Small Ruminant Research 113 (2013) 205–210

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Long cold storage influences the microbiological quality of raw goat milk

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ARTICLE INFO

Article history:
Received 4 February 2013
Accepted 7 February 2013
Available online 6 March 2013

Keywords:
Goat milk
Cold storage
Psychrotrophics
Proteolytics

ABSTRACT

Storage of goat milk production in cold temperatures is a current Brazilian legislation request, however there is no specification of a limit period for this. The present work aimed to characterize the microbiological characteristics of raw goat milk produced in a specific region of Brazil, as well as the influence of the storage system and period on its quality. Sixty-one samples from 12 goat farms were collected and subjected to analysis to enumerate hygiene indicator microorganisms, psychrotrophics and proteolytic psychrotrophics. The obtained counts were described and compared considering the system and period of storage (ANOVA, Tukey). Despite presenting low counts of mesophiles, the samples presented high counts of other groups and a relevant presence of proteolytics. Samples collected from bulk tanks presented higher counts of mesophiles and psychrotrophics when compared to immersion tanks and freezers ($p < 0.05$). When stored for a period of 48 h or longer, the counts of mesophiles, coliforms, *Escherichia coli* and psychrotrophics were also significantly higher when compared to a storage period of 24 h or less ($p < 0.05$). The results indicate specific problems in goat milk production in the studied area and the need of establishing a period limit for raw goat milk collection in Brazil.

1. Introduction

Goat milk production in Brazil has improved since the 1990s, and it is considered an important animal product to be exploited as a commodity. Despite this development, the goat milk industry in Brazil is still characterized by small scale farmers, with low daily production (around 80 L) that complicate the logistics of the dairy industry in terms of planning a proper routine for collection and processing (Costa et al., 2010; Oliveira et al., 2011). As a consequence, the storage of goat milk for a long period of time is commonplace on dairy farms. This occurs at low temperatures using a variety of equipment, such as immersion tanks (where the collected milk is stored in cans, immersed in water and kept at a low temperature using a conventional refrigeration system). Bulk tanks are not usually used despite being considered adequate equipment for proper storage (Sierra et al., 2009).

The absence of standards and specifications for the cold storage of raw goat milk has a negative effect on its quality (Goetsch et al., 2011). Storage for long periods of time, even at low temperatures, selects a specific group of microorganisms called psychrotrophics, which are responsible for the production of spoilage enzymes with a direct impact on the quality of raw goat milk and its products (Sørhaug and Stepaniak, 1997). The main enzymes produced by psychrotrophics are lipases and proteases, types of thermo-stable proteins that keep their spoilage potential even after the heat treatments usually adopted in the dairy industry (Champagne et al., 1994; Agnihotri and Pal, 1996).

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0921-4488
http://dx.doi.org/10.1016/j.smallrumres.2013.02.004

These enzymes can interfere in the adequate coagulation of milk during cheese production, and also determine the development of undesirable aroma and flavor in end products, jeopardizing their quality (McPhee and Griffiths, 2011).

Considering these characteristics, the Brazilian Agriculture Ministry established specific rules for goat milk production only in 2000, aiming to improve its quality based on animal health, milking procedures, storage, transport, and processing conditions (Brasil, 2000). However, Brazilian legislation does not specify the limit period of cold storage of raw milk on farms, nor which equipment must be used for raw goat milk storage, hampering the adoption of a standardized system for storage and jeopardizing the initial quality of this product (Delgado-Pertiñez et al., 2003; Zweifel et al., 2005). A current problem in Brazilian dairy production is deficiencies at the beginning of this food chain, mainly related to the health status of producing animals and hygienic practices during milking and storage (Nero et al., 2004, 2009; Monteiro et al., 2007). As a consequence, Brazilian authorities adopted a policy to improve the quality of milk production based mainly on establishing new microbiological parameters of quality and safety, as well as specific conditions of cold storage and transport of milk production from dairy farms (Brasil, 2000, 2011).

Even being considered an important animal product for Brazilian economy, there is a lack of scientific information about the microbiological quality of goat milk, and also concerning the effect of the storage conditions on its microbiota development. Based on this, the present work aimed to characterize the microbiological characteristics of raw goat milk produced in a specific region in Brazil, as well as to investigate of the effects of a long period of storage at low temperature on its microbiota.

2. Material and methods

2.1. Study area and raw milk samples

Twelve goat farms located in the region of the cities Viçosa and Muriaé, Minas Gerais State, Brazil, were selected for the present study, as representative of goat milk production in this region and other Brazilian regions. Raw goat milk samples ($n = 61$) were collected using sterile utensils from all selected farms, from stored milk production and with a minimum of three samples per farm. During sample collection, the storage time of goat milk at each specific farm was recorded and categorized as (1) just after milking, (2) 24 h storage, and (3) 48 h storage (or longer). The main production characteristics of the selected goat farms and the number of samples per farm are detailed in Table 1.

2.2. Sample dilution and microbiological analysis

Raw goat milk samples were transported under refrigeration until analysis and ten-fold diluted using 0.85% NaCl (w/v) according to Wehr and Frank (2004). Aliquots of 1 mL of selected dilutions were plated for aerobic mesophilic counts using Petrifilm™ Aerobic Count plates (3 M Microbiology, St. Paul, MN, USA) followed by incubation at 35 °C for 24 h, for Enterobacteriaceae using Petrifilm™ Enterobacteriaceae (3 M Microbiology) followed by incubation at 35 °C for 24 h, and for coliforms and *E. coli* counts using Petrifilm™ *E. coli* plates (3 M Microbiology) followed by incubation at 35 °C for 24 h (coliforms) and 48 h (*E. coli*). After incubation, all typical colonies were enumerated and the final results were expressed as colony forming units per mL (cfu/L).

Psychrotrophics and proteolytic psychrotrophics were enumerated according to Downes and Ito (2001). Aliquots of 0.1 mL of selected dilutions were surface plated in plate count agar (Oxoid Ltd., Basingstoke, England) added to sterile skim milk (10% v/v, Molico®, Nestlé Brasil Ltda, São Paulo, SP, Brazil) followed by incubation at 7 °C for 10 days. All colonies were enumerated after incubation, and were recorded as psychrotrophics. Then, each plate was flooded with an acetic acid solution (8% v/v) and kept at room temperature for 1 h, at which point the acid solution was discarded and the remaining proteolytic halos were recorded as proteolytic psychrotrophics. The final results were expressed as cfu/mL.

2.3. Statistical analysis

All microbiological counts were converted to $\log_{10}$ and mean values were calculated for each goat farm. Samples

Table 1
Main production characteristics from 12 goat farms located in the region of Viçosa and Muriaé, Minas Gerais State, Brazil, and raw goat milk samples collected for the present study.

Farm	Goat breed	Lactating animals (*n*)	Daily milk production (l)[a]	Milking system	Storage system	Number of samples (n)	Storage period[b]
1	Saanen	72	300	Automatic	Bulk tank	4	Up to 2 h, and 24 h
2	Saanen	42	120	Automatic	Freezing	4	Up to 2 h
3	Saanen	48	170	Handmade	Immersion tank	10	Up to 2 h, 24 h, and 48 h
4	Saanen	75	200	Handmade	Bulk tank	3	Up to 2 h
5	Saanen	45	150	Handmade	Immersion tank	9	Up to 2 h, 24 h, and 48 h
6	Saanen	19	40	Handmade	Immersion tank	5	Up to 2 h
7	Saanen	9	20	Handmade	Immersion tank	5	Up to 2 h, and 24 h
8	Saanen	39	90	Automatic	Bulk tank	5	Up to 2 h, 24 h, and 5 d
9	Saanen	44	150	Handmade	Bulk tank	5	Up to 2 h, 24 h, and 48 h
10	Saanen	24	50	Handmade	Immersion tank	5	Up to 2 h
11	Saanen	47	150	Automatic	Bulk tank	3	Up to 2 h, 24 h, and 4 d
12	Alpine	21	80	Automatic	Immersion tank	3	Up to 2 h, and 6 d

[a] Approximate value, based on records during the sampling.
[b] Storage periods of milk production, recorded during sampling.

Table 2
Mean counts ± standard deviation of hygiene indicator microorganisms from raw goat milk samples collected in 12 dairy farms located in Viçosa and Muriaé, Minas Gerais, Brazil (values in $\log_{10}$ colony forming units per mL).

Farm	Mesophilic aerobes	Enterobacteriaceae	Coliforms	*Escherichia coli*	Psychrotrophics	Proteolytic psychrotrophics
1	5.1 ± 0.3	2.9 ± 1.4	2.8 ± 1.8	2.2	4.1 ± 0.7	3.8 ± 1.1
2	4.9 ± 0.7	3.9 ± 0.5	3.6 ± 0.4	–	3.9 ± 0.3	3.9 ± 0.4
3	4.9 ± 0.6	3.9 ± 1.4	3.4 ± 1.1	1.5	4.3 ± 0.8	3.7 ± 1.1
4	5.5 ± 0.2	4.0 ± 0.7	2.9 ± 0.3	1.3 ± 0.5	4.5 ± 0.7	4.5 ± 0.0
5	5.0 ± 0.3	3.8 ± 0.6	3.0 ± 0.6	2.8 ± 0.6	4.2 ± 0.5	4.1 ± 0.6
6	4.5 ± 0.6	2.4 ± 1.0	2.5 ± 1.0	–	3.6 ± 1.0	2.4 ± 0.3
7	4.1 ± 0.6	2.0 ± 1.6	1.6 ± 0.6	1.0	2.9 ± 1.0	2.3 ± 0.5
8	6.1 ± 1.4	5.2 ± 2.3	3.5 ± 2.7	1.0	5.0 ± 0.9	5.3 ± 0.8
9	4.6 ± 0.6	3.2 ± 0.9	3.0 ± 0.9	1.0	4.3 ± 0.4	3.9 ± 0.5
10	4.7 ± 0.5	2.8 ± 0.6	2.5 ± 0.7	–	3.2 ± 1.1	3.4 ± 1.7
11	5.4 ± 0.3	4.0 ± 0.6	2.8 ± 0.6	–	4.8 ± 0.9	4.3 ± 1.5
12	5.8 ± 0.6	4.4 ± 0.7	3.8 ± 0.4	–	3.9 ± 0.4	3.5 ± 0.7
mean value	5.0 ± 0.8	3.5 ± 1.3	3.0 ± 1.1	1.7 ± 0.8	4.1 ± 0.9	3.8 ± 1.0

were also grouped according their microbiological counts and reference values from Brazilian legislation (Brasil, 2000) and scientific data (Chambers, 2002). The mean counts of raw milk samples were also calculated considering their storage system and storage period in goat farms and compared by ANOVA and Tukey test ($p < 0.05$).

3. Results and discussion

Considering the general profile of goat milk production in Brazil and the region, the selected goat farms were an adequate representative of this activity, since they fulfilled the main characteristics of Brazilian and local goat milk production (Costa et al., 2010; Oliveira et al., 2011). Based on these characteristics (Table 1), the goat milk production in the specific study area included in this study, and also in Brazil, can be considered as a promising rural activity which is undergoing significant development. Based on this, it is relevant to identify the key points of goat milk production to improve the quality and safety of this farm product, thus avoiding the current problems in cow milk production in Brazil (Nero et al., 2004, 2009; Monteiro et al., 2007; Mattos et al., 2010; Ortolani et al., 2010; Yamazi et al., 2010).

Even considering the absence of proper standardization of storage systems on goat farms in Brazil, the analysis of the mean values of hygienic indicator microorganisms presented by the goat milk samples demonstrate specific problems (Table 2). Only one farm presented a mean count of mesophilic aerobes higher than 6 log cfu/mL, a reference value usually considered as indicative of poor hygienic practices during production (Chambers, 2002). However, the mean values of enterobacteriaceae, coliforms, and *E. coli* were substantially higher when compared to reference values (Chambers, 2002); similar results were found for psychrotrophics and proteolytic psychrotrophics. These results indicate specific hygienic deficiencies during the first steps of goat milk production and problems during the cold storage of this product on goat farms. This analysis was confirmed when the data were analyzed considering the levels of contamination (Fig. 1); the majority of raw goat milk samples presented mesophilic aerobe counts lower than 6.0 log cfu/mL (Chambers, 2002), and lower than 5.7 log cfu/mL, the reference parameter of the current Brazilian legislation (Brasil, 2000). These results are also in accordance with similar studies on raw goat milk from different regions (Fonseca et al., 2006; Kondyli et al., 2012). However, when the other groups of hygiene indicator microorganisms are considered, higher frequencies of samples with counts higher than reference or literature values were found (Chambers, 2002; Picoli et al., 2006; Kondyli et al., 2012).

Currently, the logistics of goat milk collection in Brazil are highly influenced by the characteristics of goat farms; as the daily production is low, the dairy industry tends to establish collecting routes with intervals of several days between collections, supported by the absence of specification in the current Brazilian legislation (Brasil, 2000). As result, goat milk is stored for several days on dairy farms, allowing the development of psychrotrophic microorganisms (Table 2, Fig. 1). The data also revealed the relevant proteolytic activity of the psychrotrophic colonies enumerated; 22 samples presented more than 50% of the psychrotrophic counts comprised of proteolytics, confirming the spoilage potential of this group as a constituent of the raw goat milk microbiota. Psychrotrophics are highly induced to grow and produce proteolytic enzymes when present in milk stored under refrigeration for long periods or at inappropriate temperatures (Champagne et al., 1994; Sørhaug and Stepaniak, 1997; McPhee and Griffiths, 2011). The impact of such variables in the composition of the raw milk microbiota and spoilage activity has already been described, mainly in cow milk (Martins et al., 2006; Wijnands et al., 2006; Ercolini et al., 2009; Perin et al., 2012); however, considering the production characteristics of goat milk, the relevance of this group can be considered similar.

Based on the results (Table 2, Fig. 1), a direct effect of the storage routine of raw goat milk on its microbiological quality was observed; the analysis presented in Tables 3 and 4 confirms this observation. Although bulk tanks are considered as adequate for raw milk storage (Sierra et al., 2009), goat milk samples stored using this equipment presented higher counts of the majority of the researched hygiene indicator microorganisms when compared to samples stored in immersion tanks and freezers ($p < 0.05$, Table 3). Immersion tanks are widely used in milk production in Brazil, and are considered inadequate for proper cooling of this product, as temperature control and

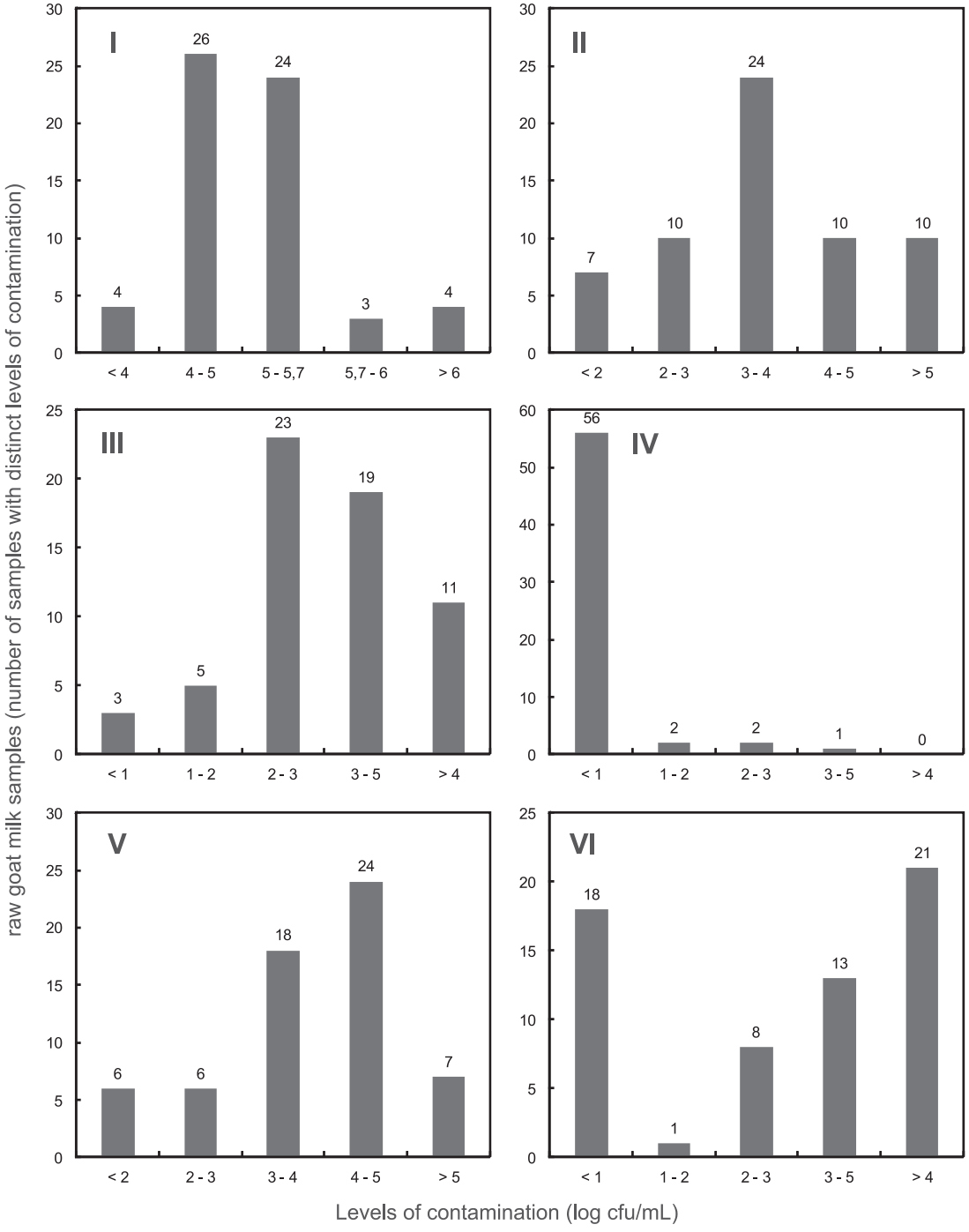

Fig. 1. Frequencies of raw goat milk samples collected in 12 dairy farms from Viçosa and Muriaé regions, Minas Gerais State, Brazil, presenting distinct levels of contamination by mesophilic aerobes (I), enterobacteriaceae (II), coliforms (III), *Escherichia coli* (IV), psychrotrophics (V) and proteolytic psychrotrophics (VI).

cold transfer are deficient (Tebaldi et al., 2008; Nero et al., 2009; Perin et al., 2012); considering this, current Brazilian legislation allows the adoption of immersion tanks only on farms with low daily milk production (Brasil, 2011). As goat milk production in Brazil is characterized by low daily production (Table 1), this system would be an alternative for proper storage of this product on farms, as observed in the analyzed samples (Table 3). The obtained counts for raw milk stored in bulk tanks were not expected, as this system is considered adequate for proper milk storage; these results were a consequence of the data from farm 8, as it presented high counts for all researched groups (Table 2) and production characteristics that suggest serious deficiencies in goat milk production. Despite this

Table 3
Mean counts ± standard deviation of hygiene indicator microorganisms from raw goat milk samples collected in 12 dairy farms located in Viçosa and Muriaé, Minas Gerais, Brazil, considering their storage system (values in $\log_{10}$ colony forming units per mL).

Storage system	*n*	Mesophilic aerobes	Enterobacteriaceae	Coliforms	*Escherichia coli*	Psychrotrophics	Proteolytic psychrotrophics
bulk tank	20	5.3 ± 0.9^{a}	3.9 ± 1.5^{a}	3.0 ± 1.4^{a}	1.4 ± 0.5^{a}	4.6 ± 0.7^{a}	4.3 ± 0.9^{a}
immersion tank	37	4.8 ± 0.7^{b}	3.3 ± 1.3^{a}	2.9 ± 1.0^{a}	2.0 ± 0.9^{a}	3.9 ± 0.9^{b}	3.5 ± 0.9^{b}
freezing	4	$4.9 \pm 0.7^{a,b}$	3.9 ± 0.5^{a}	3.6 ± 0.4^{a}	–	$3.9 \pm 0.3^{a,b}$	$3.9 \pm 0.4^{a,b}$
ANOVA		$F_{(2,58)} = 3.38$; $p = 0.041$	$F_{(2,55)} = 1.05$; $p = 0.358$	$F_{(2,56)} = 0.84$; $p = 0.436$	$F_{(1,7)} = 1.50$; $p = 0.260$	$F_{(2,51)} = 4.10$; $p = 0.022$	$F_{(2,39)} = 3.15$; $p = 0.054$

ANOVA (F): Analysis of variance, *p*: level of significance. Mean values with distinct letters in a same column are significantly different by Tukey HDS test ($p < 0.05$)

Table 4
Mean counts ± standard deviation of hygiene indicator microorganisms from raw goat milk samples collected in 12 dairy farms located in Viçosa and Muriaé, Minas Gerais, Brazil, considering their storage period (values in $\log_{10}$ colony forming units per mL).

Storage period	*n*	Mesophilic aerobes	Enterobacteriaceae	Coliforms	*Escherichia coli*	Psychrotrophics	Proteolytic psychrotrophics
Just after milking	36	4.9 ± 0.7^{b}	3.1 ± 1.0^{a}	2.7 ± 0.9^{b}	1.4 ± 0.5^{b}	3.9 ± 0.7^{b}	3.6 ± 0.9^{a}
24 h	16	4.8 ± 0.9^{b}	3.6 ± 1.6^{a}	$3.1 \pm 1.2^{a,b}$	1.2 ± 0.3^{b}	$4.0 \pm 1.0^{a,b}$	3.7 ± 1.2^{a}
48 h or longer	9	5.6 ± 0.8^{a}	4.9 ± 1.2^{a}	3.7 ± 1.5^{a}	2.8 ± 0.6^{a}	4.8 ± 0.6^{a}	4.5 ± 0.8^{a}
ANOVA		$F_{(2,58)} = 3.93$; $p = 0.025$	$F_{(2,55)} = 7.21$; $p = 0.002$	$F_{(2,56)} = 3.04$; $p = 0.056$	$F_{(2,6)} = 5.59$; $p = 0.043$	$F_{(2,51)} = 4.10$; $p = 0.022$	$F_{(2,39)} = 2.93$; $p = 0.065$

ANOVA (*F*): analysis of variance, *p*: level of significance. Mean values with distinct letters in a same column are significantly different by Tukey HDS test ($p < 0.05$).

farm presents automatic utensils and equipments for dairy production, such as automatic milking system and bulk tank, it also presented some characteristics that jeopardize the milk quality, such as low daily production with an average number of lactating animals, suggesting mastitis, and long period of storage, since one of the samples was collected after 5 days in the bulk tank (Table 1). Independent of the storage system, the poor quality of stored raw goat milk must be considered, due to the high counts of hygiene indicator microorganisms and mesophilic aerobes (Tables 2 and 3, Fig. 1).

In contrast, a direct influence of the storage period on the microbiological quality of raw goat milk was demonstrated (Table 4). Raw goat milk samples stored for periods greater than 48 h presented higher counts of mesophilic aerobes, coliforms, *E. coli*, and psychrotrophics when compared to counts from samples obtained just after milking and after 24 h storage ($p < 0.05$, Table 4). These data confirm the relevance of the storage period on the growth of the raw goat milk microbiota, enhancing the concern for the spoilage potential of specific groups (Gram et al., 2002; Perin et al., 2012). The inadequacy of long periods of storage using immersion tanks as a cooling system was already demonstrated by Perin et al. (2012), who also demonstrated the relevance of initial microbial contamination on the growth of relevant spoilage microorganisms, such as psychrotrophics. Although the present data indicate better performance of immersion tanks for raw goat milk storage related to mesophilic aerobe counts (Table 3), the long period of storage of this product on goat farms must be considered. Independent of milk storage conditions, a long period allows the growth of psychrotrophic microorganisms even at appropriate temperatures (up to 4 °C) (Champagne et al., 1994). During growth, they are able to produce heat stable proteases and lipases that will be active even after the heat treatments employed in the dairy industry for raw goat milk, jeopardizing the quality of the processed products (Griffiths et al., 1981; Fairbairn and Law, 1986; Patel and Bartlett, 1988; Owusu et al., 1991). The main psychrotrophic microorganisms usually present in raw goat milk have not been previously described, but considering its productions characteristics, this microbiota is basically similar to cow milk, which is composed mainly by *Pseudomonas* spp. and *Bacillus* spp. (Patel and Bartlett, 1988; Francis et al., 1998; Ercolini et al., 2009).

In conclusion, these data demonstrate specific failures in the goat milk production chain in Brazil, leading to the poor microbiological quality of this product identified by high counts of hygiene indicator microorganisms other than mesophilic aerobes, such as coliforms and psychrotrophics. In addition, a relevant influence of long storage periods on the microbiological quality of this product was observed, which is a consequence of the current logistics adopted by the dairy industry allowable by current Brazilian legislation. Based on these results, it is important to highlight the necessity that the Brazilian Ministry of Agriculture establish the specific equipment to be adopted on goat farms as well as a period limit for collection.

Acknowledgments

The authors are supported by CAPES, CNPq, and FAPEMIG. The present research was supported by CNPq.

References

Agnihotri, M.K., Pal, U.K., 1996. Quality and shelf-life of goat milk Paneer in refrigerated storage. Small Rum. Res. 20, 75–81.
Brasil, 2000. Instrução Normativa 37 – Aprova o Regulamento Técnico de Identidade e Qualidade de Leite de Cabra. In: MAPA (Ed.), Diário Oficial da União. Brasília, DF, Brasil.
Brasil, 2011. Instrução Normativa 62 – Aprovar 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. In: MAPA (Ed.), Diário Oficial da União. Brasília, DF, Brasil.

Chambers, J.V., 2002. The microbiology of raw milk. In: Robinson, R.K. (Ed.), Dairy Microbiology Handbook. John Wiley and Sons, Inc., New York, pp. 39–90.

Champagne, C.P., Laing, R.R., Roy, D., Mafu, A.A., Griffiths, M.W., 1994. Psychrotrophs in dairy-products – their effects and their control. Crit. Rev. Food Sci. Nutr. 34, 1–30.

Costa, R.G., Beretta Dal Monte, H.L., Pimenta Filho, E.C., Holanda Junior, E.V., Beltrao da Cruz, G.R., Carrera Menezes, M.P., 2010. Typology and characterization of goat milk production systems in the Cariris Paraibanos. Braz. J. Anim. Sci. 39, 656–666.

Delgado-Pertiñez, M., Alcalde, M.J., Guzmán-Guerrero, J.L., Castel, J.M., Mena, Y., Caravaca, F., 2003. Effect of hygiene-sanitary management on goat milk quality in semi-extensive systems in Spain. Small Rum. Res. 47, 51–61.

Downes, F.P., Ito, K., 2001. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington.

Ercolini, D., Russo, F., Ferrocino, I., Villani, F., 2009. Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. Food Microbiol. 26, 228–231.

Fairbairn, D.J., Law, B.A., 1986. Proteinases of psychrotrophic bacteria – their production, properties, effects and control. J. Dairy Res. 53, 139–177.

Fonseca, C.R., Porto, E., Dias, C.T.S., Susin, I., 2006. Qualidade do leite de cabra *in natura* e do produto pasteurizado armazenados por diferentes períodos. Cien. Tecnol. Alim. 26, 944–949.

Francis, K.P., Mayr, R., von Stetten, F., Stewart, G., Scherer, S., 1998. Discrimination of psychrotrophic and mesophilic strains of the *Bacillus cereus* group by PCR targeting of major cold shock protein genes. Appl. Environ. Microbiol. 64, 3525–3529.

Goetsch, A.L., Zeng, S.S., Gipson, T.A., 2011. Factors affecting goat milk production and quality. Small Rum. Res. 101, 55–63.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage – interactions between food spoilage bacteria. Int. J. Food Microbiol. 78, 79–97.

Griffiths, M.W., Phillips, J.D., Muir, D.D., 1981. Thermostability of proteases and lipases from a number of species of psychrotrophic bacteria of dairy origin. J. Appl. Bacteriol. 50, 289–303.

Kondyli, E., Svarnas, C., Samelis, J., Katsiari, M.C., 2012. Chemical composition and microbiological quality of ewe and goat milk of native Greek breeds. Small Rum. Res. 103, 194–199.

Martins, M.L., Pinto, C.L.O., Rocha, R.B., de Araujo, E.F., Vanetti, M.C.D., 2006. Genetic diversity of Gram-negative, proteolytic, psychrotrophic bacteria isolated from refrigerated raw milk. Int. J. Food Microbiol. 111, 144–148.

Mattos, M.R., Beloti, V., Tamanini, R., Magnani, D.F., Nero, L.A., Barros, M.A.F., Pires, E.M.F., Paquereau, B.P.D., 2010. Quality of raw milk produced in agreste region of Pernambuco, Brazil. Semina Cien. Agr. 31, 173–181.

McPhee, J.D., Griffiths, M.W., 2011. Psychrotrophic bacteria |*Pseudomonas* spp. In: Editor-in-Chief: John, W.F. (Ed.), Encyclopedia of Dairy Science, 2nd ed. Academic Press, San Diego, pp. 379–383.

Monteiro, A.A., Tamanini, R., Silva, L.C.C., Mattos, M.R., Magnani, D.F., d'Ovidio, L., Nero, L.A., Barros, M.d.A.F., Pires, E.M.F., Paquereau, B.P.D., Beloti, V., 2007. Characteristics of the milk production of the agreste region of the state of Pernambuco, Brazil. Semina: Cien. Agr. 28, 665–674.

Nero, L.A., Mattos, M.R., Beloti, V., Netto, D.P., Pinto, J.P.A.N., Andrade, N.J., Silva, W.P., Franco, B.D.G.M., 2004. Hazards in non-pasteurized milk on retail sale in Brazil: Prevalence of *Salmonella* spp. *Listeria monocytogenes* and chemical residues. Brazil. J. Microbiol. 35, 211–215.

Nero, L.A., Viçosa, G.N., Vilela Pereira, F.E., 2009. Microbiological quality of milk determined by production characteristics. Cien. Tecnol. Alim. 29, 386–390.

Oliveira, C.J.B., Hisrich, E.R., Moura, J.F.P., Givisiez, P.E.N., Costa, R.G., Gebreyes, W.A., 2011. On farm risk factors associated with goat milk quality in Northeast Brazil. Small Rum. Res. 98, 64–69.

Ortolani, M.B.T., Yamazi, A.K., Moraes, P.M., Viçosa, G.N., Nero, L.A., 2010. Microbiological quality and safety of raw milk and soft cheese and detection of autochthonous lactic acid bacteria with antagonistic activity against *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*. Foodborn Pathog. Dis. 7, 175–180.

Owusu, R.K., Makhzoum, A., Knapp, J., 1991. The thermodynamic stability of lipases and proteases from psychrotrophic bacteria. Food Chem. 39, 187–195.

Patel, T.R., Bartlett, F.M., 1988. Heat-stable proteases from psychrotrophic pseudomonads: secondary structure and heat stability. Food Microbiol. 5, 201–211.

Perin, L.M., Moraes, P.M., Almeida, M.V., Nero, L.A., 2012. Intereference of storage temperatures in the development of mesophilic, psychrotrophic, lipolytic and proteolytic microbiota of raw milk. Semina: Cien. Agr. 33, 333–342.

Picoli, S.U., Bessa, M.C., Castagna, S.M.F., Gottardi, C.P.T., Schmidt, V., Cardoso, M., 2006. Quantificação de coliformes, *Staphylococcus aureus* e mesófilos presentes em diferentes etapas da produção de queijo frescal de leite de cabra em laticínios. Cien. Tecnol. Alim. 26, 64–69.

Sierra, D., Sánchez, A., Contreras, A., Luengo, C., Corrales, J.C., de la Fe, C., Guirao, I., Morales, C.T., Gonzalo, C., 2009. Effect of storage and preservation on total bacterial counts determined by automated flow cytometry in bulk tank goat milk. J. Dairy Sci. 92, 4841–4845.

Sørhaug, T., Stepaniak, L., 1997. Psychrotrophs and their enzymes in milk and dairy products: quality aspects. Trends Food Sci. Technol. 8, 35–41.

Tebaldi, V.M.R., Oliveira, T.L.C., Boari, C.A., Piccoli, R.H., 2008. Isolation of coliforms, staphylococci, and enterococci in raw milk from communitarian expansion refrigeration tanks: identification, lipolytic and proteolytic action. Cien. Tecnol. Alim. 28, 753–760.

Wehr, H.M., Frank, J.F., 2004. Standard Methods for the Examination of Dairy Products. American Public Health Association, Washington.

Wijnands, L.M., Dufrenne, J.B., Zwietering, M.H., van Leusden, F.M., 2006. Spores from mesophilic *Bacillus cereus* strains germinate better and grow faster in simulated gastro-intestinal conditions than spores from psychrotrophic strains. Int. J. Food Microbiol. 112, 120–128.

Yamazi, A.K., Moraes, P.M., Viçosa, G.N., Ortolani, M.B.T., Nero, L.A., 2010. Práticas de produção aplicadas no controle de contaminação microbiana na produção de leite cru. Bioscience J. 26, 610–618.

Zweifel, C., Muehlherr, J.E., Ring, M., Stephan, R., 2005. Influence of different factors in milk production on standard plate count of raw small ruminant's bulk-tank milk in Switzerland. Small Rum. Res. 58, 63–70.