraná [monografia]. Curitiba: Universidade Tuiuti do Paraná; 2012.

39. Morand-Fehr P, Fedele V, Decandia M, Frileux Y. Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Rum Res. 2007;68 (1-2) 20-34. https://doi.org/10.1016/j.smallrumres.2006.09.019

Conflict of Interest

The authors report no conflict of interest with peers and institutions, political or financial.