Research, Society and Development, v. 9, n. 12, e27691210796, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i12.10796

Influence of storage temperature on the population of microorganisms in raw sheep milk and its physical-chemical profile

Influência da temperatura de armazenamento na população de micro-organismos do leite cru ovino e seu perfil físico-químico

Influencia de la temperatura de almacenamiento en la población de microorganismos de la leche cruda de oveja y su perfil físico-químico

Received: 12/01/2020 | Reviewed: 12/09/2020 | Accept: 12/18/2020 | Published: 12/24/2020

Samera Rafaela Bruzaroski
ORCID: https://orcid.org/0000-0001-5574-331X
Universidade Pitágoras Unopar, Brazil
E-mail: samera.rafaela@hotmail.com
Raquel Pinheiro de Souza
ORCID: https://orcid.org/0000-0003-1130-5646
Universidade Pitágoras Unopar, Brazil
E-mail: raquelpinheiro.vet@gmail.com
Pamela da Silva Pasquim
ORCID: https://orcid.org/0000-0003-0587-8173
Universidade Pitágoras Unopar, Brazil
E-mail: pam.machado2011@gmail.com
Rafael Fagnani
ORCID: https://orcid.org/0000-0002-7392-2087
Universidade Pitágoras Unopar, Brazil
E-mail: rafael.fagnani@unopar.br
Elsa Helena Walter de Santana
ORCID: https://orcid.org/0000-0002-7789-2575
Universidade Pitágoras Unopar, Brazil
E-mail: elsa.santana@unopar.br

Resumo

O objetivo do presente estudo foi avaliar o perfil físico-químico do leite cru ovino e a influência da temperatura de estocagem na população de micro-organismos. Amostras de leite de ovelha foram coletadas do leite de conjunto (500 mL/amostra) e avaliado a composição físico-química: gordura, proteína, lactose, teor de cinzas, matéria seca total (TDM), crioscopia (° H), acidez titulável e contagem de células somáticas (SCC). Posteriormente as amostras foram armazenadas a 4°C e 9°C por 72 horas e determinou-se a acidez titulável, micro-organismos deteriorantes e indicadores de qualidade. Petrifilm™ (3M do Brasil Ltda) foram utilizados para a contagem de aeróbios mesófilos (37°C/48horas), coliformes totais (37°C/24horas), *Escherichia coli* (37°C/48horas) e enterobactérias (37°C/24horas). Para psicrotróficos foi utilizado *Plate Count Ágar* (7º C/10 dias), para *Pseudomonas* spp. *Pseudomonas Ágar Base* com suplemento CFC (25ºC/48 horas). Os valores médios obtidos foram de 6,28% de gordura, 83,46% de umidade, 10,24% ESD, 0,92% de cinzas, 5,30% de proteína 4,43% de lactose, índice crioscópio de -0,575°H e CCS de $3{,}38 \times 10^6$ células/mL. Valores de acidez do leite foram maiores (0.24 g de ácido láctico/100 ml) com 72 horas. Quanto maior a temperatura e/ou o tempo de armazenamento, maiores as contagens de psicrotróficos, *Pseudomonas* spp., e enterobactérias, para aeróbios mesófilos apenas o tempo de armazenamento. A refrigeração a 4ºC e a redução do tempo de estocagem são importantes para o controle da multiplicação de micro-organismos que comprometem a qualidade, tempo de vida de prateleira e a segurança alimentar do leite ovino e seus derivados.

Palavras-chave: Qualidade; Derivados lácteos; Composição; Refrigeração; Vida de prateleira.

Abstract

The aim of the present study was to evaluate the physical-chemical profile of raw sheep milk and the influence of storage temperature on the population of microorganisms. Sheep milk samples were collected from the milk cans (500 mL/sample) and were evaluated the physical-chemical composition: fat, protein, lactose, ash content, total dry matter (TDM), cryoscopy index (°H), titratable acidity, and somatic cell count (SCC). After the samples were stored at 4°C and 9°C (72 h) and the titratable acidity, the population of the deteriorating microorganisms and quality indicators were determined. *Petrifilm*™ (3M do Brasil Ltda) were used for the mesophilic aerobic count (37°C/48 h), total coliforms (37°C/24 h), *Escherichia coli* (37°C/48 h), and enterobacteria (37°C/24 h). For psychrotrophic bacteria, was used *Plate Count Agar* (7ºC/10 days) and for *Pseudomonas* spp., CFC-supplemented Pseudomonas agar base (25ºC/48 h). Average values for raw milk were 6.28% fat, 83.46% moisture, 16.52% TDM, 0.92% ash, 5.30% protein, 4.43% lactose, a cryoscopy index of -0.575°H, and SCC of 3.38×10^6 cells/mL. Milk acidity values were higher (0.24 g lactic acid/100 ml) with 72 hours of storage. The higher the temperature and/or storage time, the higher the counts of psychrotrophic, *Pseudomonas* spp., and enterobacteria, for mesophilic aerobic only the storage time. Refrigeration at 4ºC and a reduction of storage time are important to control the microorganism's population that compromise the quality, shelf life, and food safety of sheep milk and dairy products.

Keywords: Quality; Dairy products; Composition; Refrigeration; Shelf life.

Resumen

El objetivo del presente estudio fue evaluar el perfil físico-químico de la leche cruda de oveja y la influencia de la temperatura de almacenamiento en la población de microorganismos. Se recolectaron muestras de leche de oveja de la leche entera (500 mL/muestra) y se evaluó la composición físico-química: grasa, proteína, lactosa, contenido de cenizas, materia seca total (TDM), crioscopia (° H), acidez titulable y recuento de células somáticas (SCC). Posteriormente, las muestras se almacenaron a 4 ° C y 9 ° C durante 72 horas y se determinó la acidez titulable, los microorganismos deteriorantes e indicadores de calidad. Se utilizó PetrifilmTM (3M do Brasil Ltda) para contar aerobios mesófilos (37 ° C / 48 horas), coliformes totales (37 ° C / 24 horas), Escherichia coli (37 ° C / 48 horas) y enterobacterias (37 ° C / 24 horas). Para pacientes psicrotróficos se utilizó Plate Count Agar (7º C / 10 días), para Pseudomonas spp. Pseudomonas Agar Base con suplemento CFC (25ºC / 48 horas). Los valores medios obtenidos fueron 6.28% grasa, 83.46% humedad, 10.24% ESD, 0.92% ceniza, 5.30% proteína 4.43% lactosa, índice de crioscopio de - 0.575 ° H y CCS de 3.38x106 células / mL. Los valores de acidez de la leche fueron mayores (0,24 g de ácido láctico / 100 ml) a las 72 horas. Cuanto mayor sea la temperatura y / o el tiempo de almacenamiento, mayor será el recuento de psicotrópicos, Pseudomonas spp. Y enterobacterias, para los aerobios mesófilos solo el tiempo de almacenamiento. La refrigeración a 4ºC y la reducción del tiempo de almacenamiento son importantes para controlar la multiplicación de microorganismos que comprometen la calidad, vida útil y seguridad alimentaria de la leche de oveja y sus derivados.

Palabras clave: Calidad; Lácteos; Composición; Refrigeración; Vida útil.

1. Introduction

Sheep milk is a functionally active dairy food composed of fatty acids, immunoglobulins, and non-immune proteins (Mohapatraa *et al.*, 2019). It is primarily used to produce dairy products, such as cheeses and yogurts, due to its high content of protein, fat, and calcium per casein unit, thus, forming an excellent matrix to produce these products (Barłowska *et al*., 2011; Mohapatraa *et al*., 2019). The physicochemical and biochemical properties of sheep milk include prebiotics and probiotics, which renders this product a suitable functional food for human health promotion and disease risk reduction (Mohapatraa *et al*., 2019).

The main deteriorating microorganisms in milk are mesophilic and psychrotrophic bacteria. Refrigeration of raw milk limits the proliferation of saccharolytic mesophiles, which are responsible for acidification and thermal instability through hydrolysis of lactose and the production of lactic acid (Mcauley *et al*., 2016). On the other hand, psychrotrophic microorganisms synthesize thermo-resistant proteolytic and lipolytic enzymes that compromise the quality of milk and its derivatives by reducing the shelf-life, turning the taste bitter and rancid, and decreasing cheese yield (Molineri *et al*., 2012). Among psychrotrophic microorganisms, the most common genus found in refrigerated raw milk are *Pseudomonas* spp., which rapidly increase and result in intense lipolytic and proteolytic activity. Few of the other contaminants, such as coliforms, *Escherichia coli*, and enterobacteria, are indicators of food hygiene quality, and their presence can compromise the shelf-life and food safety of milk and dairy products (Franco & Landgraf, 2008).

There are no parameters legally acknowledged in Brazil for sheep milk production, and there exists limited literature regarding the quality of raw milk in the country. It is important to the sheep milk market to have more information about physical-chemical and microbiological parameters, that are essential to determine the quality of milk and dairy products. Therefore, the aim of this study was to evaluate the physical-chemical profile and the influence of storage temperature on the population of deteriorating microorganisms and quality indicators in raw sheep milk.

2. Materials and Methods

Sheep milk samples were obtained from farms (3) in Southern Brazil, properties selected for the possibility of collecting and carrying out the analyzes on the same day, due to their locations. The properties perform manual milking, using a 750 ppm chlorinated solution for pre-dipping and drying the teat with a cloth.

A total of 22 sheep (Lacaune), ten fed on pasture with daily production of 4 L, and twelve confined animals (feed, corn, orange and soybean meal) with daily production of 12 L.

The samples were collected from the milk cans (500 mL/sample (Silva *et al*., 1997)), as a representative sampling from the milk production. Using a sterile stainless steel shell and sterile plastic bag, transported with a cooler box (7°C) to the microbiology laboratory.

The samples, 2 h after milk collection, had the physical chemical composition and the population of the deteriorating microorganisms and quality indicators determined. Soon after, the samples were divided into two groups and kept for 72 h under two temperatures: 4°C, which is the temperature used in bulk tank milk in the farm, and 9°C, simulating the maximum temperature allowed for milk reception in the processing industry. The titratable acidity and microbiological analysis were determinate at 24, 48, and 72 h in both storage temperatures.

In the absence of specific Brazilian legislation for sheep's milk, in this work, the parameters of storage of cow's milk were used. The time of storage of milk (72 h) in this experiment considered that, unlike cow's milk, the production of sheep milk volume is lower and thus it is more likely to be stored for longer before processing.

2.1 Physical-chemical composition

Fat content (%), protein (%), ash (%), total dry matter (TDM) (%), cryoscopy index (°H) and titratable acidity (g of lactic acid/100 mL) (AOAC, 2005) were determined in triplicates. Bentley 2000 (Bentley Instruments Inc., Chaska, MN, USA) was used for lactose (%) analysis, and Somacount 500 electronic counter (Bentley Instruments Inc.) was used for somatic cell count (SCC) (cells/mL) flow cytometry. The results were compared with the literature research's about sheep milk physical-chemical composition conducted between 2015 and 2020.

2.2 Microbiological analysis

The population of the deteriorating microorganisms and quality indicators were determined, and the results obtained were presented in a $\log_{10}$ CFU/mL. *Petrifilm*™ (3M do Brasil Ltda) were used for mesophilic aerobic (37°C/48 h), enterobacteria (37°C/24 h), total coliforms (37°C/24 h) and *E. coli* (37°C/48 h), following the manufacturer's recommendations. The Plate Count Agar (Himedia, Mumbai, India) was used to identify psychrotrophic microorganisms, incubating at 7°C/10 days (Frank & Yousef, 2004). For *Pseudomonas* spp., CFC-supplemented (cefaloridine, fusidic acid, cetrimide) Pseudomonas agar base (Himedia, Mumbai, India) was used at 25 °C for 48 h. (ISO, 2009).

2.3 Statistical analysis

To assess the influence of storage time and temperature on the microorganisms counts and titratable acidity, the results were evaluated by multiple regression analysis in a response surface design in the function of logarithmic counts for each group of microorganisms and titratable acidity. Two factors were included for all variables; time, with four levels (2, 24, 48, and 72 h) and temperature, with two levels (4°C and 9°C). The experimental design was performed with 48 runs for psychrotrophic, *Pseudomonas* spp., and titratable acidity; 22 runs for mesophiles, coliforms, and E. coli; and 15 runs for enterobacteria. The effect of each factor was evaluated by analysis of variance with 5% of significance, as assessed through the software Statistica 13.0 (Statsoft). In parallel, the influence of storage time and temperature on titratable acidity was also assessed respectively by Wilcoxon and Mann-Whitney tests.

3. Results and Discussion

In this study, the values of ash (0.92%), lactose (4.43%), cryoscopy index (0.575°H) and titratable acidity (0.23 g of lactic acid/100 mL) (Table 1) were similar of the means values described in others studies about sheep milk. (Merlin Junior *et al*., 2015; Rafiq *et al*., 2016; Balthazar *et al*., 2017; Ferro *et al*., 2017; Panayotov *et al*., 2018; Sobrino *et al*., 2018). In addition, the values of fat (6.28%), protein (5.30%) and TDM (16.52%) were lower (Table 1). The composition of sheep milk can vary according to breed, season, feeding, management, environmental conditions, stage of lactation, and udder health status (Mohapatraa *et al*., 2019).

Table 1. Average values of the physical-chemical profile of the raw sheep milk.

Physical-chemical analysis	Mean results*	Literature means results **
Fat (%)	6.28	7
Protein (%)	5.30	5.5
Lactose (%)	4.43	4.46
Ash (%)	0.92	0.92
Total dry matter (%)	16.52	18
Cryoscopy (°H)	-0.575	-0.570
Titratable acidity (g of lactic acid/100 mL)	0.23	0.23

* analysis in triplicate /sample** Source: (2015-2020) Merlin Junior *et al*., (2015); Rafiq *et al*., (2016); Balthazar *et al*., (2017); Ferro *et al*., (2017); Panayotov *et al*., (2018); Sobrino *et al*., (2018).

Regarding titratable acidity, no difference in values was observed ($P > 0.05$) between 4°C and 9°C (Table 2). However, the storage time influenced the titratable acidity ($P \leq 0.05$), with high values (0.24 g of lactic acid/100 mL) at 72 h in both storage temperatures. Therefore, both temperatures were adequate to control milk acidity, although a reduction in storage time must be considered. The increased acidity is associated with the mesophilic microorganisms and the production of lactic acid (Mcauley *et al*., 2016), which compromises the thermal stability of milk.

Table 2. Average values of titratable acidity (g of lactic acid/100 mL) of refrigerated raw sheep milk (4°C and 9°C) for 72 hours.

	Titratable Acidity (g of lactic acid/100 mL)*	
Time (h)/Temperature	4°C $\overline{X} \pm \sigma$	9°C $\overline{X} \pm \sigma$
2	0.23 ± 0.042^{aA}	0.23 ± 0.042^{aA}
24	0.23 ± 0.042^{aA}	0.22 ± 0.048^{aA}
48	0.23 ± 0.042^{aA}	0.22 ± 0.049^{aA}
72	0.24 ± 0.041^{bA}	0.24 ± 0.040^{bA}

* Means from triplicate analysis/sample; means followed by the same lower-case letters in the same column did not differ according to the Mann-Whitney test ($P \leq 0,05$). Means followed by equal capital letters on the same line did not differ according to the Wilcoxon test ($P \leq 0.05$). Source: Authors.

The average score of SCC in this study was 3.38×10^6 cells/mL. Sheep milk contains baseline levels of SCC that are higher compared to that in milk collected from healthy cows, with values that range from 200,000 to 1,000,000 cells/mL (Albenzio *et al*., 2019). In the USA, the SCC standard follows the Pasteurized Milk Ordinance of the Food and Drug Administration of 2007, which establishes a legal threshold of 1,000,000 cells/mL of milk.

As for the multiplication of microorganisms along with storage, only psychrotrophic bacteria, *Pseudomonas* spp., and enterobacteria were affected by the storage time ($P \leq 0.01$) and temperature ($P < 0.05$) (Fig. 1) (Table 3). These two variables have a linear and positive relationship on the multiplication of these groups, that is, the higher the temperature and/or storage time, the higher the counts, which directly affects the quality of milk and dairy products. Although there was an interaction effect between time and temperature, the two variables alone were enough to influence the counts of these three groups of microorganisms.

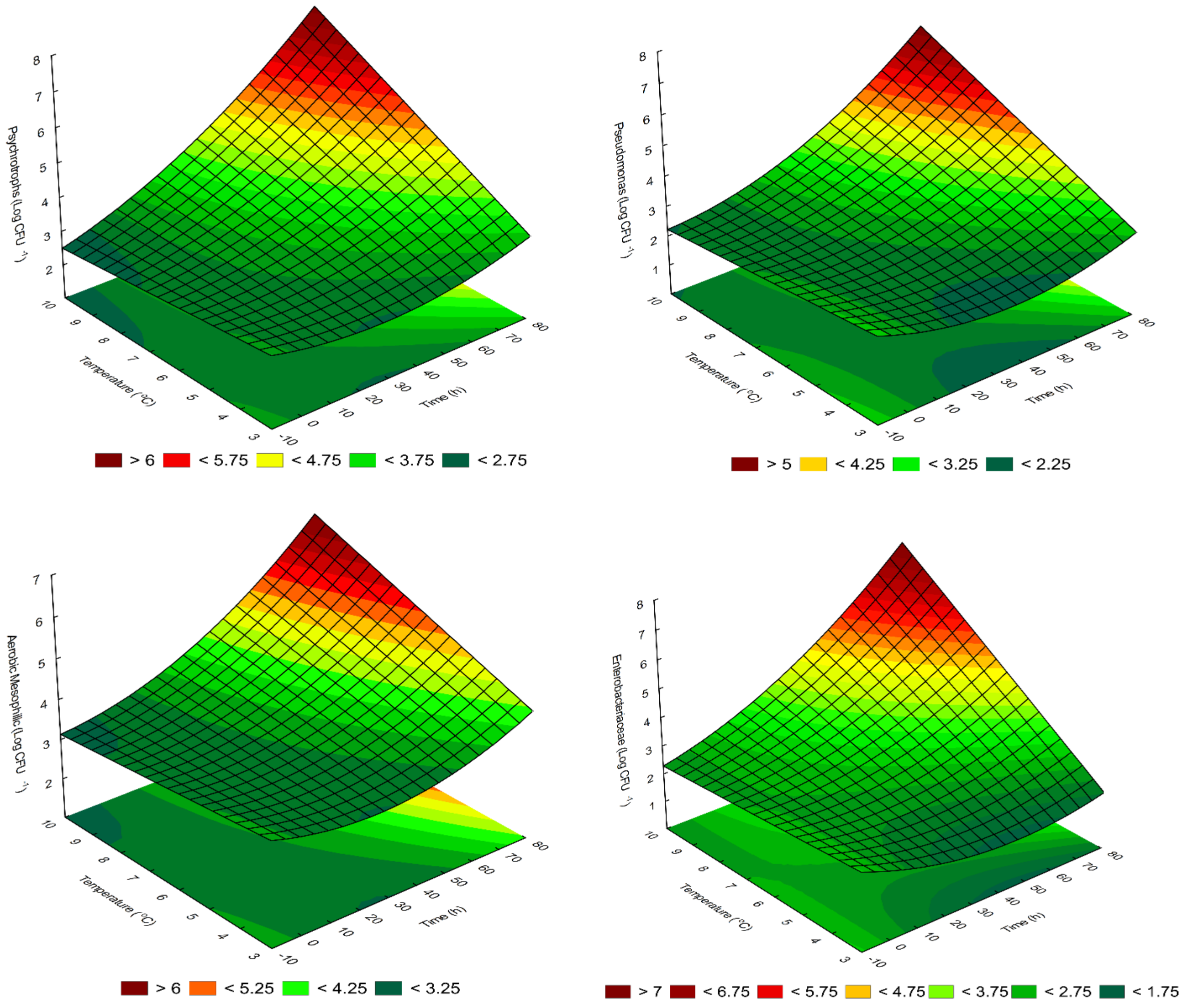
Table 3. Average count ($\log_{10}$ CFU/mL) of mesophile, psychrotrophic, *Pseudomonas* spp., total coliforms, *Escherichia coli*, and enterobacteria in raw sheep milk storage at 4°C and 9°C for 72 hours.

Storage time	2h	24h		48h		72h	
Microorganisms **/ T (°C)	*	4°C	9°C	4°C	9°C	4°C	9°C
Mesophile	4.05	4.1	4.05	4.09	4.61	4.12	6.33
Psychrotrophic	2.94	3.25	3.87	3.57	4.39	3.98	6.38
Pseudomonas spp.*	3.63	3.14	3.73	3.83	4.25	3.91	6.56
Total coliform*	2.62	2.49	3.01	1.82	4.59	3.75	5.22
*E. coli**	0.56	0.56	0.52	0	0	0	0
Enterobacteria	2.79	2.09	3.13	1.95	4.85	2.39	5.79

* sample maintained in cooler box (7°C) **$\log_{10}$ CFU/mL. Source: Authors.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i12.10796

Figure 1. Psychrotrophic, *Pseudomonas* spp., mesophilic aerobes, and enterobacteria count ($\log_{10}$ CFU/mL) in function of time (h) and storage temperature (°C) of raw sheep milk.



Source: Authors.

For enterobacteria and psychrotrophic bacteria, a strong relationship was observed with both temperature and storage time, resulting in regression coefficients (R^2) of 0.89 and 0.73, respectively. The *Enterobacteriaceae* family comprises the genera *Escherichia*, *Edwardsiella*, *Citrobacter*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, and *Yersinia*. Although these microorganisms are sensitive to heat treatment, this group is important as it includes environmental pathogens that produce enterotoxins, such as *E. coli* and *Salmonella* (Franco & Landgraf, 2008). Psychrotrophic bacteria predominantly have lipoproteolytic metabolism, which synthesis thermoresistant extracellular enzymes that influence the shelf-life and quality of milk and dairy products. Therefore, the reduction in

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i12.10796

temperature and storage time impacts milk quality, while increasing the shelf-life and food safety of dairy products. Sheep milk is mainly used to produce dairy products, such as fine cheeses and yogurts, so the presence of a deteriorating microbiota can compromise the industrial yield and sensory characteristics of these products.

For *Pseudomonas* multiplication, the intensity of the relationship with time and temperature was considered weak (R^2 = 0.31) but significant (P = 0.04), accounting for up to 31% of the variations in the counts.

For the multiplication of mesophilic aerobes, the experimental design detected a positive linear effect only for the storage time (P = 0.02 and P = 0.04). Therefore, the longer the storage time, the higher the mesophilic aerobic counts (Fig.1), regardless of the storage temperatures tested. The intensity of this relationship was considered weak (R2= 0.41), although significant for both variables thus influencing titratable acidity only with 72 hours (Table 2). In our study, it was also observed that in the samples with 72 hours of storage at 9ºC the psychrotrophic population was higher than that of the mesophilic (Table 3). Many species considered to be strictly mesophilic were included among the psychrotrophic bacteria and the high counts of these microorganisms are associated with drawbacks in good milking practices (Santana *et al*., 2001).

4. Conclusions

The values of fat, protein and total dry matter of the analyzed samples were below those mean values described in the literature. Refrigeration at 4ºC and reduction in storage time control only the multiplication of psychrotrophic, *Pseudomonas* spp., and enterobacteria of sheep milk. However, concerning mesophilic aerobes, only a reduction in storage time contributed to microbial multiplication.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES).

References

Albenzio, M., Figliola, L., Caroprese, M., Marino, R., Sevi, A., & Santillo, A. (2019). Somatic cell count in sheep milk. *Small Ruminant Research*, 176, 24-30. https://doi.org/10.1016/j.smallrumres.2019.05.013

AOAC - Association of Official Analytical Chemists (2005). Official Methods of Analysis of AOAC International, (18th ed.), Gaithersburg: MD.

Balthazar, C. F., Pimentel, T. C., Ferrão, L. L., Almada, C. N., Santillo, A., Albenzio, M., Mollakhalili, N., Nascimento, J. S., Silva, M. C., Freiras, M. Q., Santana, A. S., Granato, D., & Cruz, A. G. (2017). Sheep milk: Physicochemical characteristics and relevance for functional food development. *Comprehensive Reviews in Food Science and Food*, 16 (2), 247-262. https://doi.org/10.1111/1541-4337.12250

Barłowska, J., Szwajkowska, M., Litwinczuk, Z., & Krol, J. (2011). Nutritional value and technological suitability of milk from various animal species used for dairy production. *Comprehensive Reviews in Food Science and Food Safety*, 10, 291-302. https://doi.org/10.1111/j.1541-4337.2011.00163.x

Ferro, M. M., Tedeschi, L. O., & Atzori, A. S. (2017). The comparison of the lactation and milk yield and composition of selected breeds of sheep and goats. *Translational Animal Science*, 1 (4), 498–506. https://doi.org/10.2527/tas2017.0056

Frank, J. F., & Yousef, A. E. (2004). *Tests for groups of microorganisms*. In: Wehr, M. W., Frank, J. F. *Standard methods for the examination of dairy products*. New York: APHA, 227-247.

Franco, B. D. G. M., & Landgraf, M. (2008). *Microbiologia dos Alimentos* (2nd ed.). São Paulo: Atheneu.

ISO - International Organization for Standardization (2009). Milk and milk products: Method for the enumeration of Pseudomonas spp., norm 11059. Geneva, Switzerland (IDF/RM 225).

McAuley, C. M., Singh, T. K., Haro-Maza, J. F., Williams, R., & Buckow, R. (2016). Microbiological and physicochemical stability of raw, pasteurised or pulsed electric field-treated milk. *Innovative Food Science and Emerging Technologies*, 38, 365-373. https://doi.org/10.1016/j.ifset.2016.09.030

Merlin Junior, I. A., Santos, J. S., Costa, L. G., Costa, R. G., Ludovico, A., Rego, F. C. A., & Santana, E. H. W. (2015). Sheep milk: physical-chemical characteristics and microbiological quality. *Archivos Latinoamericanos de Nutrición*, 65 (3), 193-198.

Mohapatraa, A., Shindeb, A. K., & Singh, R. (2019). Sheep milk: A pertinent functional food. *Small Ruminant Research*, 181, 6-11. https://doi.org/10.1016/j.smallrumres.2019.10.002

Molineri, A. I., Signorini, M. L., Cuatrín, A. L., Canavesio, V. R., Neder, V. E., Russi, N. B., Bonazza, J. C. & Calvinho, L. F. (2012) Association between milking practices and psychrotrophic bacterial counts in bulk tank milk. *Revista Argentina de Microbiologia*, 44, 187-194.

Panayotov, D., Naydenova, K., Mihaylova, N., & Iliev, T. (2018). Physico-chemical and technological characteristics of Lacaune ewe's milk. *Bulgarian Journal of Agricultural Science*, 24 (sup. 1), 101-108.

Rafiq, S., Huma, N., Pasha, I., Sameen, A., Mukhtar, O. & Khan, M. I. (2016). Chemical Composition, Nitrogen Fractions and Amino Acids Profile of Milk from Different Animal Species. Asian-Australas. *Journal of animal science*, 29 (7), 1022-1028. https://doi.org/10.5713/ajas.15.0452

Santana, E. H. W., Beloti, V., Barros, A. F., Moraes, L. B., Gusmão, V. V., & Pereira, M. S. (2001). Milk contamination in different points of the process: Aerobc mesophilics and psychrotrophic aerobic microorganisms. *Semina: Ciências Agrárias*, 22 (2), 145-154. http://dx.doi.org/10.5433/1679-0359.2001v22n2p145

Silva, N., Junqueira, V. C. A., & Silveira, N. F. A. (1997). Coleta / Transporte / Estocagem / Preparação de amostras para análise. In: Silva, N. Manual de métodos de análise microbiológica de alimentos. São Paulo: Livraria Varela, 8 -20.

Sobrino, L. J., Colado, J. M. P., Sigler, A. I. G., Marín, A. L. M., Sánchez, N. N., & Romero, J. (2018). Composition and colour indices of sheep's bulktank milk are influenced by production practices. *Italian Journal of Animal Science*, 17 (2), 477–488. https://doi.org/10.1080/1828051X.2017.1383860

Percentage of contribution of each author in the manuscript

Samera Rafaela Bruzaroski – 20%

Raquel Pinheiro de Souza – 20%

Pamela da Silva Pasquim – 20%

Rafael Fagnani – 20%

Elsa Helena Walter de Santana – 20%