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Effect of temperature of storage on the composition and microbiological quality of raw milk

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The aim of the study was to evaluate the quality of refrigerated raw milk from dairy farms located in Southwestern state of Goiás during the rainy and dry seasons. Fresh milk samples were collected from bulk tank and stored for 0, 24, 48 and 72 h for the evaluation of psychrotrophic microorganisms counts, titratable acidity, chemical composition, somatic cell count (SCC) and total bacterial count (TBC). In the rainy season, the average temperature of the refrigerated raw milk samples was 17.4, 6.0, 6.1 and 5.3°C and in the dry period, the average temperature was 9.2, 2.4, 3.8 and 1.4°C for 0, 24, 48 and 72 h of storage, respectively. The physicochemical characteristics of refrigerated milk were consistent with the maximum limits established by Brazilian legislation after storage for 72 h in expansion tanks; however, in the dry period, refrigerated milk should not remain stored for more than 24 h due to the high TBC values. The results of the microbiological analyses revealed failures in the cleaning of equipment and utensils used for milking, demonstrating need for greater hygiene in the collection and maintenance of refrigerated milk at the production source.

Key words: Storage time, mastitis, refrigerated milk, hygiene.

INTRODUCTION

Aspects such as storage of refrigerated raw milk for up to 48 h at temperatures <7°C were established by Normative Instruction 51 (Brazil, 2002), in addition to somatic cell count (SCC) <750,000 SC/mL and total bacterial count (TBC) <750,000 CFU/mL, which remained until 2011. With current Normative Instruction 62, these limits have changed, for somatic cell count are allowed (SCC) maximum of 600,000 SC/mL and total bacterial count (TBC) of 600,000 CFU/mL.

In practice, it has been observed that after the implementation of granelizada milk collection, there is storage for more than 48 h at the source of production because the expansion tanks allow milk storage of the various milking, thereby reducing transportation costs.

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However, maintenance of refrigerated raw milk in expansion tanks for extended periods, provide development of psychrotrophic and proteolytic psychrotrophic count which was found in the study of Santos et al. (2009).

The storage of milk refrigerated in bulk tank is maintained at temperatures <7°C for up to 48 h but does not have enhancing effect on the milk quality since according to Guinot-Thomas et al. (1995), the changes in milk composition (decrease in pH and casein content) caused by the action of proteinases originating from psychrotrophic microorganisms begin when the microbial count reaches between 10^6 and 10^7 CFU / mL, which occurs after four days of storage at 4°C.

The objective of this study was to evaluate the quality of refrigerated raw milk stored for up to 72 h on expansion tanks from farms located in Southwestern state of Goiás during the rainy and dry seasons.

MATERIALS AND METHODS

Sampling

Refrigerated raw milk samples from bulk tank were collected during the rainy and dry seasons directly from expansion tanks installed on farms, whose owners were milk suppliers of a Dairy Industry located in Southwest Goiás, making up a total of 28 samples per season. Seven milk producing farms already established in the bulk collection program of this dairy industry were selected, so that there would not be withdrawals during the trial period. A sample was collected from each producer.

The milk remained stored for a period of 72 h in expansion tank. Milking was performed once a day, and in five farms, cows were milked by hand and in two other farms, milking was mechanical. The farms used had average production of 100 L of milk/day, had crossbred herd with access to Brachiaria pasture in the rainy season and in the dry season, in addition to grazing, animals received sugarcane to complement the diet. In all farms, animals received vaccinations provided by the board of health protection of the State of Goiás.

Milk samples were collected with 0, 24, 48 and 72 h of storage, which were characterized as mixed refrigerated raw milk (reassembly milk) from four milkings. The collection of refrigerated raw milk samples was performed after cooling for at least two hours in the expansion tank. Milk temperature (°C) was measured at the sampling time using a thermometer.

Laboratory analyses

To assess SCC and chemical composition, samples were collected in flasks containing preservative Bronopol®, and for TBC, flasks containing azidiol were used. To assess the psychrotrophic count and titratable acidity, milk samples were aseptically collected using stainless steel collector and stored in amber flasks (± 250 mL). Soon after collection, samples were placed in cool isothermal box containing ice and sent for analysis.

Chemical composition was determined using MilkoScan 4000 equipment and results were expressed as percentage. SCC was held in Fossomatic 5000 Basic equipment and the result was expressed in SC/mL. TBC was analyzed using the BactoScan FC equipment and results were expressed as CFU/ml.

For psychrotrophic count, milk samples were diluted by aseptically pipetting up 25 mL in Erlenmeyer type flask containing 225 mL of 0.1% peptone water (dilution 10^{-1}). From this dilution, decimal dilutions were prepared up to 10^{-6}. About 1 ml of dilutions was added to sterile Petri dishes in duplicate and 15 ml to 17 ml of standard agar for counting were added, molten and cooled to 45°C and homogenized (APHA, 2001). After agar solidification at room temperature, the plates were incubated at 7°C/10 days (Marshall, 1992). Counts were performed in colony counter on plates containing between 25 to 250 colonies. To calculate the number of colony forming units (CFU)/mL, number of colonies on each plate was multiplied by inverse of the inoculated dilution.

For proteolytic psychrotrophic counts, decimal dilutions were prepared as described for the psychrotrophic count. Subsequently, 1 mL of dilutions was added to sterile Petri dishes and 15 mL to 17 mL of milk agar (standard agar plus 10% skimmed milk powder reconstituted to 10%) freshly prepared, melted and cooled to 45°C. Plates were incubated at 21°C/72 h (Marshall, 1992). When plates were read, chemical precipitant was used (10% acetic acid) to identify the presence of proteolysis. Colonies with transparent halo were counted and the number of CFU/mL was calculated by multiplying the number of colonies on each plate by the inverse of the dilution.

For *Pseudomonas* spp. count, decimal dilutions were prepared as described for the psychrotrophic count. After the completion of dilutions, 0.1 ml was added to sterile Petri dishes adding 15 to 17 ml of *Pseudomonas* Agar Base plus 5 ml of glycerol, samples were inoculated in culture medium, spread with Drigalski loop, and immediately incubated at 28°C for 48 h. At the end of this period, reading and interpretation were held (King et al., 1954). The results were expressed as CFU/ml. Titratable acidity was performed according to Brasil (2006) and the results were expressed as grams (g) of lactic acid/100 ml.

Data were submitted to analysis of variance with the following factors being analyzed: season (rainy or dry) and storage time (0, 24, 48 and 72 hours) in a completely randomized design and 2 x 4 factorial arrangement. Bacterial count was analyzed by means of regression models using the Microsoft Excel software. To meet the assumptions of the analysis of variance, variables were transformed using the natural logarithm (ln x) resulting in: ln (psychrotrophic), ln (proteolytic psychrotrophic), ln (Pseudomonas +1), ln (protein), ln (SCC), ln (TBC). These transformations were performed in order to reduce the range of data. Statistical analyses were performed using the SISVAR Software (Ferreira, 2003).

RESULTS AND DISCUSSION

Table 1 show average temperature of refrigerated raw milk samples at the time of sampling, during the rainy season and dry. The temperature of fresh milk samples (zero hour) was higher in the early hours of storage in both the rainy season and in the dry season because at the collection time, the milk had not been completely cooled. According to Fagundes et al. (2004), at the second hour after milking, temperature should be 4°C. According to Brasil (2011), the storage temperature of refrigerated raw milk at the production source should be below 7°C within three hours after milking.

The average titratable acidity results (Table 2) significantly differed between seasons. In the dry season, titratable acidity was higher than in the rainy season; being 0.16; 0.17; 0.17 and 0.17 for 0; 24; 48 and 72 h, respectively, however, during the storage time of up to 72 h at the production source, no significant difference in the

Table 1. Temperature of refrigerated raw milk samples at the time of sampling, during the rainy and dry season.

	Storage (hours)	Rainy (°C)	Dry (°C)
Temperature	zero	17.4	9.2
	24	6.0	2.4
	48	6.1	3.8
	72	5.3	1.4

Table 2. Mean titratable acidity and chemical composition values of refrigerated milk stored for up to 72 h at the production source during the rainy and dry seasons.

Season	Storage (hours)	Titratable acidity	Fat (%)	Protein (%)	Lactose (%)	EST (%)	ESD (%)
Rainy	0	0.16	3.66	3.25	4.50	12.41	8.75
	24	0.16	3.65	3.26	4.46	12.38	8.73
	48	0.15	3.69	3.26	4.45	12.40	8.71
	72	0.16	3.70	3.25	4.42	12.38	8.68
Mean		0.16b	3.68b	3.26a	4.46a	12.39b	8.72b
Dry	0	0.16	4.27	3.30	4.55	13.10	8.83
	24	0.17	3.99	3.35	4.67	13.00	9.01
	48	0.17	3.96	3.35	4.66	12.95	9.00
	72	0.17	3.99	3.29	4.58	12.85	8.86
Mean		0.17a	4.05a	3.32a	4.62a	12.98a	8.93a

Same letters in the same column do not differ statistically from each other at 5% significance. Titratable acidity results are expressed in grams of lactic acid/100 ml of milk.

titratable acidity was found. The results found are within limits established by Brazilian legislation from 0.14 to 0.18 g of lactic acid/100 mL of milk (Brasil, 2011).

During storage of milk for 72 h, no significant changes in the titratable acidity results were found. The collection of reassembly milk resulted in samples with different characteristics every 24 h, but with no changes in the titratable acidity results. Although the titratable acidity results had not differed between seasons, the higher acidity observed in the dry season may be related to the higher bacterial count observed in this period, which resulted in a significant increase in titratable acidity.

There was a significant difference in the fat content (Table 2) of refrigerated milk samples according to the season. During the dry season, the fat content was higher than in the rainy season; however, no significant differences were observed during the storage period. The average fat content obtained in the rainy season may be related to the diet offered to animals, which consisted of *Brachiaria*.

To maintain stable rumen function and prevent depression in content milk fat, NRC (2001) recommends minimum 25% dietary fiber, measured as detergent fiber neutral, with 75% of the total diet being supplied by forage.

The mean protein values (Table 2) observed in this study were higher than those obtained by Noro et al. (2006) (3.10% in the rainy period and 3.17% in the dry season) and Gonzalez et al. (2004) (2.98% in the rainy period and 2.87% in the dry season).

There was no significant difference for the lactose content (Table 2) during storage and between seasons. The average lactose results observed in this study were similar to those obtained by Noro et al. (2006), who reported mean values of 4.46 (rainy season) and 4.55% (dry season).

The average EST results (Table 2) during storage of refrigerated milk for up to 72 h did not differ significantly. The mean EST values were significantly different between seasons, with higher results in the dry season. The mean EST values of this study were higher than the results obtained by Gonzalez et al. (2004), with mean of 12.08% for the rainy period and 12.04% for the dry period and Martins et al. (2006), with mean of 11.41% for the rainy period and 11.24% for the dry season; however, these researchers observed greater EST during the rainy season.

The mean ESD values (Table 2) significantly differed between seasons. There was no significant difference during storage in refrigeration tanks for up to 72 h at the production source. The ESD values of the present study were higher than the results obtained by Gonzalez et al. (2004), which were 8.39 (rainy season) and 8.42% (dry season) and by Martins et al. (2006) who obtained 8.3 (rainy season) and 8.0% (dry season).

The mean chemical composition results of refrigerated

Table 3. Mean SCC and TBC values of refrigerated milk stored for up to 72 h at the production source during the rainy and dry seasons.

Season	Storage (hours)	SCC (CS/mL)	TBC (CFU/mL)
Rainy	0	295.857	136.143
	24	286.857	115.429
	48	305.000	237.143
	72	286.857	421.571
Mean		293.643a	227.572a
Dry	0	498.429	359.429
	24	487.857	1.966.429
	48	472.571	3.371.429
	72	498.857	1.858.286
Mean		489.429a	1.888.893a

Same letters in the same column do not statistically differ from each other at 5% significance.

raw milk samples stored for 72 h at the production source in the different seasons are in line with Brasil (2011), who found minimum fat, protein, EST and ESD contents of 3.0; 2.9, 11.4 and 8.4%, respectively.

Similar results were found by Andrade et al. (2014) where medios fat values (3.48%); protein (3.29%); EST (12.13%) and ESD (8.65%) were seen in the dry hazard. Already in the rainy hazard were found the following results for fat (3.59%); protein (3.31%); EST (12.25%) and ESD (8.66%).

Brazilian legislation for raw milk quality determines storage time of refrigerated milk of up to 48 h in the farm and recommends 24 h as maximum storage time; however, some dairies collect refrigerated milk stored for more than 48 h in expansion tanks. Longer storage time can be attributed to factors such as storage capacity that allows the storage of several milkings and reduction of freight costs due to higher milk volume collected on the farm.

As there are no studies that evaluate the characteristics of refrigerated raw milk stored for more than 48 h on the farm, comparisons used in this study refer to the different seasons. In assessing the milk quality in production systems in Southern state of Rio Grande do Sul, Zanela et al. (2006) reported that only 41.8% of milk samples were within limits established by law, and that the chemical composition standards of milk required by Brazilian law should be revised, considering regional variations.

The average SCC results (Table 3) of refrigerated milk samples did not differ between seasons and storage time; however, higher SCC values can be observed in the dry season, which agrees with Bueno et al. (2005) in the State of Goiás.

The mean SCC values obtained in this research for the rainy and dry seasons are in line with Brasil (2011), who established maximum limit of 600,000 SC/mL. These values were lower than those described by Machado et al. (2000), who evaluated the quality of milk stored in expansion tanks of some regions. These researchers obtained mean value of 641,000 SC/mL, with standard deviation of 767,000 SC/mL, and standard deviation larger than the mean value was attributed to the large variation among herds analyzed. The high SCC values in milk obtained from expansion tanks suggest loss of milk production and low SCC is indicative of good health status of the mammary gland.

Although the TBC values showed a wide variation (Table 3), the results did not differ between seasons and storage time. However, TBC values were higher in the dry season. The mean TBC values in the rainy season, regardless of storage time, were within limits established by Brazil (2011), but in the dry season, the values found from the 24 h of storage were higher than the limit of 600,000 CFU/ml of milk allowed by law. In the dry season, the mean TBC result was high, which would make milk not to be in compliance with requirements of Normative Instruction 62/2011. Similar results were found by Bozo et al. (2013) where the values of SCC and TBC were higher in the dry season.

The TBC values obtained in this study are related to the study by Silveira et al. (2000), who reported that the microbial load present in fresh milk is influenced by the season, production and handling practices on the farm, geographic location, milk temperature and distance between farm and dairy industry.

Storage of raw milk under refrigeration for long periods at the dairy farm and bulk transportation to the processing industry can increase milk TBC because according to Baruffaldi et al. (1984), the bacteriological quality of freshly milked milk are specific to each region, and the mixture of milks of various origins can compromise the quality of the final blend due to the introduction of various microbial levels.

The mean psychrotrophic count (Table 4) significantly differed between seasons, and higher count was observed in the rainy season, but the results were not significant during the storage period. The mean proteolytic psychrotrophic count (Table 4) did not differ

Table 4. Mean psychrotrophic, proteolytic psychrotrophic and *Pseudomonas* spp. count of refrigerated milk stored for up to 72 h at the production source during the rainy and dry seasons.

Season	Storage (hours)	Psychrotrophic	Proteolytic psychrotrophic	*Pseudomonas* spp.
Rainy	0	77.843	42.171	20.496
	24	594.857	66.286	48.371
	48	201.143	96.429	19.906
	72	328.857	400.571	99.344
Mean		300.675a	151.364a	47.029a
Dry	0	13.929	32.786	17.286
	24	35.314	43.929	4.286
	48	530.157	138.129	3.571
	72	500.614	53.286	1.857
Mean		270.004b	67.033a	6.750b

Same letters in the same column do not statistically differ from each other at 5% significance. Results are expressed as CFU/mL.

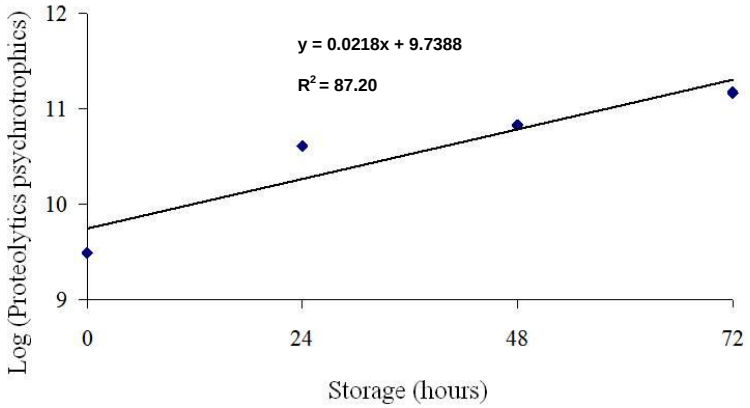

Figure 1. Proteolytic psychrotrophic count of refrigerated milk stored for up to 72 h at the production source during the rainy and dry seasons.

significantly between seasons; however, higher proteolytic psychrotrophic count was observed during the rainy season. The mean *Pseudomonas* spp. count (Table 4) significantly differed between seasons, with higher counts during the rainy season.

The results obtained in this study differ from those obtained by Santos et al. (2013), with higher temperature (4, 7 and 10°C) and storage time (24, 48, 72 and 96 h) greater the counts of psychrotrophic.

The results obtained in this study differ from those obtained by Pinto et al. (2006), who reported a variation from 2.0×10^2 to 1.0×10^7; 5.0×10^1 to 1.2×10^6 and 1.0×10^1 to 3.8×10^6 CFU/mL for psychrotrophic, proteolytic psychrotrophic and *Pseudomonas* spp. count, respectively. According to Fox (1989), psychrotrophic bacteria are apparently not significant as to proteolysis unless the population exceeds 10^6 CFU/mL. The increased proteolytic psychrotrophic bacteria count observed in this study (Table 4) can lead to increased proteolysis in milk and dairy products. According to Vidal-Martins et al. (2005), during storage of UHT milk, increased proteolysis index and apparent viscosity during storage was observed, which could be related to the presence of proteases produced by psychrotrophic bacteria in raw milk.

The psychrotrophic count of milk stored under refrigeration for up to 72 h during the rainy season was higher than the limit of 10% stipulated for this type of microorganism in milk (Brasil, 1980). Among psychrotrophic bacteria, *Pseudomonas* spp. are the predominant spoilage bacteria in refrigerated raw milk, particularly *Pseudomonas fluorescens*. According to Muir (1996), in newly milked milk, *Pseudomonas* spp., are present in about 10% of the total microbiota, but in milk kept under refrigeration, these bacteria have predominance over the other species present in both fresh and processed milk.

The proteolytic psychrotrophic count (Figure 1) resulted in increasing linear behavior (R^2 = 87.20%) during the storage time of refrigerated raw milk at the production

source in the rainy and dry seasons. The result obtained for the proteolytic psychrotrophic count allowed identifying that after milking, this group of microorganisms showed significant growth in the first 72 h of storage.

Firstly, psychrotrophic bacteria are responsible for producing thermostable proteases and lipases, causing significant damage to the dairy chain. Although, the proteolytic activity in milk may be due to enzymes originating from somatic cells (Santos et al., 2003), because according to Santos et al. (2006), milk with high SCC had higher proteolysis rate during storage.

Pedrico et al. (2009) reported that, to meet the requirements of Brazilian legislation, quality policies involving public agencies, technicians and industry should be disseminated due to the need to develop activities aimed at improving the quality of milk.

Conclusions

The average titratable acidity of milk differed significantly between seasons. There was a significant difference in the fat content according to the season, and in the dry season, the fat content was higher than in the rainy season. The mean SCC values obtained in this study for the rainy and dry seasons were within standards required by Brazilian legislation. In the dry period, refrigerated milk should not remain stored for more than 24 h due to the high TBC values. The high occurrence of psychrotrophic bacteria during the rainy season may be related to poor hygiene practices during milking. Thus, for refrigerated milk to meet the requirements of Brazilian legislation regarding TBC, measures aimed at the explanation of the milk production chain in relation to the need for producing milk with adequate sanitary quality and that does not result in public health problems should be adopted.

Conflict of interests

The authors did not declare any conflict of interest.

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