MICROBIOLOGICAL AND PHYSICAL-CHEMICAL PROFILE OF GOAT MILK IN THE SEMIARID REGION OF THE SAN FRANCISCO VALLEY*

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ABSTRACT

In Brazil, the largest goat herd is in the Northeast, especially in Bahia and Pernambuco, which are the first and second states in number of goats. The semiarid region of San Francisco Valley still lacks studies on determination of parameters for the goat milk produced. The aim of this study was to characterize the microbiological and physical-chemical profile of goat milk produced in the semiarid region of the San Francisco Valley in accordance with current legislation. Forty-two samples of goat milk were obtained from six municipalities in the semiarid region of the San Francisco Valley (Juazeiro, Uauá, Senhor do Bonfim, Curaçá, Jaguarari and Petrolina), with seven samples per municipality, and the microbiological and physical-chemical parameters were analyzed. The microbiological analyses were Total Aerobic Mesophilic Bacteria (TAMB) and Lactic Acid Bacteria (LAB) counts, enumeration of total and thermotolerant coliforms and *Escherichia coli* research. Indirect measurement of Somatic Cell Count (SCC) was also performed. The physical-chemical tests performed were acidity, Freezing Point Depression (FDP) and quantitative determination of fat by the Gerber method. There were significant differences for microbiological analyses in the cities studied. In the samples, LAB was predominant among the microorganisms examined and *E. coli* was detected in all municipalities. For physical-chemical parameters, there was no significant difference only for FDP. The microbiological and physical-chemical characteristics were markedly separate by Principal Component Analysis (PCA). The cities of Petrolina and Juazeiro were characterised by greater quantification of fat and indirect SCC, respectively. In Uauá, Curaçá, Jaguarari and Senhor do Bonfim, the parameters for total coliforms, FDP, *E. coli* and acidity stood out, respectively. The microbiological results are indicative of the need for hygienic practices during milking to obtain better milk quality.

Keywords: Microbiota. Quality. Legislation of goat milk. Principal Component Analysis.

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INTRODUCTION

Goat milk is an important nutritional component in semiarid regions. Brazil is the largest milk producer in South America at 141.000 tons per year. The Northeast region accounts for 90% of the total goat population. This fact show a grated potential of this part of Brazil. According to Braga et al. (2010) and Oliveira et al. (2011), goat raising has socioeconomic importance for employment and income generation in this brazilian region. However, private market access is needed for the long-term sustainability of smallholder in the goat milk value chain (MEDINA et al., 2011; OLIVEIRA et al., 2011).

New markets and uses for goat milk and goat milk products are also being proposed, for example, as a base for medicinal and infant foods. It is essential that the quality and safety of goat milk be optimized to ensure consumer confidence within the context of growing interest in existing and new goat dairy products worldwide (SILANIKOVE et al. 2010). In Brazil, there is legislation to ensure the quality of goat milk, which establishes physical-chemical and microbiological requirements for human consumption of the product (BRASIL, 2000). However, for the San Francisco Valley in the semiarid brazilian region, studies to determine the physical-chemical and microbiological parameters for goat milk are still lacking. In addition, local producers do not know if the milk meets the requirements of brazilian legislation, a crucial factor to introduce the product in the market.

The research on the physical-chemical and microbiological characteristics of goat milk allows to kwon the quality of raw milk , changes during storage and how is the management on the goat farms. The aim of this study was to investigate the microbiological and physical-chemical profile of goat milk from the semiarid region of the San Francisco Valley, Brazil.

MATERIALS AND METHODS

Samples of raw goat milk were collected from extensively small-scale dairy farms with mixed breed goat in six municipalities (Juazeiro, Uauá, Senhor do Bonfim, Curaçá, Jaguarari and Petrolina) of the San Francisco Valley, Northeast of Brazil:. Seven farms were randomly selected from each county for a total of 42 milk samples. Trained research personnel aseptically collected the milk samples (150 mL) from containers, in which the product was stored after the daily milking, using a stainless steel ladle (alcohol-sanitized) and sterile plastic bottles. Samples were kept in a cooler for storage at 4ºC until delivery to the laboratory for physical-chemical and microbiological analyses on the same day.

In microbiological analysis for enumeration of total and thermotolerant coliforms and *E. coli* research, Fluorocult LMX broth was used according to miniaturized technique proposed by Franco et al. (2008) In brief, a 100 µL aliquot was taken from each sample and inoculated in an Eppendorf microtube containing 900 µL of 0.1% peptone solution until the dilution 10^{-8} was obtained. Dilutions were performed in a series of three Eppendorf tubes containing 1000 µL of the Fluorocult broth. After incubation at 37°C for 24 to 48 h, the Eppendorf tubes that showed change in color of the medium to bluish green were considered positive for total coliforms. Under ultraviolet light (366 nm wave length), the Eppendorf tubes with blue color and which presented fluorescence were considered positive for thermotolerant coliforms. The Indole test was performed for confirmation of *E. coli*. Positive Eppendorf tubes for each dilution were noted for subsequent calculation of the Most Probable Number (MPN) per mL of sample, using the McCrady Table (MERCK, 2002).

The Total Aerobic Mesophilic Bacteria (TAMB) counts were carried out as described by Brasil (2003). The samples (10^{0}) and dilutions (10^{-1} to 10^{-8}) were plated out in Plate Count Agar (PCA, Merck®). Plates were incubated at 37°C for 48 hours. For Lactic Acid Bacteria (LAB) analysis, 0.1 mL aliquots of goat milk (10^{0}) and dilutions (to 10^{-8}) were transferred to plates with a specific medium, de Man, Rogosa and Sharpe (MRS, Himedia®). The plates were incubated at 37°C for 72 - 96 hours in aerobic conditions according to Lima et al. (2009). Indirect

measurement of Somatic Cell Count (SCC) was performed using the Somaticell® kit (Verus Madasa, São Paulo, Brazil), according to manufacturer's instructions.

For physical-chemical analyses, the following tests were performed, according to Brasil (2006): titratable acidity in degrees Dornic (ºD – Dornic Solution - Cap Lab®), quantitative determination of fat (Gerber method) and Freezing Point Depression (FDP) using a previously calibrated electronic cryoscope (Mod. MK 540 Flex) and values were expressed in degrees Hortvert (ºH).

All tests were performed in triplicate, except for the indirect somatic cell count. In statistical analyses, the data were analyzed through analysis of variance (ANOVA), and mean values were compared using the Scott-Knott test ($p < 0.05$) The software used for statistical analysis was the SISVAR® (Lavras, Brazil) software, version 4.5. The microbiological and physical-chemical profile of goat milk in the municipalities of the semiarid region was analyzed by Principal Component Analysis (PCA), using the software XLSTAT 7.5.2 (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

The TAMB count was significant among the counties surveyed (Table 1). The average TAMB count was 3.77 log cfu mL^{-1}. All farms showed conformity with current Brazilian legislation (BRASIL, 2000), which establishes a threshold of 5.69 log cfu mL^{-1} of TAMB for raw goat milk. The municipalities of Jaguarari and Curaçá presented a high count. For total coliforms, there was detection in all samples, which ranged from 1 to 2 log NMP mL^{-1}. Among the municipalities studied only Uauá presented high detection for total coliforms ($p <0.05$). The thermotolerant coliform-positive samples were also checked for the presence of *E. coli*. In Jaguarari, there was significantly higher detection of *E. coli* in relation to other places.

Different results were reported in similar areas. Oliveira et al. (2011) reported a high count of TAMB and total coliforms in goat milk produced by family farmersin Cariri (Paraiba, Brazil), where 62.5% of the properties exhibited a count greater than 5.69 log cfu mL^{-1} of TAMB and a mean value for coliform of 6.07 log NMP mL^{-1}. In addition, Gottardi et al. (2008) evaluated the quality of milk produced by eight dairy goat farms located in the Taquari Valley (Rio Grande do Sul, Brazil) and found a total coliform counts up to 6.14 log cfu mL^{-1} , as well as thermotolerant coliforms on two farms.

This research, there was low detection of TAMB, coliforms and *E.coli*. However, according to standard of bacteriological quality in milk, the presence of *E. coli* is indicative of failure in hygienic procedures during milking and represents a potential public health threat (ALTALHI and HASSAN, 2009). Thus, this practice should be carried out more carefully on the farms, especially since goat dairy products in South America are manufactured in craft conditions (MEDINA et al., 2011).

Lactic Acid Bacteria (LAB) was predominant among the microorganisms examined with an average of 3.93 log cfu mL^{-1} (Table 1). Delavenne et al. (2012) reported similar results for the population of LAB in goat, cow and sheep. The authors affirmed that raw cow and goat milk, in contrast with ewe's milk, can be considered as a reservoir of antifungal lactic acid bacteria. According to Medina et al. (2011), the main microorganisms in goat milk and goat dairy products are lactic acid bacteria as *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus*. There are many studies reporting the benefits of LAB in dairy products, such as antifungal properties (DELAVENNE et al., 2012), bacteriocin production (LAVILLA-LERMA et al., 2013), conjugated linoleic acid production (VAN NIEUWENHOVE et al., 2009), folate synthesis (SANNA et al., 2005) and generation of bioactive peptides - ACE-inhibitory peptides (MINERVINI et al., 2009). Thus, LAB contributes to an increase in the functional value of goat milk.

Table 1. Mean count of TAMB, total coliforms, *E. coli* and LAB in raw goat milk from six municipalities in the San Francisco Valley.

County	TAMB[1] log cfu mL^{-1}	Total Coliform[2] log NMP mL^{-1}	*E. coli*[3] log NMP mL^{-1}	LAB[4] log cfu mL^{-1}
Juazeiro	3.17^{b}	1.75^{a}	0.00^{a}	3.84^{b}
Curaçá	3.35^{b}	1.65^{a}	0.39^{a}	3.81^{b}
Senhor do Bonfim	3.00^{a}	2.06^{a}	0.11^{a}	3.72^{b}
Uauá	3.05^{a}	2.79^{b}	0.57^{a}	4.19^{c}
Jaguarari	3.31^{b}	1.67^{a}	1.15^{b}	3.47^{a}
Petrolina	2.85^{a}	1.36^{a}	0.45^{a}	3.59^{a}
Average	3.77	1.88	0.44	3,93

Within each column, mean values with different letters are significantly different (P<0.05) according to the Scott-Knott test. [1] [1]Standard error =0.09; [2]Standard error =0.02; [3]Standard error =0.03; [4]Standard error =0.06

The average of indirect Somatic Cell Count (SCC) was ranged from 415,000 to 3,065,357 cells/mL (Table 2). According to manufacturer's instructions for the Somaticell® kit, counts from 400,000 to 1,200,000 cells/ml indicate that the probability of infection in the mammary gland is quite high, and in counts above 1,200,000 cells/mL, the presence of infection in the mammary gland of the animal is practically certain. Thus, the county of Juazeiro presented herds with udder infection. Oliveira et al. (2011) and Han et al. (2010) found higher values, around 2,494,097 and 1,500,000 respectively in raw goat milk. In Brazilian legislation, there is no limit for SCC in raw goat milk. According to Oliveira (2011), other countries generally show 1 million somatic cells per ml as threshold values.

Table 2. Average of the indirect somatic cell count in raw goat milk from six municipalities of the San Francisco Valley.

County	Indirect SCC cells/mL (average)
Juazeiro	3,065,357
Curaça	1,169,000
Senhor do Bonfim	667,857
Uauá	517,357
Jaguarari	415,000
Petrolina	1,889,215
Average	931,686

Table 3 shows the average of acidity, freezing point depression (FDP) and fat in raw goat milk. The physical-chemical parameters of goat milk vary as a result of multiple factors, including genetic characteristics, stage of lactation, diet, physiology and climate (COSTA et al., 2009; SCHONFELDT et al., 2012).

Table 3. Average of physical-chemical parameters of raw goat milk from six municipalities of the San Francisco Valley.

County	Acidity (°D) [1]	FDP (-°H)	Fat % [2]
Juazeiro	17.36^{b}	0.568	5.46^{b}
Curaçá	15.94^{a}	0.591	4.35^{a}
Senhor do Bonfim	17.57^{b}	0.570	3.79^{a}
Uauá	17.61^{b}	0.522	4.59^{a}
Jaguarari	17.90^{b}	0.562	4.15^{a}
Petrolina	16.19^{a}	0.574	3.96^{a}
Average	17.09	0,564	4.38

Within each column, mean values with different letters are significantly different (P<0.05) according to the Scott-Knott test.
[1]Standard error = 0.544; [2] Standard error= 0.316.

There were significant differences for acidity among the samples analyzed. All samples met the threshold values of 13 to 18ºD (BRASIL, 2000). The average acidity of all samples of the counties was 17.09°D. This value was higher than those reported by Andrade et al. (2008) and Almeida et al. (2009), which were 15.8°D and 15.6°D, respectively. The acidity of the milk increases through hydrolysis of lactose by microbial enzymes, which leads to the formation of lactic acid. Therefore, high acidity indicates high microbial activity. Microbial activity depends on the state of conservation of the samples or the hygiene procedures during the milking process (SCHMIDT et al., 2008).

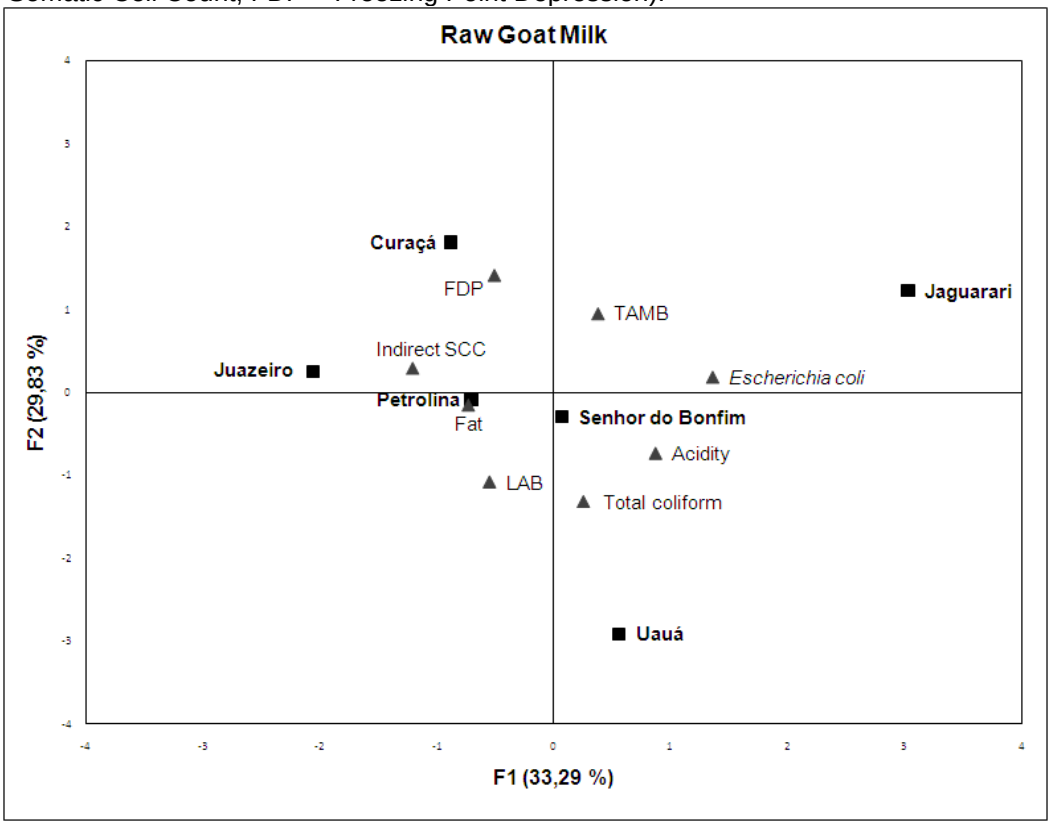
The FDP of samples did not differ significantly (*P*>0.05) in this study (Table 3). Two samples did not meet brazilian legislation, which establishes values between -0.550 and -0.585°H (BRASIL, 2000). The average of the samples in our survey, -0.564°H, was similar to that found by Mayer and Fiechter (2012) who evaluated goat milk in Austria and found the mean of -0.561°H. In Brazil, Andrade et al. (2008) found an average of -0.553ºH in a study of 15 Alpine goats in the state of Minas Gerais, Brazil. For routine estimation of milk FDP, it is important to prevent adulterated milk from reaching the market. Henno et al. (2008) demonstrated that breed, lactation stage, interaction of year and period and rumen protein balance group affect the freezing point. Moreover, lactose, chloride salts and other water-soluble constituents (such as calcium, potassium, and magnesium) contribute to the FDP of milk.

The average determination of fat obtained in raw goat milk by the Gerber method was 4.38% (Table 3). The average determination of fat among the herds varied significantly ($p < 0.05$) and all samples met the value required by Brazilian law, a minimum of 2.9%. Oscillations in fat content are caused by factors such as milking shift, the breed, the lactation period, the type of diet provided and seasonality (QUEIROGA et al., 2007).

Mestawet et al. (2012) established that fat was the only component that showed high significant variation in different stages of lactation of four goat breeds from Ethiopia, with mean values of 4.70%, 3.65%, 5.15% and 4.90%. Similarity, Mayer and Fiechter (2012) reported a mean value for fat of 3.74%. However, Almeida et al. (2009) found an average value of 2.6% for fat in goat herds in Minas Gerais and Rio de Janeiro, Brazil.

To discriminate the microbiological and physical-chemical parameters found in the raw goat milk from each of the counties investigated in this study, PCA was assessed, based on quantification of each of these parameters (Figure 1). The first two components explained 63.12% of the total variance; PC1 and PC2 accounted for 33.29% and 29.83% of the raw goat milk, respectively. The microbiological and physical-chemical characteristics were markedly separate in the plane of the biplot. Petrolina and Juazeiro were characterized by greater quantification of fat and indirect SCC, respectively. In Uauá, Curaçá, Jaguarari and Senhor do Bonfim, the parameters for total coliforms, FDP, *E. coli* and acidity stood out, respectively. The parameters for acidity and FDP remained in opposite planes in Figure 1 because the measures are inversely proportional.

Figure 1. Principal component analysis (PCA) based on quantification of microbiological and physical-chemical parameters of the raw goat milk obtained from six municipalities in the semiarid region of the San Francisco Valley. (TAMB = Total Aerobic Mesophilic Bacteria; LAB = Lactic Acid Bacteria; indirect SCC = Somatic Cell Count; FDP = Freezing Point Depression).



CONCLUSION

The detection of *E. coli* in raw goat milk in the San Francisco Valley, Brazil, indicates the need for better hygiene during milking, as well as the implementation of Good Dairy Farming Practices and the improvement of handling procedures. It is necessary to intensify studies on the quality of goat milk for the purpose of product conformity with the parameters established in legislation, and for it to enter in the national market. Standardization of goat milk within the minimum requirements imposed by law will lead to better acceptance of the product by consumers, especially in relation to microbiological safety, with a positive reflection on goat raising, the main activity in the semiarid Northeast.

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PERFIL MICROBIOLÓGICO E FÍSICO-QUÍMICO DO LEITE CAPRINO NA REGIÃO SEMIÁRIDA DO VALE DO SÃO FRANCISCO

RESUMO

No Brasil, o maior rebanho de caprinos encontra-se no nordeste, com destaque para Bahia e Pernambuco, no qual são a primeira e segunda Unidade de Federação com o maior efetivo de caprinos. A região semiárida do Vale do São Francisco ainda é carente de estudos para fixar parâmetros para o leite caprino produzido. O objetivo deste trabalho foi caracterizar o perfil microbiológico e físico-químico do leite caprino produzido na região semiárida do Vale do São Francisco em acordo com a legislação

vigente. Quarenta e duas amostras de leite caprino foram obtidas de seis municípios da região semiárida do Vale do São Francisco (Juazeiro, Uauá, Senhor do Bonfim, Curaçá, Jaguarari e Petrolina), sendo sete amostras por município, e pesquisados parâmetros microbiológicos e físico–químicos. As análises microbiológicas consistiram na contagem de: Bactérias Aeróbias Mesófilas Totais (BAMT) e Bactérias do Ácido Lático (BAL), enumeração de coliformes totais e termotolerantes e detecção de *Escherichia coli.* A contagem indireta de célula somáticas também foi realizada. Os testes físico-químicos realizados foram acidez titulável, depressão do ponto de congelamento e teor de gordura pelo método de Gerber. Houve diferença significativa para as análises microbiológicas nos municípios estudados. Nas amostras, as BAL foram predominantes entre os microrganismos examinados e *E. coli* foi detectada em todos os municípios. Para os parâmetros físico-químicos, apenas não houve diferença significativa para a depressão do ponto de congelamento. As características microbiológicas e físico-químicas foram marcadamente separadas através da Análise de Componentes Principais. Petrolina e Juazeiro foram caracterizadas pela maior quantificação da gordura e contagem indireta de células somáticas, respectivamente. Em Uauá, Curaçá, Jaguarari e Senhor do Bonfim destacaram-se os parâmetros: coliformes totais, FDP, *E. coli* e acidez, respectivamente. Os resultados microbiológicos são indicativos da necessidade de uma melhor prática higiênica durante a ordenha para a obtenção de um leite de melhor qualidade.

Palavras-chave: Análise de Componentes Principais. Legislação de leite caprino. Microbiota. Qualidade.

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