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Influence seasons of the year and somatic cell counts on physical-chemical and sensory parameters of milk and *coalho* cheese

Edine Roberta de Lima, Juliana Paula Felipe de Oliveira*, Maria de Fátima Bezerra, Luis Henrique Fernandes Borba, Joadilza da Silva Bezerra and Adriano Henrique do Nascimento Rangel

[1]Unidade Acadêmica Especializada em Ciências Agrárias, Universidade Federal do Rio Grande do Norte, Avenida RN 160, Km 03, 59280-000, Macaíba, Rio Grande do Norte, Brazil. *Author for correspondence. E-mail: jupaula.oliv@yahoo.com.br

ABSTRACT. The objective of this study was to evaluate the influence seasons of the year and somatic cell counts (SCC) on physical-chemical and sensory parameters of cow's milk from individual and collective refrigeration tanks with different milking systems. In addition to analyzing the sensory acceptance of pasteurized milk and *coalho* cheese developed with high (> 800,000 cells mL^{-1}) and low SCC levels (<200,000 cells mL^{-1}). Seasonality did not affect fat content, casein, lactose, total solids, defatted dry extract and SCC, but lower protein levels than May and June were observed from July to September. Based on the SCC results, milk from refrigeration tanks was classified as low SCC and high SCC. The samples were used to obtain pasteurized milk and *coalho* cheese which were sensorially evaluated for general appearance, odor and flavor by a panel of untrained tasters using a hedonic scale of 9 points. Pasteurized milk with high and low SCC presented pleasant sensory characteristics to the consumer up until two days of storage. The same behavior was observed for *coalho* cheese at 20 and 40 days of shelf life, respectively.

Keywords: dairy products; mastitis; milk composition; milk quality; refrigeration tank; shelf life.

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Introduction

One of society's current requirements is for the productive sector to provide food with high biological value, which is safe and has health benefits. Thus, it is essential to focus on quality parameters in milk production systems and effective knowledge of the factors that affect raw materials with regard to the health of the mammary gland that directly interferes with the quality of the final product. Therefore, monitoring milk quality adds value to the entire production chain and increases food safety for consumers, as well is being directly related to competitiveness, profitability, maintenance and market conquests (Henrichs, Macedo, & Karam, 2014; Rangel et al., 2013).

The physical-chemical characteristics of milk as well as its composition can vary due to various factors such as lactation stage, feeding, animal health status and genetic factors. In addition, seasonal variations also have influence (Bernabucci et al., 2015; Larsen et al., 2010). Larsen et al. (2010) found lower fat in summer milk compared to winter milk and attributed these differences to climatic conditions.

SCC levels may increase due to high environmental humidity and temperature (Nasr & El-Tarabany, 2017), and milking procedures (Reyes et al., 2017). The quality of the milk that arrives at the dairy is mainly evaluated by the SCC, an important health indicator of the mammary gland (Li, Richoux, Boutinaud, Martin, & Gagnaire, 2014). High SCC are associated with reduction in yield, production of derivatives and organoleptic changes in milk and milk products, as well as a reduction in shelf life (Andrade et al., 2007; Barbano, Ma, & Santos, 2006). Such alterations occur through the action of proteolytic and lipolytic enzymes present in somatic cells, which can cause rancidity and a bitter taste in dairy products that result in lower sensory acceptance and reduced shelf life (Barbano et al., 2006; Vianna, Mazal, Santos, Bolini, & Gigante, 2008), as well as higher water retention, higher coagulation time, higher moisture content (Bobbo, Cipolat-Gotet, Bittante, & Cecchinato, 2016; Mazal, Vianna, Santos, & Gigante, 2007).

Therefore, the objective of this study was to evaluate the influence of seasonality and SCC on physical-chemical and sensory parameters of cow's milk from individual and collective refrigeration tanks with different milking systems and to analyze the sensory acceptance of pasteurized milk and *coalho* cheese developed with high and low SCC.

Material and methods

Ethics committee

All volunteers signed the Free and Informed Consent Form (TCLE) and answered a questionnaire on the consumption habits of dairy products. All procedures were conducted in accordance with the guidelines set out by the Ethics Committee of the UFRN under license number CAAE – 67250417.9.0000.5537.

Experiment location and sample collection

The raw milk was collected from tanks of producers from the Associação dos Pequenos Agropecuaristas do Sertão de Angicos (APASA), located in the municipality of Angicos, in the semi-arid region of Rio Grande do Norte, at latitude 5° 39' 56" south, 36° 36' 04" west longitude, and 110 meters high. According to the Köppen and Geiger (1928) classification, the climate of the region is BSh, arid, presenting temperatures between 25 and 33°C and average annual rainfall of 753 mm. A data set was developed of Holstein x Zebu lactating dairy cows of different degrees of breeding (1/2 and 3/4).

The collection procedure was performed after homogenization by mechanical stirring, and later aliquot removal from the milk tank was performed using a stainless steel sanitary ladle. Samples were identified and stored in 40-mL plastic bottles containing the preservative Bronopol® (2-bromo-2-nitro-1,3-propanediol), stored at 2°C to 6°C and sent to a laboratory integrated in the Brazilian Milk Quality Network (RBQL) for the purposes of SCC analysis performed by flow cytometry using Somacount 300® equipment (Bentley Instruments Inc., Chasca MN, USA).

The samples were submitted to Fourier transform infrared (FTIR) absorption using Dairy spec® equipment (Bentley Instruments Inc Chasca MN, USA) for determining the fat, protein, casein, lactose, total solids and defatted dry extract (DDE). The composition analyzes were carried out at the Milk Quality Laboratory of the Federal University of Rio Grande do Norte (LABOLEITE-UFRN).

Milk processing and *coalho* cheese production

Milk cooling tanks were classified as low SCC (less than 200,000 cells mL^{-1}) and high SCC (greater than 800,000 cells mL^{-1}) from the SCC results, and the respective samples were pasteurized (72°C/15 s). The milk was then stored under refrigeration until sensory evaluation.

For *coalho* cheese production, milk samples with low SCC (less than 200,000 cells mL^{-1}) and high SCC (greater than 800,000 cells mL^{-1}) were used. Milk was submitted to slow pasteurization (65°C/30 min) and then the temperature was adjusted to 35°C. The rennet was added and maintened for around 40 min after homogenization. When the curding point was reached, it was cut and he curd heated under stirring to 45°C. Next, the whey was partially removed and the mass was salted, pre-pressed and shaped. In the proper form, the mass was subjected to pressing and turning. The cheeses were packaged and stored below 4°C.

The raw material, ingredients and packaging used to produce the cheese were handled according to the good manufacturing practices (GMPs) for dairy products. Pasteurized milk and *coalho* cheese were wrapped in black plastic bags, which were used to prevent oxidation from light components, and stored in a refrigerator at temperature below 4°C until the day of the respective sensory analyzes, according to the adapted methodology in Ma et al. (2000).

Sensory analyses

An affective acceptance test was performed for the sensory analysis. A total of 92 untrained panelists aged 18 to 60 years were invited. The panelists were recruited on the basis of voluntary consent and reported not allergic reactions to milk or dairy products. They were subsequently instructed regarding the test procedure and how to fill in the answer sheet before starting the evaluation. The test was conducted at the Academic Unit Specialized in Agrarian Sciences (EAJ-UFRN, in Macaíba, Rio Grande do Norte state).

At the time of the sensory analysis, the samples were conditioned in iceboxes and kept under refrigerated temperature until they were served to the tasters to ensure food safety, consumer health and ideal conditions for sensory tests.

The panelists judged the appearance, odor and flavor attributes of pasteurized milk after two days of storage (4°C) and cheese at 20 and 40 days of shelf life. The sensory evaluation of the milk samples and *coalho* cheese was performed using a 9-point verbal hedonic scale structured with extremes of 1 (I really disliked it) to 9 (I liked it very much) to evaluate the overall acceptance, aroma and flavor according to Meilgaard, Civille, and Carr (1991).

Samples were made available randomly in a 50 mL white disposable cup, coded with three-digit random numbers. Room temperature water and crackers without salt were provided to the tasters to remove the residual flavor between samples.

Statistical analysis

The data was submitted to analysis of variance, descriptive analysis and the differences between the treatments were compared using the Tukey test, adopting 0.05 as critical level of probability for the type I error. Statistical Analysis System (SAS, 2004) statistical package was used for the analyses.

Results and discussion

Descriptive statistics of physical-chemical parameters and milk SCC

Table 1 presents a descriptive analysis of the milk's physical-chemical characteristics and SCC. The SCC presented a high coefficient of variation which indicated great variability of this count in the analyzed samples. Among the milk components, fat and casein had the highest coefficients of variation. The components that showed the lowest variation were lactose, total protein and DDE. The variation source of these components may be genetic, physiological and environmental, and therefore are very variable (Machado, Pereira, & Sarríes, 2000).

Observing the average values of milk composition and SCC (419,070 cells mL^{-1}), it can be verified that values were compatible with the current legislation for the Brazilian Northeast region regulated by IN-62 of the Ministry of Agriculture, Livestock and Supply (MAPA) (Table 1). Milk with high solids content is of great interest to the industry because it increases the yield of dairy products (Montanhini, Moraes, & Montanhini Neto, 2013).

Seasonal effects on physical-chemical parameters and SCC of milk

Total protein levels in the months of August and September were lower than May and June (Table 2). No variations were observed during the evaluated period for the components fat, casein, lactose, total solids and defatted dry extract (Table 2), which is compatible with the results found by Silva et al. (2016) in herds of the same region.

Such behavior may be a reflection of the climatic conditions of the semi-arid region during the hottest seasons, with low precipitation (Menezes, Sampaio, Giongo, & Pérez-Marin, 2012) during the collection months. Even with this reduction in total protein, it is important to note that the mean values observed were in accordance with the standards required by the legislation, which stablish more than 2.9% total protein in raw milk.

In order to observe the seasonality influence on the milk composition, (Roma Júnior, Montoya, Martins, Cassoli, & Machado, 2009) also observed variation in total protein levels in cattle located in the states of Minas Gerais and São Paulo. In state of Rio Grande do Sul, Noro, González, Campos, and Dürr (2006) observed that the protein content was lower in the summer months.

Table 1. Descriptive statistics with the of physical-chemical characteristics and somatic cells counting (SCC, in 1000 mL^{-1}) in cow's milk.

Characteristics	N	mean ± *SD*	CV	Range
Fat (%)	101	3.51 ± 0.46	13.11	2.33 - 5.51
Total protein (%)	101	2.92 ± 0.18	6.17	2.24 - 3.38
Casein (%)	101	2.29 ± 0.34	14.99	1.17 - 5.25
Lactose (%)	101	4.73 ± 0.20	4.28	3.85 - 5.05
Total Solids (%)	101	12.06 ± 0.61	5.04	10.24 - 13.73
DDE (%)	101	8.55 ± 0.35	4.14	7.13 - 9.35
SCC	95	419.07 ± 287.50	68.60	30.00 - 1338.00

DDE = Defatted Dry Extract. Number of information (N), standard deviation (SD), coefficient of variation (CV, in%), minimum (MIN) and maximum (MAX) values (range).

Table 2. Comparison of physical-chemical parameters and somatic cell counts of cow's milk between May and September.

Parameters	Months				
	May	June	July	August	September
Fat (%)	3.55 ± 0.55^{a}	3.58 + 0.36^{a}	3.47 ± 0.35^{a}	3.41 ± 0.46^{a}	3.57 ± 0.55^{a}
TP (%)	3.00 ± 0.24^{a}	3.01 ± 0.13^{a}	2.94 ± 0.12^{ab}	2.84 ± 0.14^{b}	2.84 ± 0.15^{b}
Casein (%)	2.30 ± 0.29^{a}	2.48 ± 0.68^{a}	2.28 ± 0.10^{a}	2.20 ± 0.11^{a}	2.20 ± 0.13^{a}
Lactose (%)	4.68 ± 0.25^{a}	4.74 ± 0.18^{a}	4.76 ± 0.22^{a}	4.73 ± 0.19^{a}	4.75 ± 0.18^{a}
TS (%)	12.13 ± 0.74^{a}	12.22 ± 0.53^{a}	12.05 ± 0.47^{a}	11.86 ± 0.61^{a}	12.08 ± 0.64^{a}
DDE (%)	8.58 ± 0.47^{a}	8.65 ± 0.28^{a}	8.59 ± 0.35^{a}	8.45 ± 0.32^{a}	8.48 ± 0.32^{a}
SCC (mil mL^{-1})	361.33 ± 339.25^{a}	490.89 ±256.99^{a}	435.58 ±290.23^{a}	411.59 ±281.08^{a}	405.75±270.73^{a}

Results are presented as means ± standard deviation. a,b: Means in the same line followed by different letters are significantly different by HSD Tukey's test ($p < 0.05$). TP = Total Protein; TS = Total Solids; DDE = Defatted Dry Extract; SCC = Somatic Cell Count.

The mean SCC concentration did not differ across the evaluation months (Table 2), however, Alberton et al. (2012) also did not find significant difference in the SCC analysis in relation to the season.

The SCC from the tank is an important resource for monitoring milk quality and the mammary gland health in the herds, since it indicates the occurrence of subclinical mastitis and possible economic losses resulting from them (Hogeveen, Huijps, & Lam, 2011). It is important to reiterate that the values can reach a maximum of 400,000 cells mL^{-1}, according to the current legislation regulated by the IN-62.

Physical-chemical evaluation and SCC in milk cooling tanks from manual and mechanical milking systems

Storage tanks and different milking systems influenced the concentrations of total protein, lactose, total solids, defatted dry extract and SCC. The milk from individual tanks with manual milking (ITMA) presented ~~a~~ higher total protein and total solids ($p < 0.05$) than that stored in collective tanks with manual milking (CTMA), and similar to individual tanks with mechanical milking (ITME) (Table 3). The milk samples from collective CTMA and ITME presented similar DDE percentage to each other and lower than ITMA.

The SCC in the individual refrigeration tanks in farms that use mechanical milking reached levels considered high by the literature (Emanuelson & Nielsen, 2017; Rodrigues, Lima, Canniatti-Brazaca, & Bicalho, 2017). The mean SCC values for the ITMA and CTMA were within the value recommended by the current legislation. These findings oppose studies that pointed out that SCC in refrigerated tanks with manual milking cattle is superior to mechanically milked (Alhussien & Dang, 2018; Reyes et al., 2017). The SCC of tank milk is a general indicator of udder health in a herd and is also considered as an indirect method of measuring milk quality (Schukken, Wilson, Welcome, Garrison-Tikofsky, & Gonzalez, 2003).

In a similar study, Campos et al. (2016) concluded that the tank milk composition was within the regulated specifications, but the SCC exceeded the described limits. Similarly, Rangel et al. (2013) described average SCC values of 750,000 cells mL^{-1}, when studying tank milk characteristics. This result can be attributed to deficiencies in milking hygienic procedures, cleaning milking equipment and/or the absence of programs to improve milk quality and mastitis control in the studied region.

Table 3. Physical-chemical parameters and somatic cell counts of cow's milk according to the type of refrigeration tanks (individual and collective) in different milking systems (manual and mechanical).

Parameters	Milking system	Refigeration Tank	
		Individual	Collective
Fat (%)	Mechanical,	3.45 ± 0.28^{a}	-
	Manual	3.52 + 0.55^{a}	3.54 ± 0.37^{a}
Total Protein (%)	Mechanical	2.96 ± 0.10^{a}	-
	Manual	2.96 ± 0.16^{a}	2.83 ± 0.21^{b}
Casein (%)	Mechanical	2.30 ± 0.08^{a}	-
	Manual	2.35 ± 0.42^{a}	2.17 ± 0.24^{a}
Lactose (%)	Mechanical	4.64 ± 0.12^{b}	-
	Manual	4.83 ± 0.13^{a}	4.60 ± 0.26^{b}
Total Solids (%)	Mechanical	11.94 ± 0.43^{ab}	-
	Manual	12.23 ± 0.59^{a}	11.84 ± 0.67^{b}
DDE (%)	Mechanical	8.48 ± 0.19^{b}	-
	Manual	8.70 ± 0.25^{a}	8.30 ± 0.46^{b}
SCC (1000 mL^{-1})	Mechanical	803.16 ± 326.94^{a}	-
	Manual	381.96 ± 183.78^{b}	266.33 ± 110.11^{b}

Results are presented as means ± standard deviation. a,b: Means in the same line followed by different letters are significantly different by HSD Tukey's test ($p < 0.05$). DDE = defatted dry extract; SCC = somatic cell count.

Sensory evaluation of pasteurized milk and *coalho* cheese

The parameters related to sensory acceptance of pasteurized milk (general appearance, odor and flavor) was not influenced by SCC levels (Table 4), where the averages remained between 6.74 (slightly liked) and 7.12 (moderately liked). These results corroborate with Corassin, Rosim, Kobashigawa, Fernandes, and Oliveira (2013), who reported that different SCC levels did not alter sensory parameters in milk.

On the other hand, the *coalho* cheese samples (Table 5) presented similar values for the appearance and odor attributes. Only cheese with low SCC at 40 days of shelf life presented a significant difference ($p < 0.05$) for the flavor, with a lower value (6.05-slightly liked) when compared to cheese samples with high SCC (moderately liked). Flavor changes are probably due to lipolysis and proteolysis which became more intense over the storage period, thereby producing undesirable flavors and low sensory acceptance, as observed in pasteurized milk by Ma et al. (2000) and in prato cheese by Vianna et al. (2008).

Studies indicate that high SCC in milk can affect the composition of dairy products, and their technological and sensory properties. For example, Ma et al. (2000) found that pasteurized milk with high SCC concentration had faster lipolysis and proteolysis rates, and functional and sensory changes such as bitter and rancid taste were observed under refrigeration after 14 days of storage. Sert, Mercan, Aydemir, and Civelek (2016) observed that high SCC reduced the solubility, dispersibility and wettability of milk powder. In cheeses, studies indicate a longer time of coagulation, greater syneresis and less sensory acceptance due to defects in texture and taste (Bobbo et al., 2016; Vianna et al., 2008).

However, the present work shows that pasteurized milk with high SCC maintained its sensory qualities. In addition, *coalho* cheese made from high SCC milk remained sensorially enjoyable for consumption even after 40 days of refrigerated storage. Pasteurization possibly reduced the damage caused in the milk quality and *coalho* cheese, not presenting alterations in the evaluated sensory aspects. These findings are in agreement with results obtained by Li et al. (2017). The authors checked different amounts of somatic cells using microfiltration technology to monitor their presence in the cheese matrix. The study showed that although there is an increase in lipolysis and proteolysis, SCC did not reduce the final cheese quality.

Table 4. Sensory evaluation of pasteurized of cow's milk with low and high somatic cell count (SCC).

Group	General appearance	Odor	Flavor
Low SCC	7.03 ± 1.25^{a}	$6.97 + 1.41^{a}$	6.76 ± 1.71^{a}
High SCC	7.12 ± 1.41^{a}	6.74 ± 1.58^{a}	6.88 ± 1.78^{a}

Results are presented as means ± standard deviation.

Table 5. Mean and standard deviation obtained from *Coalho* cheese of cow's milk with low and high somatic cell count (SCC) in a sensory evaluation.

Group	Shelf life (days)	General appearance	Odor	Flavor
Low SCC	20	7.25 ± 1.26^{a}	$6.12 + 1.43^{a}$	6.68 ± 1.73^{ab}
	40	7.00 ± 1.42^{a}	$6.36 + 1.69^{a}$	6.05 ± 2.06^{b}
High SCC	20	7.38 ± 1.20^{a}	6.62 ± 1.45^{a}	7.29 ± 1.49^{a}
	40	7.06 ± 1.40^{a}	6.13 ± 1.65^{a}	7.28 ± 1.23^{a}

Results are presented as means ± standard deviation. a,b: Means in the same column followed by different letters are significantly different by HSD Tukey's test ($p < 0.05$). Low SCC < 200,000 cells mL^{-1}; High SCC > 200,000 cells mL^{-1}.

Conclusion

The season of the year did not affect most of the physical-chemical parameters or SCC, only causing small reductions in total protein in the warmer months. Regarding the milking method, milk quality was influenced by presenting higher SCC values in mechanical milking. Pasteurized milk with high and low SCC presented pleasant sensory characteristics to the consumer up until two days of storage. The same behavior was observed for *coalho* cheese at 20 and 40 days of shelf life, respectively.

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