



Influence of milking method, storage conditions and somatic cell counts on the milk quality form tanks



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Abstract:

The objective of this study was to evaluate the influence of the milking method, the storage conditions and the SCC (Somatic Cell Count) increase on the quality of raw milk. Monthly evaluations were performed out over a year in 21 tanks by monitoring the refrigeration temperature and the storage time of the milk in the tank. The tanks were grouped into three temperature levels. Milk storage time intervals were established in each tank: up to 24 h of storage; between 24 and 48 h; and above 48 h. The effect of SCC on the composition was evaluated in three categories: Low SCC; Medium SCC; High SCC. In the analyzed period, 10.8 % presented low SCC, followed by 46.5 % with medium SCC, while 42.7 % had high SCC. There was a positive correlation between

SCC and protein, and a negative correlation between SCC and lactose. It is concluded that the milking method does not influence the microbial contamination of the milk; however, longer storage time and increased temperature influenced an increase in microorganism counts in milk. In evaluating the hygienic/sanitary quality of the milk, 42.7 % had high SCC and the total bacterial counts presented values above the values recommended by legislation.

Key words: TBC, Chemical composition, Hygiene, Temperature.

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Introduction

One of the current requirements of society is for the productive sector to provide food of high biological value, and that is safe and healthy. This requirement directs legislation, research and technology transfer so that these demands are fulfilled, and is directly linked to the competitiveness and profitability of the sector, being of fundamental importance for being able to enter and maintain products in markets⁽¹⁾.

Milk from all mammalian species is a heterogeneous mixture of milk secretion that contains numerous components and exhibits a wide variety of chemical and functional activities⁽²⁾. Its characteristics are associated with physical-chemical parameters and adequate hygienic-sanitary milking standards⁽³⁾. The general health of the herd and in particular that of the mammary gland associated to the milking and milk storage conditions influence milk quality⁽⁴⁾.

The raw milk production process with refrigeration and storage in tanks, facilitates collection logistics and reduces economic losses by acidifying activity of mesophilic bacteria⁽⁵⁾. Mastitis is a common disease in dairy herds and causes high economic losses due to alterations in the secretory tissue of the mammary gland, causing a reduction in the productive life of the animals and modifications to the main milk components⁽⁶⁾.

SCC is considered to be one of the main parameters for assessing milk quality, having a direct relationship with composition, industrial yield and food safety. At higher levels, SCC and TBC are associated with increased enzyme activity which are potentially harmful to milk constituents, resulting in compromised quality and product characteristics⁽⁷⁾.

In view of the relevance of these variables to milk characteristics, the objective of this study was to evaluate the influence of the milking method (manual and mechanical) and the effects of storage conditions (temperature and storage time) and the SCC on the quality of raw milk stored in tanks.

Material and methods

Experiment location and sample collection

All procedures have been conducted in accordance with the guidelines set out by the Ethics Research Committee under CEP/UFRN, license number 2.054.761. Animal experiments were not performed in the present study, as described in Law No. 11.794 of October 8, 2008, which regulates item VII of § 1 of art. 225 of the Federal Constitution establishing procedures for the scientific use of animals; revokes Law No. 6.638, of May 8, 1979; and makes other provisions.

Monthly evaluations were carried out over a year in 21 milk storage tanks (15 individual and 6 collective tanks). Cooled raw milk was collected from tanks of producers linked to the *Associação de Pequenos Agropecuaristas do Sertão de Angicos (APASA)*, located in the semi-arid region of Rio Grande do Norte state, at 5° 39' 56" S, 36° 36' 04" W and 110 m altitude. Dairy animals were crossbred bred in semi-intensive production system. The climate of the region is BSh (arid) according to the Köppen-Geiger classification, presenting temperatures between 25 and 33 °C and average annual rainfall of 753 mm.

The tanks were classified regarding temperature, storage time, milking method (manual and mechanical) and storage form (individual and collective). In order to measure the tank's milk temperature, a previous homogenization was carried out for 5 min, and then the temperature was measured using a digital thermometer with a Mt-350-Minipa laser marker. To standardize the diagnosed temperature ranges, a three-level scaling was performed: Scale 1: 0 °C to 4 °C (ideal temperature); Scale 2: between 4.01 and 7 °C

(intermediate temperature); and Scale 3: above 7 °C (high temperature). According to the obtained temperature measurements, the tanks were grouped into the defined temperature scales and represented by values expressed as a percentage of the tank total and tank type.

In order to standardize the milk storage period, the tanks were grouped into three intervals and represented by values expressed as a percentage of the tanks' total: Interval 1 (up to 24 h of storage); interval 2 (between 24 and 48 h); and interval 3 (over 48 h). The amount of milk collected was checked directly in the tank at the collection time using a stainless steel ruler specifically for this purpose. Information regarding milking method (manual or mechanical) and type of tank (individual or collective) used on the properties were obtained from the database provided by the beneficiary company. The milk collection procedure was performed after homogenization by mechanical stirring. Samples were withdrawn from the tank using a sanitized stainless steel ladle. The milk samples were duly identified, kept at a temperature between 2 °C and 6 °C and then sent to the laboratory.

Physico-chemical and microbiological analysis

Aliquots for evaluating SCC and TBC were packed in 40-mL plastic bottles with Bronopol[®] (2-bromo-2-nitro-1,3-propanediol) and Azidol[®] preservatives, respectively. In order to correlate SCC values with chemical composition, a classification was made according to the values found, giving rise to three categories: Low SCC: SCC <200.000 cells L⁻¹; Medium SCC: 201.000<SCC<400.000; High SCC: SCC>400.000 cells mL⁻¹. SCC and TBC analyzes were carried out in a laboratory integrated to the Brazilian Milk Quality Network (*RBQL*). SCC was determined using the flow cytometry method through a SomaScope[®] electronic counter [Delta, ISO 13366/International Dairy Federation - IDF - 148-2⁽⁸⁾] and the results expressed in one thousand somatic cells per milliliter. TBC was obtained by flow cytometry through a Bactocount[®] electronic counter [Bentley Instruments Inc., ISO 21187/International Dairy Federation - IDF-196], with the results expressed in number of colony forming units per ml. The Fourier Transform Infrared Absorption (FTIR) method through a LactoScope[®] device [Delta, ISO 9622/International Dairy Federation -IDF- 141C⁽⁹⁾] was used in order to determine the fat, protein, lactose, total solids, casein and urea nitrogen contents.

Statistical analysis

To achieve normal data distribution, SCC was also analyzed using the somatic cell count score (SCS) as a result of log transforming the $SCC^{(10)}$, using the equation $SCS = \log_2(SCC/100) + 3$. TBC was transformed into a logarithm (\log_{10} CFU mL), and logarithmic transformation of TBC ($\times 1000$ CFU mL⁻¹) to logTBC (\log_{10} CFU mL) was performed⁽¹¹⁾.

The general mathematical model used was:

$$y_{ij} = \mu + t_i + \varepsilon_{ij}$$

Where:

Y = dependent variables, composition characteristics or milk quality indicator;

μ = general mean;

t = independent variable, SCC classes, where $i = 1$ to 3 ($1 =$ SCC lower than 200.000; $2 =$ SCC between 200.001 to 400.000; and $3 =$ SCC higher than 400.000; or the milking method, with $i = 1$ for manual or $2 =$ for mechanical milking).

ε = random error.

The following analyzes were performed in applying the general mathematical model: analysis of variance and Tukey's test for comparison of means. In addition, Pearson correlation coefficient was also performed between the milk components. The MEANS, GLM and CORR procedures of the SAS version 9.1 statistical package were used for statistical analysis.

Results and discussion

No influence of the milking method was observed on the TBC ($P > 0.05$). The results suggest that both production methods can be used to produce quality milk. However, Franca *et al*⁽¹²⁾ verified greater bacterial contamination in milk obtained by the mechanical milking method when compared to the manual. Even with the results obtained in the present study, it can be inferred that failures in the operation of the milking equipment can cause lesions in the epithelium of the mammary gland, causing mastitis and increased microbial contamination.

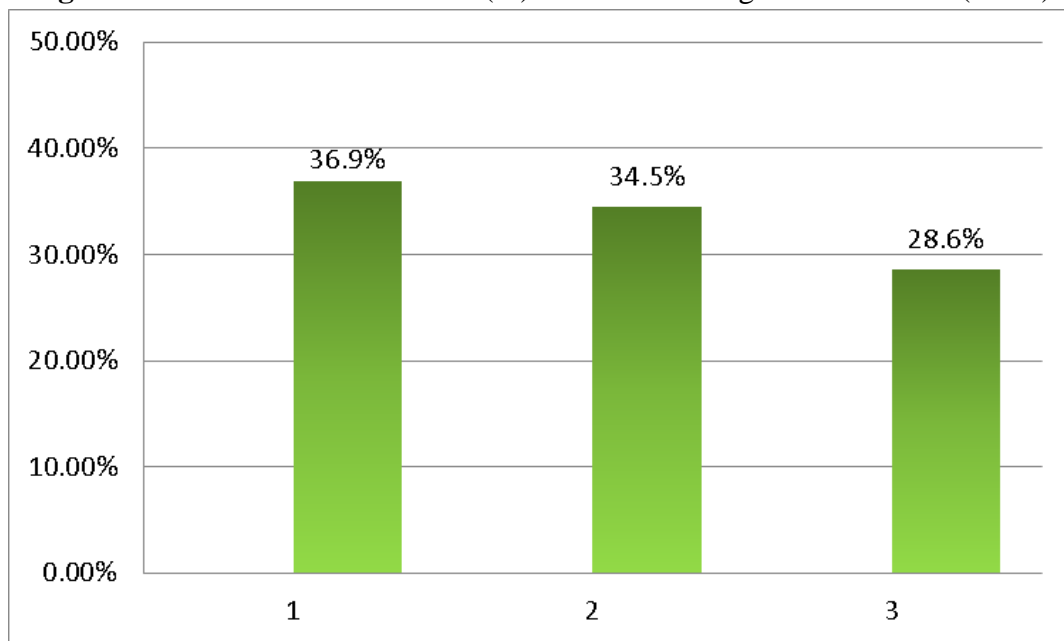
However, regardless of the milking type, the evaluated milk samples presented TBC above the limit recommended by the current legislation⁽¹³⁾ for the region and period of the study. This fact may be a reflection of several factors such as the presence of mastitis, inadequate milking hygiene practices and/or inefficient storage/cooling conditions on the farm, as well as the use of poor quality water⁽¹⁴⁾.

Some workers adopted good milking practices and sanitary measures to control mastitis in dairy farms, and achieved a reduction of more than 90 % TBC and 74.3 % SCC, highlighting the influence of adequate production and health techniques on milk quality⁽¹⁵⁾. According to the established scale, the results showed that the collective tank suffered a lower temperature oscillation than the individual tank.

It was found a positive correlation between temperature and milk bacterial counts and observed that the proliferation of bacteria was higher in tanks with high storage temperature, which shows the importance of maintaining the milk in the tanks at low temperature after milking in order to minimize the microbial growth⁽¹⁶⁾.

Keeping the temperature of the milk at the maximum allowable level represents a critical point for the quality and may have future consequences, since the temperature of the milk in the case of an isothermal truck may increase before reaching its destination. Therefore, even if the legislation allows milk to be stored up to 7 °C, it is important that it be kept at temperatures below 4° C, safeguarding its quality, yield and profitability in the industry. The distribution of the tanks in percentage within the storage time intervals is described in Figure 1.

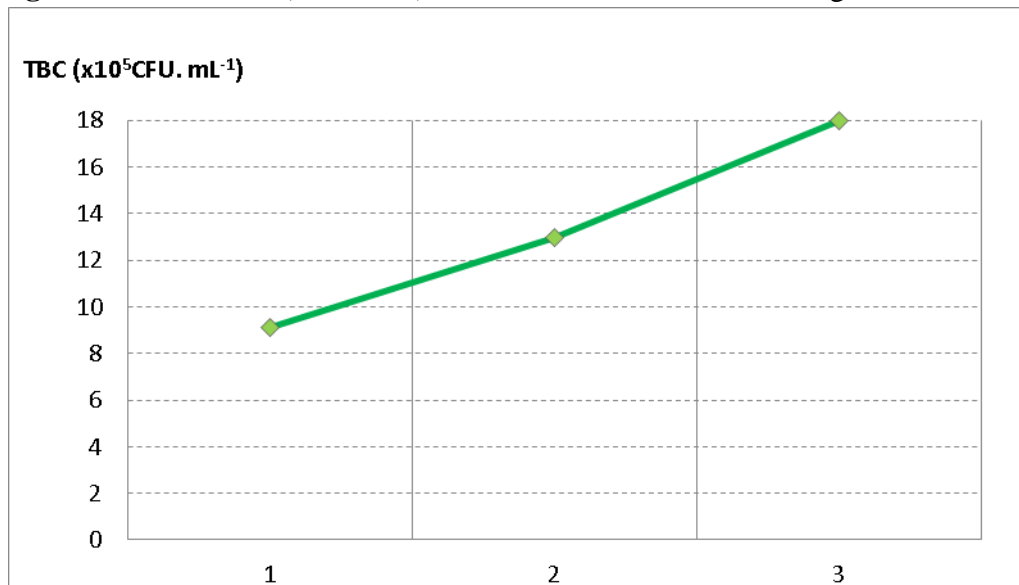
Figure 1: Distribution of the tanks (%) within the storage time intervals (hours)



Intervals 1: up to 24 h of storage (ideal); 2: between 24 and 48 h (intermediate time) and 3: above 48 h (high).

The mean TBC in raw milk from tanks at storage time intervals is shown in Figure 2. There was a significant difference ($P<0.05$) in the TBC of the raw milk according to the storage time. In addition, a positive correlation ($r= 0.24$; $P<0.05$) between storage time and milk TBC was also observed, indicating that the longer storage time results in an increase in microorganism counts in milk.

Figure 2: Mean TBC (CFU mL^{-1}) in raw milk from tanks at storage time intervals



TBC= TBC (x10⁵CFU mL⁻¹); Time intervals - 1: up to 24 h of storage (ideal); 2: between 24 and 48 h (intermediate time) and 3: above 48 h (high).

No difference was observed in the TBC between the first two storage intervals, but there was a significant difference ($P<0.05$) between intervals 1 and 3, demonstrating the importance of a reduced storage period on reduced bacterial multiplication. It is important to note that the TBC values at the three times observed are considered high. The appropriate storage procedure for milk in tanks can minimize the risks of qualitative losses of the raw material that may reflect on the properties and durability of its derivative products⁽¹⁷⁾.

Thus, even when refrigerated at a suitable temperature, the milk storage time in the tank is a determining factor for psychrotrophic microorganism multiplication⁽⁴⁾. CEMPIR These may lead to a decrease in the shelf life of pasteurized milk and derivatives due to the production of thermoresistant microbial lipases and proteases, which is an important control point to be verified by the industry⁽¹⁸⁾.

The chemical composition of milk from tanks is shown in Table 1, which presents descriptive statistical analysis and is in compliance with current legislation.

Table 1: Descriptive statistics of the chemical composition data of tank milk (%)

Variable	N	Mean \pm SD	Minimum value	Maximum value	CV (%)
Fat	273	3.59 \pm 0.55	1.51	7.37	15.24
Protein	269	3.00 \pm 0.20	2.21	3.84	6.72
Casein	269	2.33 \pm 0.36	1.17	5.25	15.26
Lactose	238	4.61 \pm 0.25	3.38	5.05	5.48
Total solids	257	12.11 \pm 0.70	9.32	15.63	5.84
DDE	257	8.51 \pm 0.40	6.57	9.41	4.64

N= number of observations; SD= standard deviation; CV= coefficient of variation; DDE = defatted dry extract (%).

The hygienic-sanitary quality of tank milk in relation to SCC, SCS and TBC is demonstrated in Table 2, which presents a descriptive statistical analysis of the data showing that the TBC is high and the average SCC is in accordance with the current legislation.

Table 2: Hygienic sanitary quality of tank milk in relation to SCC, SCS and TBC

Variable	N	Mean \pm SD	Minimum value	Maximum value	CV (%)
SCC (cells mL ⁻¹)	267	457.0 \pm 314.0	30.0	2532.0	69.0
SCS (cells mL ⁻¹)	267	5.93 \pm 0.64	3.40	7.84	10.73
TBC (CFU mL ⁻¹)	124	1.35 x10 ⁶ \pm 1. 32 x10 ⁶	6.2x10 ⁴	6.23x10 ⁶	97.98

SCC= Somatic cell count; SCS= [$\log_2(\text{SCC}/100.000) + 3$]; TBC= Total bacterial count.

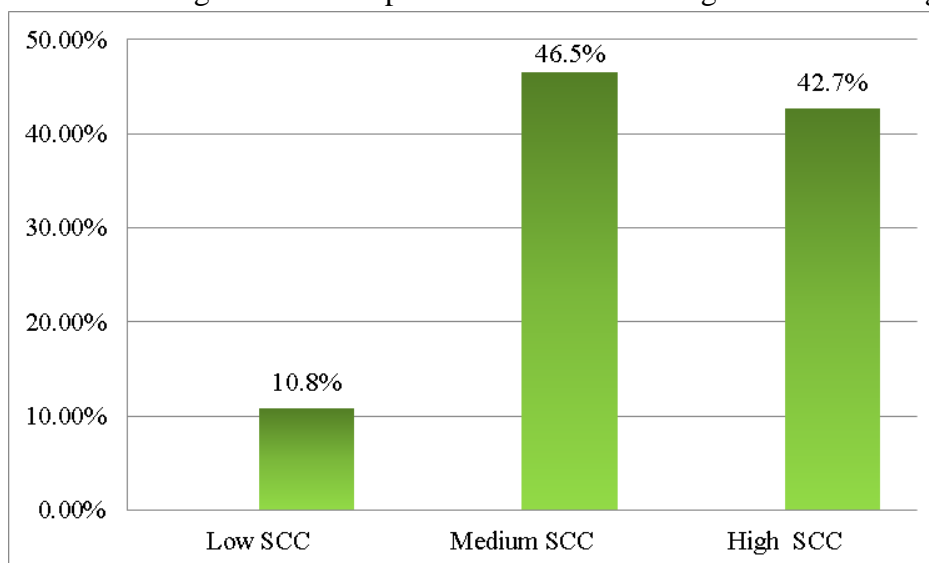
N= number of observations; SD= standard deviation; CV= coefficient of variation.

Data from 44,000 herds throughout Brazil, noted that 62 % had a SCC value of up to 500.000 cells L⁻¹ and approximately 23,760 herds (54 %) had TBC up to 300,000 CFU mL⁻¹(19), with SCC and TBC being in accordance with the legislation in force for the period(20). However, according to the authors, even representing the highest percentage these values indicate that SCC and TBC are high, which leads to losses in industrial yield, losses in the processing and shorter shelf life of the derivatives.

In a similar study(21), they concluded that the tank milk's composition was within the regulated specifications, but the SCC exceeded the allowed limits. Similarly, in studying the characteristics of tank milk(22) found an average SCC of 750,000 cells/mL.

These results demonstrated deficiencies in mastitis control in the studied region in relation to the hygiene procedures for milking, the instruments and the equipment used. In bulk tanks, SCC values are routinely used as indicators for milk quality, the herd health and for managing production practices, and are also related to changes in milk and milk products⁽²³⁾. The SCC analysis results exhibited 10.8 % of samples with low SCC (Figure 3). SCC in tank milk is a general indicator of udder health in a herd and is also considered as an indirect method of measuring milk quality⁽²⁴⁾. An animal is considered infected when SCC in milk is higher than 200,000 cells mL⁻¹. SCC values greater than 200,000 cells. L⁻¹ change the milk components⁽¹⁹⁾.

Figure 3: Percentage of milk samples from tanks according to the SCC categories



Low SCC - $SCC < 200,000$ cells mL⁻¹; Medium SCC - $200,001 < SCC < 400,000$; High SCC - $SCC > 400,000$ cells mL⁻¹.

Among the samples analyzed in this study, more than 42.7 % had high SCC, reflecting inefficiencies in relation to mastitis control and mammary gland hygiene. In addition, producers may not be aware of the extensive damage caused by high SCC, which includes the herd, the raw material, the final product and the consumer. This high count may also be related to a lack of monitoring by the industry, not establishing SCC criteria for receiving milk.

High SCC is related to the type of protein, changes in the composition of fatty acids, lactose, ions, mineral concentration and higher pH of raw milk⁽²⁵⁾, as well as causing a decrease in milk production⁽⁶⁾. These changes in milk components are due to mastitis-induced lesions in the glandular epithelium resulting in reduced synthesis in the breast alveoli and increased influx of blood components into milk such as sodium, chlorine, immunoglobulins, and other serum proteins.

Some authors studied two values of SCC in raw milk and did not find significant effects on the physico-chemical characteristics (pH, titratable acidity, fat, protein, lactose and total solids). In this study it is noteworthy that SCC categories were high despite the difference in milk types⁽²⁶⁾.

In the present study, there was no variation ($P>0.05$) in the chemical composition of the milk among the different SCC categories (Table 3). These data are contrary to others⁽²⁷⁾, who clarified that there is a decrease in the lactose, fat, casein, calcium and phosphorus contents in milk with high SCC due to the increase of proteolytic and lipolytic enzyme activity which are milk degradation processes. The concentration of milk components may occur, leading to an increase in their percentages. This effect is caused by a significant reduction in the volume of produced milk.

Table 3: Mean and coefficient of variation (CV) for milk quality indicators associated with different somatic cell counts (SCC) classes (%)

Variable	N	Low SCC	Medium SCC	High SCC	CV (%)
		Mean	Mean	Mean	
Fat	267	3.61	3.60	3.57	15.34
Protein	267	2.97	2.97	3.03	6.66
Casein	236	2.31	2.32	2.34	15.37
Lactose	255	4.63	4.62	4.59	5.50
Total solids	255	12.14	12.09	12.12	5.88
DDE	255	8.53	8.48	8.54	4.65

N= number of observations; SCC= Low <200.000 cells mL⁻¹; Medium - 200.001<SCC<400.000; High: SCC>400.000 cells mL⁻¹. N= number of observations; DDE= defatted dry extract.
 $P<0.05$).

In a study developed by Savic *et al*⁽²⁸⁾, the values found for the somatic cell quantities of the tank milk in the interval between the minimum value of 58,000 cells mL⁻¹ and the maximum of 516.000 did not significantly affect the protein and lactose content. Different effects from the present research were revealed in other work⁽²⁹⁾ in which they observed the influence of the SCC on the lactose and defatted dry extract percentages when analyzing milks with four levels of SCC (less than 400.000 cells L⁻¹ to greater than 1'000,000 cells L⁻¹).

It was observed an increase in the fat percentage with the increase in SCC in working with tank milk samples collected in the *agreste* region of Rio Grande do Norte, which

may be related to a marked decrease in milk production, increasing the total solids concentration with evidence for the fat, according to the authors⁽⁷⁾.

The results expressed in Table 4 demonstrate that SCC has a positive correlation with protein and negative with lactose. This effect can be explained due to lesions in the alveolar cells, which impaired lactose synthesis and altered the epithelium permeability of the mammary gland, increasing the passage of serum proteins from the blood to the milk, thereby provoking a change in the protein characteristics of milk as reflected by the increase in protein⁽³⁰⁾.

Table 4: Pearson correlation coefficients ($P < 0.05$) between parameters of fat, protein, casein, lactose, total dry extract, defatted dry extract and somatic cell count (SCC)

Variables	Fat	Protein	Casein	Lactose	TS	DDE	SCC
Fat	1.00	ns	ns	ns	0.81	ns	ns
Protein		1.00	0.56	0.38	0.44	ns	0.14
Casein			1.00	0.25	0.28	0.41	ns
Lactose				1.00	0.48	0.84	-0.13
Total solids					1.00	ns	ns
DDE						1.00	ns
SCC							1.00

TS= total solids; DDE = defatted dry extract (%); ns= non-significant.

Conclusions and implications

Inadequate milking procedures, refrigeration and the milk storage time in tanks reflect in negative effects on milk characteristics and hygienic sanitary conditions. Adopting efficient production and management practices aimed at controlling mastitis associated with adequate storage can contribute to improving raw milk quality and increased profitability of the industry.

Acknowledgments

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