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Seasonal variation, method of determination of bovine milk stability, and its relation with physical, chemical, and sanitary characteristics of raw milk

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ABSTRACT - The objective of this research was to determine the variation of milk stability evaluated with ethanol, boiling, and coagulation time tests (CTT) to identify milk components related with stability and verify the correlation between the three methods. Bulk raw milk was collected monthly at 50 dairy farms from January 2007 to October 2009 and physicochemical attributes, somatic cell (SCC), and total bacterial counts (TBC) were determined. Milk samples were classified into low, medium, and high stability to ethanol test when coagulation occurred at 72 °GL, between 74 and 78 °GL, and above 78 °GL, respectively. Univariate analysis was performed considering the effects of year, months, and interaction in a completely randomized design. Principal factor analysis and logistic regression were done. There was an interaction between months and years for stability to the ethanol test and coagulation time. All samples were stable at the boiling test. Boiling test was not related to ethanol and coagulation time tests. Coagulation time was weakly but positively correlated with ethanol test. Broken line analysis revealed that milk stability measured with CTT and ethanol tests decreased sharply when SCC attained 790,000 or 106 cell/mL of milk, respectively. Milk stability measured with ethanol test decreased when TBC was higher than 250,000 cfu/mL, while there was no inflexion point between TBC and stability measured with CTT. Milk with high stability presented lower values for acidity, TBC, and SCC but higher values for pH, lactose, protein, and CTT compared with low-stability milk. Due to the execution easiness, single-point cut-off result and low cost, we do not recommend the replacement of ethanol test for boiling or coagulation time test.

Key Words: milk composition, seasonality, somatic cell count

Introduction

Stability of milk is a multifactorial phenomenon, which can be influenced by milk acidity, pH, composition of casein micelles, ionic calcium concentration, among others (Lewis and Deeth, 2009; Fischer et al., 2012; Horne, 2015). Milk stability has been accessed by tests, such as ethanol, boiling, sedimentation and viscosity, and coagulation time. In Brazil, milk stability is estimated by the ethanol test performed at the farm and dairy plant platform (Brasil, 2011).

Milk with low stability to ethanol test (coagulation at 72 °GL) is considered unsuitable for industrial procedures involving heating and should not be transported to the industry (Brasil, 2011). Although usage of single-point ethanol test for grading milk to thermal process is not used in western developed countries, it is still used in Latin America, Africa, and Far East (Lin et al., 2009; Kassa et al., 2013; Horne, 2015; Rathnayake et al., 2016). The main reason is because it represents a simple, rapid, practical, and low-cost test in regions characterized by large number of dairy farms and low technology, rendering other more sophisticated methods impractical (Horne, 2015).

Discussion about the suitability of indirect test to estimate milk stability still persists. Previous studies have shown that milk ethanol stability is weakly related to coagulation time test (CTT) (Molina et al., 2001) and milk traits associated to them are quite different (Chavez et al., 2004). Milk ethanol stability is related to the ionic strength, potassium, and chloride contents while coagulation time is related to pH, urea, and phosphorus contents. Both tests are related to ionic calcium (Negri et al., 2001). These authors

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collected milk samples in autumn and spring and only considered milk with less than 100.000 cfu/mL.

It might be worth evaluating how milk stability varies during the whole year and how milk stability varies when milk with wider range of sanitary and hygienic-related traits is considered, even more considering the high values of somatic cell count and total bacterial count in milk produced in Brazil and other developing countries. Therefore, it may be worth studying how milk stability changes between and within years, if this variability is related to other milk traits, and how the ethanol test correlates with coagulation time and boiling tests. The study was carried out to determine the seasonal variation of milk stability evaluated with ethanol, boiling, and coagulation time tests, identify milk components related with the results, and verify the correlation among the three methods.

Material and Methods

The experiment was conducted between January 2007 and October 2009 in the northeast region of Rio Grande do Sul, Brazil. Raw bulk milk samples from 50 dairy farms were evaluated once a month, totaling 1700 milk samples. All producers sold their milk to a local industry and were randomly selected within all milk production extracts. Approximately, a volume of 200 mL of raw milk was collected from cooling bulk tanks at each milk supplier into 250-mL milk sampling bottles, and immediately transferred to the laboratory under cooling conditions for further analysis. Milk samples were divided into two aliquots. The first one was used to determine total bacterial count (TBC) and somatic cell count (SCC) by flow cytometry and milk chemical composition (lactose, protein, fat, and total solids) by infrared radiation spectrophotometry (Fonseca and Santos, 2000). The second aliquot was analyzed for: a) titratable acidity and pH by potenciometry; b) boiling test: 3 mL of milk was placed into a test tube and heated until the boiling point, for three times for each sample, followed by the visual inspection of clot formation. Clot formation was considered as a positive result in the test, otherwise the result would be considered negative; c) stability to the ethanol test: equal amounts of milk and solution with ethanol concentration varying from 68 to 80 ºGL were mixed in a Petri dish until visual clot detection. Milk stability was settled as the minimal ethanol concentration, which induced clot formation. Milk samples with absence of clot formation when mixed with solution with 80 °GL ethanol were considered unstable at 81 °GL; and d) coagulation time test, executed between November 2008 and October 2009. Each milk sample was inserted into an individual glass capillary with 120 mm of length, 0.15 mm of external diameter, and 0.08 mm of internal diameter. Both ends of the capillary were closed. After that, the capillary was immersed in glycerin at 145 °C; the coagulation time was the time elapsed between the immersion and the visualization of clots inside the capillary (Negri et al., 2001).

Farms were considered as experimental units. Data about ethanol test were analyzed according to a completely randomized design in a factorial arrangement (months and years) with repeated measurements, using the SAS software (Statistical Analysis System, version 9.3) and applying the procedure PROC MIXED (ANOVA), considering the effects of months and tests of means (LSmeans) for significant variables. P-values (<0.05) were considered significant. Year and months were considered as fixed effects, while farms were the random term and months the repeated measurements.

The statistical model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + f_k + e_{ijk},$$

in which Y_{ijk} is observation made for each farm k on each month i on year j; μ is overall mean; α_i is the effect of the months i; β_j is the effect of the year j; $(\alpha \times \beta)_{ij}$ is the effect of the interaction between years and months; f_k is the random effect of farm k; and e_{ijk} is random error associated with each observation.

Because of missing data, analysis for CTT was performed just considering the year of 2009 and the effect of months.

The statistical model used was:

$$Y_{ij} = \mu + \alpha_i + f_j + e_{ij},$$

in which Y_{ij} is observation made for each farm j on each month i; μ is the overall mean; α_i is the fixed effect of the months i; f_j is the random effect of farm; and e_{ij} is random error associated with each observation.

The boiling test values were registered as a binomial variable (presence or absence of coagulated milk) and further analyzed using NPAR1WAY procedure of SAS. Values of SCC and TBC were converted using logarithmic transformation ($\log_{10}$) to homogenate variance and fit normal distribution.

Complementarily descriptive analysis was done using UNIVARIATE and FREQ procedures of SAS to calculate mean, mode, median, maximal, and minimal values. Correlation among methods of stability determination was performed using PROC CORR (coefficient of Spearman) of SAS.

The principal factor analysis (PROC FACTOR) was performed with seven milk traits and the msa option to measure the adequacy of the traits was used in the statistical procedure to select the variables.

Logistic and broken line regressions were calculated to determine the limiting ethanol concentrations used to

test milk stability, in which inflexion in the milk traits such as pH, TBC, and SCC occurred. Logistic and broken line regressions were performed using NLIN and LOGISTIC procedures of SAS, respectively.

The model used for the broken regression line was:

$$Y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 (x_{i1} - x)\delta_i + \varepsilon_i,$$

in which $\delta_i = 1$, if $x_{i1} > x$ and 0, if $x_{i1} < x$; y_i is ethanol stability; x is a milk trait such as pH, TBC, or SCC; and β is the regression coefficient.

The model used for the logistic regression was:

$$\text{logit}(p) = \beta_0 + \beta_1 x_{i1},$$

in which (p) is the probability of occurrence of unstable milk (LINA); x is the independent variable; and β is the regression coefficient. Milk was considered unstable when it precipitated in solution with 72 °GL or less.

To access the differences of composition between milk samples with distinct stability, milk samples were classified into low, medium, and high stability to ethanol test when coagulation occurred at 72 or less than 72 °GL, between 74 and 78 °GL, and above 78 °GL, respectively. Milk attributes were subjected to analysis of variance according to stability classification using GLM procedure of SAS and tests of means (LSmeans) for significant variables. P-values were considered significant at <0.05.

The statistical model used was:

$$Y_i = \mu + \alpha_i + e_i,$$

in which Y_i is observation made for each stability i; μ is the overall mean; α_i is the effect of the stability class; and e_i is the experimental error.

Results

Descriptive statistics of the raw data revealed that, from the 50 dairy farms, 78.4% of the farmers had low level of formal education as they studied until the eighth school grade, 72% of the farms allocated less than 15 ha to the dairy activity, and all herds were composed by predominantly Holstein breed. Milking was done manually in 25% of the farms. Most of the farmers (91.3%) executed both milking and feeding practices. Feeding practices were diversified, but it could be noticed that in 97% of the farms, corn silage was used, 82% of the farmers used commercial concentrate sold by the dairy industry with 20 to 22% crude protein, 68% of the farms cultivated warm (pearl millet) and cold season pastures (black oats), but only 5% used hay. Practices for prevention against mastitis, such as pre- dipping and post-dipping were adopted by 12 and 74% of the farmers, respectively, while clinical (stripping milk into a strip cup) and subclinical mastitis tests were adopted by 45% of farmers. Milk was cooled in bulk tanks in all farms evaluated.

Overall milk stability to the ethanol test was 74.8 °GL (Tables 1 and 2). However, approximately 35% of milk samples were unstable in 72 °GL ethanol and 81% were unstable in 78 °GL ethanol. Milk stability to ethanol test varied among months within the years 2007 and 2009 (P<0.05), but did not significantly vary in 2008. In 2007, minimal values were registered in January (73.86 °GL), February (74.06 °GL), April (74.84 °GL), and November (74.02 °GL), while maximal values were found in August (78.12 °GL), July (76.76 °GL), and September (76.08 °GL). In 2009, minimal values were registered in January (73.53 °GL) and February (73.47 °GL), while maximal values were observed in July (76.15 °GL), August (76.13 °GL), and September (76.31 °GL).

All samples were stable in the boiling test regardless of year and months within year of evaluation. Overall mean for milk CTT was 5 min but it varied from 0.2 to 30.0 minutes (Table 1).

Stability of milk to the ethanol test was moderately and positively correlated with CTT (r = 0.28, P<0.0001,

Table 1 - Descriptive analysis of the physical-chemical and sanitary attributes of milk produced by 50 farmers in the northeast region of Rio Grande do Sul from 2007 to 2009

Attribute	n	Mean	Median	Mode	Amplitude	CV (%)
pH	1583	6.8	6.8	6.8	6.3-7.0	1.2
Titratable acidity (°D)	1583	15.9	16.0	15.0	13-19	8.6
Ethanol (°GL)	1700	74.8	76.0	78.0	68-81	5.6
CTT (min)	588	5.04	4.42	3.45	0.2-30.0	56.2
Boiling test[1]	1700	0	0	0	0	0
Log_{10} (TBC)[2]	1569	6.0	6.0	5.5	4.4-7.2	10.0
Log_{10} (SCC)[2]	1583	5.8	5.8	5.7	4.5-6.6	5.6
Lactose (g/kg)	1583	44.5	43.8	44.5	32.0-48.0	4.2
Protein (g/kg)	1583	31.0	30.9	30.1	21.0-39.0	6.2
Fat (g/kg)	1583	38.5	37.4	37.8	21.0-57.0	23.2

CTT - coagulation time test; TBC - total bacterial count; SCC - somatic cell count; CV - coefficient of variation.
[1] Boiling test: frequency of precipitation following boiling.
[2] Values of total bacterial count and somatic cell count following log_{10} transformation.

n = 588), pH (r = 0.28, P<0.0001, n = 1700), lactose (r = 0.31, P<0.0001, n = 1621), and protein (r = 0.1, P<0.0001, n = 1621), but moderately and negatively correlated with titratable acidity (r = –0.22, P<0.0001, n = 1700) and log_{10}TBC (r = –0.20, P<0.0001, n = 1615) and weakly related with log_{10}SCC (r = –0.07, P = 0.0069, n = 1621). Coagulation time test was positively related with pH (r = 0.20, P<0.0001, n = 591) and lactose (r = 0.2, P<0.0001, n = 591), but negatively correlated with titratable acidity (r = –0.17, P<0.0001, n = 591), log_{10}TBC (r = –0.15, P<0.0003, n = 591), and log_{10}SCC (r = –0.13, P = 0.0019, n = 591).

Principal factor analysis confirmed previous correlations mentioned before, as it highlighted a positive association between stability measured with ethanol test, CTT, pH, and concentration of lactose and a negative association of milk stability (ethanol and CTT) with acidity and TBC (Figure 1).

Broken line analysis revealed that milk stability values measured with CTT and ethanol test decreased sharply when SCC values attained 790,000 or 1,000,000 cell/mL of milk, respectively. Besides, milk stability measured with ethanol test decreased when TBC was higher than 250,000 cfu/mL, while there was no inflexion point between TBC and stability measured with CTT. In addition, inflexion points for milk stability measured with CTT and ethanol test were observed at pH value of 6.65, decreasing with lower pH values. There was not an inflexion point between CTT and ethanol test, which was confirmed by the linear relation between the two methods of predicting milk stability (CTT = 0.12x – 4.1, R^2 = 0.05, X = concentration of ethanol, °GL; P<0.0001).

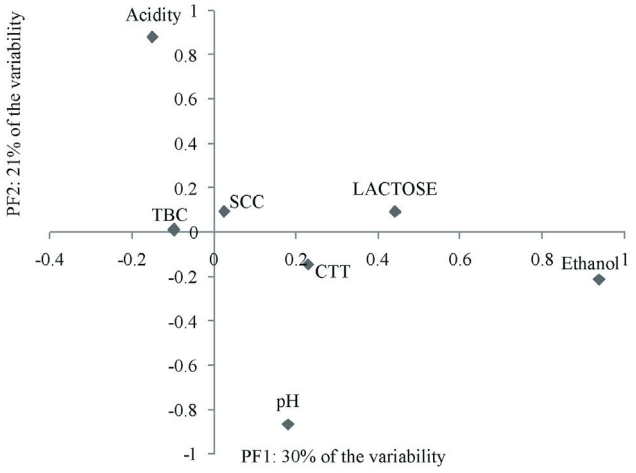
Logistic analysis showed that an increase in each unit of log_{10}TBC or log_{10}SCC increased the odds of occurrence of unstable milk by 2.23 and 1.6 times, respectively (P<0.01). The increase in each minute of CTT test increased the odds of occurrence of ethanol stable milk by 1.15 times (P<0.01).

Milk with higher stability in the ethanol test (coagulated at ethanol above 78 °GL) presented lower values for acidity, TBC, and SCC but higher pH, levels of lactose, and protein and lasted longer in the CTT without the formation of clots when compared with unstable milk samples (coagulated when mixed with ethanol at 72 °GL or less), which makes it more suitable for dairy industry (Table 3).

Table 2 - Frequency of occurrence of values within and out the range accepted by the normative instruction 62 for the physical-chemical and sanitary characteristics of milk produced by 50 farmers in the northeast region of Rio Grande do Sul from 2007 to 2009

Attribute	Frequency of samples in each range (%)		
	<6.6	6.6-6.8	>6.8
pH[1]	<6.6	6.6-6.8	>6.8
	0.4	91.7	7.8
Titratable acidity (°D)[1]	<14	14-18	>18
	0.1	99.8	0.1
Stability to alcohol test (°GL)[1]	≤72	73-78	>78
	35.2	46.2	18.6
TBC (cfu/mL × 10^3)[1]	≤200,000	200,000-600,000	>600,000
	5.8	32.1	37.9
SCC (SC/mL × 10^3)[1]	≤100,000	100,000-600,000	>600,000
	8.3	40.1	51.6
Lactose (g/100 g)[1]	<4.3	-	≥4.3
	30.1	-	699
Protein (g/100 g)[1]	<2.9	-	≥2.9
	9.9	-	90.1
Fat (g/100 g)[1]	<3.0	-	≥3.0
	2.1	-	97.9

TBC - total bacterial count; SCC - somatic cell count.
[1] Reference value according to normative instruction 62, IN62 (Brasil, 2011).



PF - principal factor analysis; TBC - total bacterial count; SCC - somatic cell count; CTT - coagulation time test.

Figure 1 - Orthogonal plan with principal factors for milk components and stability tests.

Table 3 - Mean values for attributes in raw milk samples from 50 dairy farms presenting high (>78 °GL), intermediary (from 72 to 78 °GL), or low (≤72 °GL) stability to the ethanol test with corresponding significance levels

Attribute	Ethanol stability			P>F	CV (%)
	≤72 °GL	72>X≤78 ºGL	>78 ºGL		
pH	6.7a	6.8b	6.8b	<0.0001	1.2
Acidity (°D)	16.2a	15.7b	15.5c	<0.0001	8.4
Lactose (g/100 g)	4.3a	4.4b	4.4c	<0.0001	3.6
Protein (g/100 g)	3.08a	3.12b	3.11b	0.0043	5.9
Fat (g/100 g)	3.7	3.8	3.8	0.1171	10.2
CTT (minutes)	4.5a	5.2b	5.6b	<0.0001	49.1
Log_{10} (SCC)	5.8a	5.8b	5.8b	0.0159	5.5
Log_{10} (TBC)	6.1a	5.9b	5.9b	<0.0001	9.8

CTT - coagulation time test; SCC - somatic cell count; TBC - total bacterial count; CV - coefficient of variation.
a,b,c - means in the same row followed by different letters are significantly different (lsmeans; P<0.05).

Discussion

Milk stability is a complex issue (Horne, 2015) and some milk characteristics have been consistently related to low ethanol stability, such as acidity (Rathnayake et al., 2016) ionic calcium concentration (Tsioulpas et al., 2007; Lewis, 2011; Nian et al., 2012), ionic strength (Chavez et al., 2004), phosphate (Gaucheron, 2005), and citrate contents (Tsioulpas et al., 2007). Other milk characteristics were not consistently related to stability. Some studies have reported significant effects on stability related to the casein composition (Barbosa et al., 2012), while other have not reported significant effects (Marques et al., 2011; Botaro et al., 2007, 2009). Somatic cell count (SCC) has been controversially related to low stability, as some studies reported a relation between high SCC and low stability (Oliveira et al., 2011; 2013), while others have shown no relation (Kolling, 2012).

Factors related to the animals, such as extended lactation period (Marques et al., 2010a; Tsioulpas et al., 2007), affect milk stability. Feed restriction (Gabbi et al., 2013, 2016), excess of fiber in the diet (Barchiesi-Ferrari et al., 2007), or nutrient imbalance (Marques et al., 2010b) negatively affect milk stability, although the underlying mechanisms are not completely determined; however, it seems that metabolism disturbance leading to metabolic acidosis (Fagnani et al., 2014; Marques et al., 2011) and higher permeability of the tight junctions of epithelial mammary cells (Stumpf et al., 2013) is probably enrolled as a causal factor of the low milk stability.

The present study aimed to highlight the suitability of indirect test to estimate milk stability and identify the milk traits related to milk stability. We might consider some limitations of our study. We monitored only 50 dairy farms and, although they were representative of the range of farms of this region concerning milk production, feeding, and milking practices, they might be not representative of dairy farms located in other regions. On the other hand, as these farms were monthly monitored for 36 months, a considerable number of milk samples were collected and analyzed, giving robustness to the results about the effect of seasonality and correlation between methods. The farm characteristics were surveyed twice a year but they were not taken into consideration in the multivariate analysis. We are also aware that the mineral components such as ionic calcium, ionic magnesium, chlorides, potassium, sodium, citrate, phosphate, and ionic strength were not measured. These measurements are expensive and laborious and are not usually performed by dairy industries. Extrapolations of the results and considerations and inferences should be made with caution and with these limitations in mind.

Overall ethanol stability value (74.8 °GL) was higher than 72 °GL, which is the minimal threshold required by the IN62 for raw milk (Brasil, 2011), and approached the value indicated for UHT process (Farahnik, 1982). High occurrence of milk ethanol instability, not related to excessive acidity, was previously reported in other regions by Marques et al. (2007) in Rio Grande do Sul, Brazil; Oliveira et al. (2011) in São Paulo, Brazil; Fagnani et al. (2016) in Paraná, Brazil; and Lin et al. (2009) in the eastern Taiwan.

Low and high ethanol stability values observed in different months, depending on the year, might be partially explained by variation in feed supply or feed quality with consequent changes in mineral composition (Tsioulpas et al., 2007; Lewis 2011; Stumpf et al., 2013; Horne, 2015). The overall low stability value observed in 2008 was probably related to the occurrence of the La Niña phenomenon, which led to shortages in rainfall, mostly on spring and summer, reducing the production and quality of feeds offered to the animals. In 2007 and 2009, high values for milk stability were observed when cool season pastures were actively growing (July to October) and when silage was supplied to the animals (March to June); low stability was registered in the beginning of summer, when the land is used for corn cultivation, and in the beginning of autumn, when a forage gap is very frequent.

The absence of coagulation in the boiling test might be due to the fact that no sample presented pH below 6.5 or acidity above 20 °D and confirmed its low sensitivity compared with ethanol test, as previously reported by Fonteh et al. (2005) and Silva et al. (2012).

The huge variation observed for CTT was already noticed (Chavez et al., 2004), but one of the limitations of this test is the lack of a clear definition of the value for the low threshold for milk stability (Molina et al., 2001); therefore, its comparison with the ethanol test remains inconclusive (Negri et al., 2001). It is only possible to assume that, the longer milk resists to the treatment without the formation of clots, the greater is its thermal stability, but there are doubts about the reliability of this test to estimate thermal stability at the dairy industry (Lewis and Deeth, 2009; Horne, 2015). Values of coagulation time test were lower than those previously reported by Molina et al. (2001) and Chavez et al. (2004), who observed the formation of clots between 10 and 20 min and after 20 min in immersion, respectively. This fact might partially be related to the saline imbalance, as 51.6% of the samples of the present study presented SCC higher than 600,000

cells/mL, in contrast with Chavez et al. (2004), who did not analyze milk with more than 500,000 cells/mL. As we used the same kind of oil bath and glass capillaries described by Negri et al. (2001) and Chavez et al. (2004), differences concerning personal and laboratory facilities should have been of minor importance.

The positive, although moderate, correlation between CTT and ethanol test observed in the present study (linear correlation and principal factor analysis) has already been reported (Molina et al., 2001; Chavez et al., 2004) and might be due to the different challenges they impose to milk components, i.e. dehydration and increase of the dielectric constant promoted by ethanol test and heating by CTT. Although the relation was positive and significant, the coefficient of determination was very low, as already noticed (Singh, 2004). To the knowledge of the authors, despite the results of Negri et al. (2011), there is no advantage to choose CTT instead of ethanol stability test to estimate milk stability (Horne, 2015).

Milk stability is negatively related to acidity, because the reduction in pH reduces calcium phosphate caseinate and enhances ionic calcium concentration, which in turn decreases repulsion forces among caseins, favoring coagulation (Lewis, 2011; Horne, 2015). Horne and Muir (1990), Chavez et al. (2004), and Lewis (2011) reported that results in the ethanol test and CTT were adversely affected by ionic calcium concentration. The output of calcium phosphate from the micellar structure leads to the exposition of the hydrophobic portion of the caseins, enhancing the propensity for aggregation (Philippe et al. 2003).

In the present study, 47.3% and 52.3% of samples presented pH between 6.5 and 6.7 and between 6.7 and 7.0, respectively. These variations, although minimal, can affect the stability of milk. Within the range of 6.5 to 6.7, as pH increases, the concentration of ionic calcium reduces and stability of casein micelles is enhanced. Between 6.7 and 7.0, the κ-caseins separate from the micelles, reducing the stability of milk. Above 7.0, the concentration of ionic calcium is lowered and the stability increases (Horne and Muir, 1990; Singh, 2004; Horne, 2015).

The negative association between SCC and milk stability observed in the correlation analysis, but not in the principal factor analysis, was also found by Marques et al. (2010a,b) and Oliveira et al. (2011; 2013). In the present study, the sanitary quality of milk was low, as 51.7% of samples presented SCC levels above 600,000 cells/mL. High values for SCC are probably related to low milk stability due to proteolysis, low casein contents, high values of sodium and chlorine contents, and micelle destabilization (Chavez et al., 2004; Horne, 2015); on the other hand, high values for TBC are related to low milk stability, probably due to proteolysis and lactose degradation, which leads to lower pH and calcium phosphate values and further increase in ionic calcium concentrations and micelle destabilization (Auldist and Hubble, 1998; Chavez et al., 2004; Bueno et al., 2008; Lewis and Deeth, 2009). The increase in the paracellular flow of components between the blood stream and the milk when SCC is elevated (Stelwagen et al., 2000) leads to elevated levels of sodium, chloride, and phosphorus in milk (Smyth et al., 2004) and might be related to low stability. However, Donatele et al. (2003) and Kolling (2012) found no relationship between TBC, SCC, and milk stability.

These relations between milk stability and milk traits are supported by differences in composition presented by milk with low, medium, and high ethanol stability. Differences in lactose and protein contents between milk with high stability and unstable milk are partially due to inadequate supply of nutrients, feeding of low-quality forages, metabolic or ruminal disturbances, and poor hygienic-sanitary characteristics of milk (Zanela et al., 2006; Barchiesi-Ferrrari et al., 2007; Marques et al., 2010a; Fagnani et al., 2014; Gabbi et al., 2016; Werncke et al., 2016).

Until now, factors that can be used to predict the behavior of raw milk in stability tests and in industrial procedures involving heating have not been completely identified (Horne, 2015). Lewis (2011) suggested that levels of ionic calcium are involved and recommended its measurement. However, it requires expensive ion-selective electrodes and demands training of personal engaged in routine tests at the industry. The absence of a defined minimal threshold for coagulation time, besides the time spent in its application (that can last for few seconds until several minutes) and personal health issues (smoke produced by glycerin heating) impair the implementation of CTT in the industry or on the farm as a routine test of milk stability. Boiling test presents some favorable aspects such as easiness, clear cut-off point, and low cost, but is much less sensitive and predictive than CTT and ethanol test to evaluate milk stability, as it did not vary in the present study despite huge changes in milk composition.

Conclusions

Stability varies among years and months within year and according to the method used. Milk is stable in the boiling test. Indirect methods of milk stability evaluation are poorly correlated. High ethanol stability milk presents better chemical composition than low stability milk. Among the indirect methods for estimating milk stability, ethanol test remains as the most suitable.

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