Chapter 12

Characterization of enzymatic coagulation of milk from Holstein cows

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ABSTRACT

The milk coagulation capacity of fourteen Holstein cows was analyzed in three different periods of the year, using a low amplitude dynamic oscillatory rheological analysis. In most of the samples analyzed, the modules G' and G" increased with the time of coagulation and the gels were characterized as weak. However, the rheological results in real-time of the coagulation showed that the milk of two animals, one for presenting slow coagulation (greater than 36 minutes) and the other for not coagulating are unsuitable for cheese production by enzymatic coagulation. The values of the Temperature and Humidity Index were less than 72 and did not influence the rheological properties. Significant differences between fat and lactose contents and between pH and somatic cell score values were found between the analysis periods. Evaluating the milk coagulation capacity and the strength of the gel formed are essential to assess the appropriate destination of milk, that is, milk for cheese production or milk for the production of derivatives that do not depend on enzymatic coagulation.

Keywords: milk coagulation properties, rennet coagulation, rheology.

1 INTRODUCTION

The transformation of milk into cheese involves physical, chemical, and biochemical steps, and coagulation is fundamental. Enzymatic coagulation has a direct influence on the curd firmness and consequently on the yield obtained (Frederiksen et al., 2011). In recent years, several studies have been reported regarding milk coagulation properties (MCP) (Cassandro et al., 2008; Manuelian et al., 2018; Poulsen et al., 2017). Milk coagulation properties are parameters that define the ability of milk to react with an enzymatic coagulant (chymosin) during cheesemaking, forming a curd with proper consistency between 11 and 18 minutes (Zannoni; Annibaldi, 1981; Cassandro et al., 2008). Milk with desirable characteristics of MCP results is greater than when compared with milk with a slow coagulation time or even with no coagulation at the ideal time (Frederiksen et al., 2011; Pretto et al., 2013). Several factors such as chemical composition (protein and fat), mainly concerning casein and its fractions, concentration of the coagulant enzyme, temperature of coagulation, somatic cell content, pH, and environmental factors may influence coagulation (O'Callaghan et al., 2001; Politis; NG-Kwai-hang, 1988). The milk composition may change seasonally. In general, a higher concentration of fat in milk occurs in colder seasons (Fox, Mcsweeney, 1998). Thermal stress, associated with high temperature and relative humidity, harms milk production and composition (Zimbelman et al., 2013) and it may affect milk capacity of coagulation. Some physical and optical methods have been used to monitor milk coagulation. The systems or devices used range from traditional ones such as knife test (Del Prato, 2001) to others such as the *Berridge* method (Berridge, 1952), lactodinamographic parameters (Annibaldi et al., 1977), near (Callaghan et al., 2000) and medium infrared (Dal Zotto et al., 2008) and rheometry (Hussain et al., 2012). Rheological studies allow the evaluation and monitoring of curd formation during the milk enzymatic coagulation through the formation of a gel-type system. The curd, formed by enzymatic coagulation, is rheologically characterized as a gel and its rheological properties in small deformation can be determined by low-amplitude dynamic oscillation, with the determination of the elastic modulus (G') and the loss modulus (G") allowing to evaluate the formation of the gel and its stiffness (Lucey et al., 1998; Schramm, 2000). Therefore, a direct way to measure gel formation is to monitor the time evolution of rheological properties during coagulation. Esteves et al. (2002) comparatively evaluated the rheological properties of coagulated milk by the action of chymosin (animal curd) and vegetable coagulants. The last one presented a slightly more proteolytic behaviour than chymosin, which may have contributed to a lower firmness of the curd gel obtained. Hussain *et* al. (2011) evaluated the curd obtained from buffalo and cow's milk at different pH values. They observed that curd rheology was adversely affected at lower pH (5.8 to 5.6) in both types of milk, which was attributed to the solubilization of casein-bound calcium from the micelles. The maximum stiffness of the curd was obtained at pH 6.0 in both types of milk. Hussain et al. (2012) also investigated the effects of different temperatures (28, 34 and 39°C) on the rheological properties of cows' milk and buffalo milk. The maximum curd strength (G') was obtained at a gelation temperature of 34 °C in both types of bovine milk. The curd coagulation time was reduced with the increase in gelation temperature in both types of milk.

Dairies are non-seasonal products, therefore their production and consumption occur during the whole year. Thus, the objective of this study was to evaluate the enzymatic coagulation behaviour of fresh bovine milk from a herd of fourteen Holstein animals in three distinct periods of the year by low amplitude dynamic oscillatory rheological analysis. The chemical composition of fresh bovine milk and the somatic cells of the animals are been also determined.

2 MATERIALS AND METHODS

Samples, Data Collection

Samples were collected from 14 Holstein-Friesian animals belonging to dairy herds of Fazenda Escola Capão da Onça, State University of Ponta Grossa, Ponta Grossa - Paraná, Brazil (990 m altitude under the geographic coordinates 25°05'26 "LS and 50° 03'37" LW). The milk was collected in three distinct periods during the year (2016), and all the collections were performed during milking. The first collection in May (P1), the second in August (P2) and the third in December (P3). The animals were identified with numbers from 1 to 14. Animals 1, 3, 11 and 14 were collected in two periods because in one of the periods the animals were in the prepartum period. Samples were collected in sterile vials containing bronopol (2-Bromo-2-nitropropane-1,3-diol) preservative in the form of tablets giving a final milk concentration of 0.02 to 0.05%. The vials were identified and maintained at 6 to 7°C until the moment of analysis.

Rheological measurements

The rheological analyses were conducted on a Haake Mars II rheometer (Thermo Electron GmbH, Germany) coupled to a Haake K15 thermostatic bath, Haake DC5B3 water thermocouple, and a Peltier temperature controller (TC 81, Haake). The sensor used was a cone-plate type with a diameter of 60 mm and a cone angle of 2.0° (C60 2.0°/TiL). To identify the rennet coagulation time (RCT, min) of milk samples, dynamic oscillatory measurements were performed at a fixed frequency of 0.1 Hz, and 1% deformation. The elastic modulus (G’) and the loss modulus (G’’) were determined over time for 45 minutes at a fixed temperature of 35°C (Hussain et al., 2011). The RCT was defined as the time when the storage module G’ reached a value of 1.0 Pa (Lucey et al., 2000). After 45 minutes of the time scanning, a frequency sweep between 0.1 Hz and 10 Hz (in CD mode, 1.0% constant deformation at 35 °C) was performed to evaluate the viscoelastic behaviour of curds formed after enzymatic coagulation. The experiments were performed at least in duplicates. Before starting the experiments, each (fresh) milk sample was heated to 35°C. Then it was added 100.0 µl of the liquid (bovine) coagulant (20.0% chymosin and 80.0% pepsin with 320 IMCU/ml previously diluted in distilled water) to obtain a final concentration of 0.053 IMCU/ml of milk, to 3.0 ml of milk. The sample was thoroughly stirred for 30 seconds and the mixture was immediately transferred to the rheometer plate (Esteves et al., 2002).

Component analysis, pH, and somatic cell count

Fat contents, total protein (TP) and casein (CN) were determined from fresh milk samples (preheated at 40°C for 15 minutes in a thermostated bath) using medium infrared in Bentley 2000 equipment (Bentley Instruments- USA), according to International Dairy Federation standards - IDF Standard: 141C: 2000 (ISO/IDF, 2000). Somatic cell count (SCC) was performed by flow cytometry on Somacount 300 equipment (Bentley Instruments-USA) according to International Dairy Federation

Standard, IDF Standard 148-2: 2006 (ISO/IDF, 2006). The SCC values were transformed, on a logarithmic scale for Somatic Cell Score [$SCS = 3 + \log_2 (SCC/100)$], to reach the normality and homogeneity of the variances. The pH determinations of the fresh milk samples were performed using a QUIMIS® benchtop pH meter.

Temperature and Humidity Index (THI)

The temperature and humidity index (THI) were calculated according to equation (1) of the National Research Council (NRC, 1971):

$$THI = (1{,}8 \times T + 32) - (0{,}55 - 0{,}0055 \times RH) \times (1{,}8 \times T - 26) \quad (1)$$

Where: T (temperature, °C) and RH (relative humidity, %). The temperature and relative humidity data were provided by the Paraná Meteorological System (SIMEPAR). The THI was calculated with the average values of environmental temperature and relative humidity. The average was obtained with the values of these parameters regarding the week of sampling.

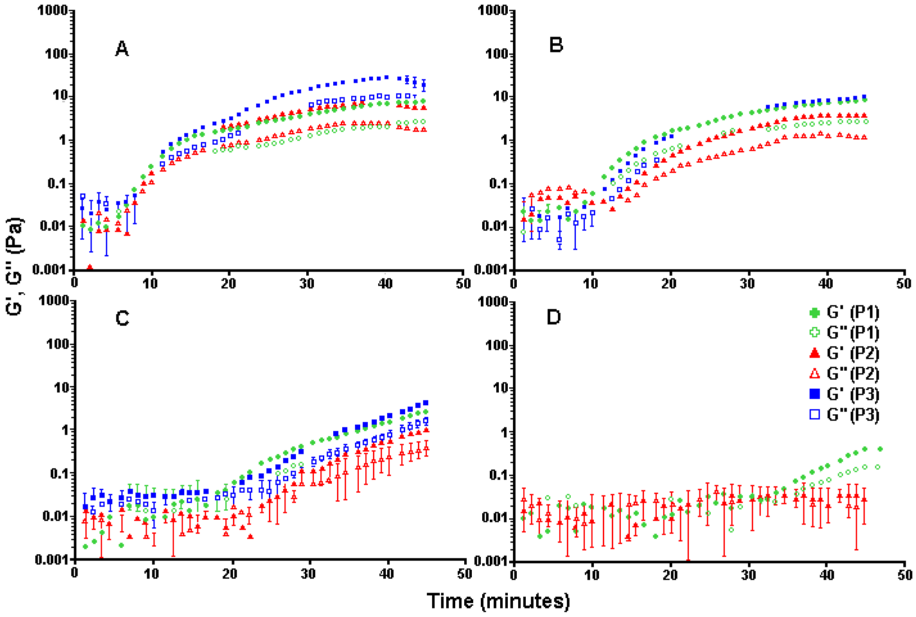
Statistical Analysis

The influence of the fresh milk collection period on the fat, total protein, casein, pH, and somatic cell count was evaluated by generalized equation estimation (GEE) analysis. The dependent variables that presented normal distribution were analyzed using the identity binding function. The periods (months of collection) were considered as a factor, and the analyses were performed with the mean values of each period (P): May (P1), August (P2) and December (P3). GEE analyses were performed in SPSS Software version 22.

3 RESULTS AND DISCUSSION

Figure 1 shows the G' and G'' moduli of milk samples collected from animals 5, 10, 13 and 14. A pronounced increase in the values of G' and G'' which expresses the RCT was observed and the time at which this increase occurs was different among the samples evaluated (Table 1).

Figure. 1 - Dynamic oscillatory rheological analysis. The elastic modulus (G’, full symbols) and viscous modulus (G’’, open symbols) as a function of the time (45 minutes) of the samples collected from animals 5 (A), 10 (B), 13 (C) and 14 (D) after the addition of coagulant, evaluated in three different collection periods: P1-May; P2-august; P3-December; fixed frequency of 0.1 Hz; deformation of 1% (35°C).



According to the classification of Frederiksen et al. (2011), the coagulation profiles obtained were considered: adequate with RCT <15 min (milk samples from animal 5; Figure 1-A); intermediate with RCT > 15 min (milk samples from animal 10; Figure 1-B) and non-coagulable (NC) with RCT > 40 min (milk samples from animal 14; Figure 1-D). The milk that presented RCT > 36 min (milk samples of animal 13 - Figure 1-C) was considered an NC sample because, after 45 min incubation with the coagulant, it was presented in a liquid state. The rheological parameters determined for the other investigated milk samples are also presented in Table 1.

Table 1 - Results of the analyses from milk samples in the three periods of May (P1), August (P2) and December (P3) and the corresponding THI.

Animal	Period	RCT*, min	G'**, Pa	G"**, Pa	G'/G"**	Fat,%	PT*,%	CN*,%	SCS*	pH	THI*
1***	P1	9.92 ± 0.59	33.07 ± 0.42	9.57 ± 0.15	3.11 ± 0.26	4.09	3.63	2.90	2.14	7.09	58.37
	P2	25.82 ± 3.41	16.40 ± 8.33	5.07 ± 2.51	3.13 ± 0.57	3.71	3.73	2.99	5.49	7.07	58.9
2	P1	9.92 ± 1.08	18.73 ± 2.13	4.88 ± 1.21	3.66 ± 0.49	4.43	2.98	2.29	0.44	7.01	58.37
	P2	12.48 ± 0.02	62.66± 7.84	15.07 ±2.00	3.68 ± 0.31	3.87	3.48	2.74	0.06	6.99	58.90
	P3	9.92 ± 0.01	43.53 ± 8.07	10.72 ±1.91	3.48 ± 0.49	3.93	3.52	2.76	4.70	6.75	66.62
3***	P1	11.46 ± 1.08	29.70 ± 5.34	7.47 ± 1.02	3.02 ± 0.28	4.79	3.95	3.17	3.44	6.93	58.37
	P3	18.12 ± 0.03	28.30 ± 0.03	7.90 ± 0.06	3.24 ± 0.38	3.40	2.71	2.15	5.68	6.95	66.62
4	P1	12.49 ± 0.01	37.62 ± 0.90	8.62 ± 0.50	3.75 ± 0.47	3.88	3.56	2.84	0.26	6.90	58.37
	P2	11.46 ± 0.03	23.29 ± 0.47	5.88 ± 0.14	4.00 ± 1.52	4.22	3.55	2.82	1.21	7.14	58.90
	P3	13.50 ± 0.69	31.49 ± 0.05	8.27 ± 0.14	3.51 ± 0.27	4.14	3.83	3.02	1.71	6.87	66.62

5	P1	14.57 ± 2.52	12.53 ± 0.09	3.33 ± 0.08	3.46 ± 0.38	3.85	2.41	1.80	3.28	7.04	58.37
	P2	14.53 ± 1.56	8.49 ± 0.84	2.24 ± 0.22	3.95 ± 1.55	3.48	2.56	1.93	3.46	7.12	58.90
	P3	13.6 0± 0.70	15.29 ± 0.63	4.22 ± 0.04	4.00 ± 1.43	3.25	3.24	2.51	2.35	6.78	66.62
6	P1	14.54 ± 0.10	31.55 ± 6.10	8.95 ± 1.79	3.22 ±0.24	2.12	3.24	2.59	1.88	6.95	58.37
	P2	21.21 ± 0.73	9.62 ± 0.83	2.90 ± 0.14	3.77 ± 3.03	1.44	3.22	2.56	2.58	7.08	58.90
	P3	22.23 ± 1.80	14.89 ± 1.06	4.84 ± 0.37	2.89 ± 0.25	2.95	3.29	2.58	3.80	6.86	66.62
7	P1	18.13 ± 0.88	34.56 ± 1.69	9.15 ± 0.37	3.37 ± 0.33	3.54	3.27	2.60	2.53	7.02	58.37
	P2	12.48 ± 1.03	19.91 ± 6.53	5.08 ± 1.74	3.70 ± 0.49	2.10	3.16	2.48	3.73	6.93	58.90
	P3	12.49 ± 0.74	71.43 ± 23.33	20.25 ±8.03	3.23 ± 0.21	3.42	2.68	2.02	0.44	6.77	66.62
8	P1	14.59 ± 0.72	16.25 ± 0.12	4.66 ±0.083	3.29 ± 0.49	6.50	2.58	1.90	3.58	7.00	58.37
	P2	21.20 ±1.56	20.61 ± 1.86	5.44 ± 0.63	3.43 ± 0.37	2.63	3.03	2.37	1.11	6.95	58.90
	P3	11.48 ± 0.02	17.02 ± 0.53	4.73 ± 0.05	3.57 ± 0.54	3.32	3.30	2.60	3.07	6.81	66.62
9	P1	18.13 ± 0.01	16.88 ± 1.03	4.47 ± 0.30	3.59 ± 0.50	3.05	2.58	1.98	4.28	6.94	58.37
	P2	18.13 ± 1.28	14.42 ± 0.30	3.96 ± 0.08	3.33 ± 0.40	4.09	2.68	2.02	4.19	7.06	58.90
	P3	13.50 ± 0.74	29.44 ± 3.34	7.80 ± 1.24	3.40 ± 0.30	3.00	3.11	2.46	3.78	6.86	66.62
10	P1	18.13 ± 1.29	12.43 ± 1.05	3.55 ± 0.25	3.38 ± 0.63	2.65	2.65	2.06	1,32	7.08	58.37
	P2	24.75 ± 1.02	5.51 ± 0.33	1.61 ± 0.05	3.35 ± 1.56	2.87	2.98	2.36	-1,64	6.99	58.90
	P3	19.15 ± 0.02	15.26 ± 1.72	4.57 ± 0.68	3.13 ± 0.35	2.95	3.05	2.37	-0,06	6.82	66.62
11***	P2	9.92 ± 0.59	22.40 ± 6.21	5.07 ± 1.37	4.24 ± 1.39	3.4	2.71	2.09	-2,06	6.93	58.90
	P3	12.48 ± 0.01	67.00 ± 1.78	16.88 ±0.51	3.60 ± 0.29	3.06	2.86	2.20	0.26	6.73	66.62
12	P1	20.18 ± 1.02	21.04 ± 0.55	5.87 ± 0.15	2.71 ± 0.49	1.37	2.84	2.24	-0,47	6.94	58.37
	P2	18.13 ± 0.72	21.30 ± 4.39	6.40 ± 1.20	3.18 ± 0.41	1.54	3.02	2.38	1.26	7.05	58.90
	P3	14.53 ± 0.73	15.94 ± 0.88	3.87 ± 0.05	3.75 ± 0.48	2.34	3.15	2.46	2.14	6.80	66.62
13	P1	36.09 ± 2.35	5.48 ± 0.88	1.83 ± 0.38	2.71 ± 1.06	3.76	3.26	2.59	6.57	7.02	58.37
	P2	44.81 ± 1.45	2.19 ± 0.69	0.76 ± 0.25	2.44 ± 1.01	2.26	3.51	2.83	5.81	7.16	58.90
	P3	36.08 ± 1.29	10.38 ± 1.62	3.42 ± 0.55	3.20 ± 1.11	2.92	3.32	2.64	4.92	6.95	66.62
14***	P1	n.c	0.54 ± 0.15	0.27 ± 0.04	n.c	2.79	3.37	2.68	5.01	7.12	58.37
	P2	n.c	0.19 ± 0.03	0.05 ± 0.02	n.c	2.79	3,44	2.74	5.12	7.34	58.90

Among the milk samples of the animals evaluated in this study, the majority presented an increase of both moduli (G', G'') during the time sweep, after the addition of the coagulant. This behaviour is consistent with changing from a liquid to a more viscoelastic gel system (Hussain et al., 2012). The same behaviour was evidenced by other authors as described by Esteves et al. (2002); Hussain et al. (2011) and

Hussain et al. (2012). The exception among the samples evaluated was the milk sample collected from animal 14. The moduli values (G’, G’’) stable throughout the time scanning, after the addition of the coagulant (Figure 1 D), characterize a liquid behaviour, where the coagulation did not occur. The real-time rheological results of coagulation showed that the milk collected from animals 13 (Figure 1 C) because of slow coagulation and 14 (Figure 1 D) are not suitable for cheese production through enzymatic coagulation. According to Cecchinato et al. (2011) slow-coagulating milk curd formation has less time to stiffen, and it is, therefore, more fragile, resulting in protein and fat losses to the serum at the cutting and press stages, reflecting yield reduction.

Besides, the dynamic oscillatory analysis presented in Figure 1 and Table 1 by the analyzed samples collected from animals 5 (Fig 1A), 10 (Fig 1B), 13 (Fig 1C) and 14 (Fig 1D) show similar behaviour when compared individually to the collected samples at different periods of the year (May - P1; August - P2; December - P3). According to (Beux et al., 2017) the influence of milk collection in different periods of the year on milk coagulation capacity can be affected by changes in temperature and humidity throughout the year. However, these authors reported that for values of temperature and humidity (THI) below 75, milk does not present significant changes regarding this characteristic. As can be observed in Table 1, the THI in the three collection periods was less than 70, a factor that may have contributed to the fact that the milk presented the same coagulation profile in the different collection periods.

In the same experiment, the analysis of the dynamic moduli (G’, G”) as a function of time, frequency sweep (elastic (G’) and viscous (G’’) moduli as a function of frequency) were performed (Figure 2) to evaluate the viscoelastic properties of the milk samples collected from animals 5, 10, 13 and 14, in the three periods of the year, formed after adding the coagulant. For the samples of animals 5 and 10 (Figure 2A and 2B), both moduli had a slight frequency dependence, with G’ higher than G’’ at all the frequencies analyzed (0.1-10 Hz) in all of the 3 collection periods evaluated. The values of G’ higher than G’’ characterize the milk samples of animals 5 and 10 as a material with gel behaviour (Steffe, 1996; Chamberlain, Rao, 2000). The milk sample of animal 13 showed a weaker gel behaviour (Figure 2C) when compared with the samples of animals 5 and 10, but both moduli had also a slight frequency dependence, with G’ higher than G’’ at all the frequencies analyzed. At high frequencies (7 Hz) a crossover of the modules occurred. This behaviour can be attributed to the lower consistency and stability of these samples. Else more, it is in agreement with the behaviour demonstrated in figure 1 C which shows milk samples coagulated at high RTC (> 36 min). This behaviour was reflected in the visual appearance of the samples after 45 min of incubation, which became more fluid.

Figure. 2 - G' and G" modules as a function of frequency (0.1-10 Hz with deformation of 1%) in the collection periods for the milk of animals 5 (A); 10 (B); 13 (C) and 14

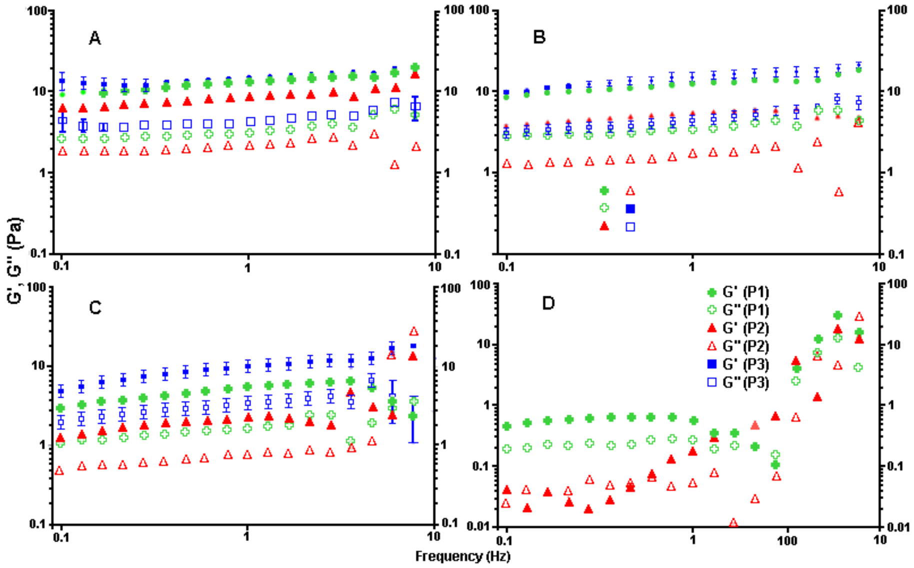


Figure 2D shows the frequency sweep of milk samples collected from animal 14 (non-coagulable, NC, with RCT > 40 min). For the P1 sample a crossover can be observed between the G' and G" modules at 2 Hz and for P2 at 0.12 Hz. This behaviour is characteristic of a viscoelastic system or concentrated solution without gel formation. Cassandro et al. (2008) and Cecchinato et al. (2011) also found NC milks from Holstein and Brown Swiss cows. However, the authors used latodinamography as a technique to evaluate coagulation. The other milk samples collected from animals 1, 2, 3, 4, 6, 7, 8, 9, 11 and 12 were also subjected to frequency sweeps. The values of G’, G’’ and the ratio G’/G’’ at 1 Hz are shown in Table 1. The profiles obtained for all the above-mentioned samples presented a viscoelastic behaviour similar to the samples shown in Figures 2A and 2B. G’ values greater than G’’ in every frequency range analyzed the slight frequency dependence, and the G’/G’’ ratio less than 10 (Table 1) allows them to be characterized as weak gels (Steffe, 1996). The lowest value for the G’/G’’ ratio was 2.44, obtained for the sample in animal 13 in December (P3) is justified by its highest RCT (44.81 ± 1.45 min). On the other hand, the highest ratio found was for animal sample 11 in August (P2), which was 4.24 and with RCT 9.92 ± 0.59 min being one of the shortest coagulation times.

The ability of milk coagulation plays an essential role in the production of hard cheeses, such as Grana Padano and Parmigiano-Reggiano (Pretto, et al., 2013). The literature demonstrates that milk coagulation in a shorter time presents a greater firmness of the curd, producing a higher yield with lower losses of milk components in the serum compared to the milk that presents late coagulation and with low firmness of curds (Aleandri, et al., 1989; Wedholm, et al., 2006).

Milk composition and temperature and humidity index

The results obtained for the analysis of components of the milk samples evaluated are presented in Table 1. The minimum and maximum values for fat were between 1.37% and 6.50%; for total protein (PT) between 2.41% and 3.95% and casein (CN) 1.80% and 3.17%. The literature reports that the average fat, total protein and casein in Holstein cow milk is about 3.00%, 3.20% and 2.65%, respectively (Botaro, et al., 2009; Cassandro, et al., 2008; Ribas, et al., 2004). However, variations in the chemical composition of milk, considering the same breed or different breeds, are quite common due to factors such as management, nutrition, genetics, and environmental conditions (Del Prato, 2001; Goddard, 2001). Frederiksen et al. (2011) reported indexes of 4.12% for fat, 3.47% for total protein and 2.69% for casein in Holstein cow milk. The month of milk collection is a factor associated with the quality (chemical composition) and quantity of the milk produced, and this is related to the nutrition of the animals, more specifically with the pasture, which undergoes variations throughout the year (Heck, et al., 2009).

The results for fat, somatic cell score (SCS) and pH in the milk samples were statistically different ($p < 0.05$) due to the collection period which was not observed with a protein (TP) and casein content (CN) (Table 2). Fat was significantly different ($p < 0.05$) between August (P2) and December (P3) as shown in Table 3. The lowest mean value of 2.99% was obtained for the milk samples collected in August (P2) and 3.55% for those collected in December (P3). The pH values were higher in August (P2) than in December (P3) ($p < 0.05$). The values of pH considered normal in the milk of freshly milked animals are between 6.4 and 6.8 (Del Prato, 2001). However, Cassandro et al. (2008) found pH values for Holstein cow milk between 6.33 and 7.18. Somatic cells (SC) have a reference value, according to IN-76 (BRASIL, 2018) which is at most 4.0 x 10^5 cells mL^{-1} which corresponds to the somatic cell score (SCS) equal to 5.0. Out of the milk samples analyzed, five presented values higher than the reference values.

Table 2 - Test of the effects model for the dependent variables fat, TP, CN, SCS, and pH

Effects	Fat, %		TP*,%		CN*,%		SCS*		pH	
	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.
Periods	15,11	<0,05	3,07	0,21	4,41	0,11	16,05	<0,05	219,36	<0,05

*TP (total protein); CN (casein); SCS (somatic cell score)

Milk sample from animal 1 in August (P2) with SCS equal to 5.49; of animal 3 in December (P3) with SCS equal to 5.69; of animal 13 in May (P1) and August (P2) with 6.57 and 5.81 respectively, and the sample of August (P2) with SCS equal to 5.12, as it can be observed in Table 1. The mean results were statistically different ($p < 0.05$) between August (P2) and December (P3) with values of 2.62 and 3.88, respectively (Table 3).

Table 3 - Comparison between the variables analyzed between May (P1), August (P2), and December (P3)

	Variables				
Periods**	Fat,%	TP*,%	CN*,%	SCS*	pH
P1	$3,26^{a,b}$	$3,40^{a}$	$2,70^{a}$	$3,23^{a,b}$	$6,98^{a}$
P2	$2,99^{b}$	$3,33^{a}$	$2,64^{a}$	$2,62^{b}$	$7,08^{a}$
P3	$3,55^{a}$	$3,15^{a}$	$2,46^{a}$	$3,88^{a}$	$6,84^{b}$

[a, b] Means with different letters in the same column, considering separately the periods of the breeds, they differ statistically ($p < 0.05$) according to the marginal means estimated by GEE. *TP (total protein), CN (casein), SCS (somatic cell score).

August (P2) was identified by the lowest mean value for SCS. According to the literature, milk collected in winter is characterized by a lower content of somatic cells (Fonseca; Santos, 2000). The temperature-humidity index (THI) is widely used to assess the impact of thermal stress on dairy cows, as this can affect productivity and change milk composition (Bohmanova, et al., 2007; Gantner et al., 2011). The THI values of milk samples collected in May (P1), August (P2) and December (P3) were 58.37; 58.90 and 66.62, respectively. The indexes obtained are below 72 which is the characteristic index of an environment free of heat stress (Armstrong, 1994) and the proven consequences such as the change in the chemical composition and the quantity of milk produced (Zimbelman, et al., 2013). Beux et al. (2018) analyzed the milk clotting of Italian Holstein-Friesian cows, collected in the summer and autumn and 56.6% of the samples collected in the summer and 20.9% of the samples collected in the autumn did not coagulate. In the summer, the mean was 73.24 ± 6.74 (values between 64.66- 81.53) and during the autumn was 57.43 ± 4.99 (values between 50.04-70.40). However, according to the authors, more studies should be done to understand the real relationship between THI and milk coagulation.

4 CONCLUSIONS

Among the different milk samples analyzed, it was possible to observe variations in rheological parameters, chemical composition and somatic cells. During the three periods of year samples analyzed, there was no significant variation of the temperature and humidity index (THI), as values below 72 which is the characteristic index of an environment free of heat stress and which affect milk clotting. However, the milk of two animals was considered unsuitable for cheese production. The other milk presented weak gel behaviour. There were variations in the time of enzymatic coagulation as well as in the storage modules between the samples of the animals in the different periods of analysis but additional research in particular on non-coagulable milk is needed.

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