Behavior of the Physiochemical Parameters of Raw Milk Stored in Temporary Horizontal Storage Tanks

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Abstract—Milk is a product with a high nutritional value, and it may appear in daily meals in its natural form or processed and transformed into various milk products. To be processed in industry, milk must have the proper quality conditions for its consumption, and the levels of its constituents must fall within the standards indicated by Normative Instruction no. 62 (2011). Due to its composition, its constituents tend to separate when at rest. In this context, the objective of this work was to evaluate the behavior of the physiochemical parameters of chilled raw milk stored in temporary horizontal storage tanks. With the milk at rest in the tank, collections were made of the milk at the times of 0, 60, 120, 180, 210, 240, 270 and 300 minutes. These collections were performed at two points of the tank: at the top collection point (Ps) and the bottom collection point (Pi) of the tank. After the collection of the samples, the following parameters were determined: fat (G), non-fatty solids (SNF), density (D), cryoscopic index (SI), protein (P), lactose (L) and solids (SI). After completion of the tests, it was possible to verify that the solid constituents of the milk showed different behaviors, since the fat separated completely at 210 minutes promoting a stabilization in the separation of the fat. This same behavior was found for Density and the Cryoscopic Index. The other solid constituents of the milk didn't separate, maintaining themselves stable in both the bottom and top of the tank. As such, the conclusion can be drawn that fat is the only physiochemical compound that separates from milk at rest, thus affecting its Density and Cryoscopic Index.

Keywords - Fat, milk, rest, storage, temporary horizontal tank.

I. INTRODUCTION

Milk is a white and opaque liquid. It’s appearance is the result of the reflection of light by fat globules, insoluble phosphates and casein, with variations ranging from cream to bluish. It has a slightly sweet taste because of the presence of lactose, sodium chloride, fats and proteins. It is a homogeneous mixture with a high nutritional value and it plays a fundamental role in human nutrition, in addition to providing energy and nutrients for subsistence (Koblitz, 2011; Gonzaga et al., 2015).

According to the Normative Instruction no. 62/2011 of the Ministry of Agriculture, Livestock and Food Supply (MAPA), milk is the product arising from the complete and uninterrupted milking of healthy, well-nourished and rested cows in hygienic conditions (Brazil, 2011).

Controlling the physiochemical and microbiological quality of the milk arriving in industrial and processing units is crucial to ensure the health of the population and should be a routine procedure (Tronco 2008; Azevedo 2014).

To be processed in industry, the milk must have the proper quality and consumption conditions and levels within the standards indicated by the Normative Instruction No. 62 of December 29, 2011 from the Ministry of Agriculture, Livestock and Food Supply (MAPA), whose parameters are used as indicators to provide the conditions in which the milk was obtained and processed or to identify any fraud of the product (Brazil, 2011).

When the milk arrives at the reception platform of the dairy industry, milk samples should be collected directly in the truck and go through a series of analyses through rapid tests. Standing out as one of the main analyses required by legislation are the acidity, density, color, smell and texture tests, but the most important factor to be analyzed is the temperature of the milk. This temperature should remain in a range of 7ºC to 10ºC (Brazil, 2011). The minimum quality requirements that refrigerated raw milk in rural properties must comply with, and which can be considered as the acceptable contamination limits of milk, are: a fat content of at least 3% titratable acidity between 0.14 and 0.18 g of lactic acid, density at 15ºC between 1.028 and 1.034 g/cm³, dry degreased extract...
Milk samples supplied by the dairy producer were of milk collected on the same day as the analysis. These samples were transported in a cold chamber to the test laboratories of the Universidade Comunitária da Região de Chapecó - Unochapecó. The official analyses were performed in the Food Technology Laboratory of the Chapecó Campus. The samples were kept in the temporary horizontal tank, in a controlled temperature environment of 7.00 °C ± 2.0, as determined by the Normative Instruction no. 62 (Brazil, 2011) of the Ministry of Agriculture, Livestock and Food Supply - MAPA, which establishes that chilled raw milk in Brazil must pass through an analysis to assess its quality. All analyses were performed in triplicate.

2.2 STORAGE TANK
In order to carry out this experiment, an isothermal, horizontal temporary storage tank was designed and built with a capacity of 33 liters of milk, with similar characteristics as the truck tanks used for the transport of milk in bulk to the dairy industries. The tank was built in stainless material and jacketed according to Figure 1, allowing it to maintain the temperature of the milk under ideal conditions, thus complying with IN 75/2003 and IN 62/2011 of the Ministry of Agriculture (Brazil, 2003; Brazil, 2011).

The tank was constructed with two collection points for the milk samples, called: Top Collection Point (Ps) and Bottom Collection Point (Pi). These points allowed for the collection of milk samples during the evaluation times. At each collection point, a faucet was installed to perform the collection without contaminating the rest of the stored milk. The faucets used are in accordance with sanitary legislation.

2.3 BEHAVIOR OF THE MILK’S SOLID CONSTITUENTS
With the milk at rest in the tank, collections were made of the milk at the times of 0, 60, 120, 180, 210, 240, 270 and 300 minutes. These collections were performed at two points of the tank: at the top collection point (Ps) and the bottom collection point (Pi). After the collection of the samples, the following parameters were determined: fat (G), non-fatty solids (SNF), density (D), cryoscopic index (95), protein (P), lactose (L) and solids (SI). The centesimal composition of the milk's constituents was determined through the Master Mini milk analyzing equipment from AKSO, which uses ultrasound
technology to analyze the samples. The method is based on an ultrasound spectroscopy based on the undulating movement that propagates in the medium where the product is inserted.

The deformations of the milk molecules indicate if it was altered in its composition. In addition, the physiochemical analysis of the milk through ultrasound spectroscopy has advantages over traditional methods since samples don't need to be prepared, minimal volumes of the intact samples need to be used, no chemical reagents or specific glassware are necessary and results can be obtained in few minutes.

Before the analyses, the equipment was properly calibrated and cleaned according to the manufacturer's instructions. After adding a milk sample to a 25 ml cuvette, the device sucks in the quantity of milk needed for analysis and shows the values of the constituents of the milk under analysis on the screen (Ponsano et al., 2007).

2.4 STATISTICAL ANALYSIS

The Microcal Origin software, version 7.0 (Microcal Software Inc., Northampton, MA, USA), was used for the statistical data analysis, ANOVA using the Tukey test, with a significance level of 5%, was used for the analysis of variance. The physiochemical parameters were evaluated by the difference of the sample means according to the Normative Instruction no. 62 (2011). All activities were performed in triplicate.

III. RESULTS AND DISCUSSION

The behavior of the physiochemical parameters of the milk stored in a temporary horizontal tank is presented in Table 1, 2 and 3. In Table 1 and Figure 2 the fat behavior can be seen.

Table 1: Behavior of the fat in storage tanks in 8 evaluation times.

| Time (Minutes) | Mean Top Collection Point | Mean Bottom Collection Point |
|----------------|---------------------------|-----------------------------|
| 0              | 3.85±0.05bA               | 3.75±0.05aA                 |
| 60             | 4.95±0.05abA              | 3.85±0.05aA                 |
| 120            | 9.70±0.60bA               | 3.05±0.05aB                 |
| 180            | 13.60±0.20bA              | 3.15±0.05aB                 |
| 210            | 15.75±0.15cA              | 2.75±0.05bB                 |
| 240            | 15.85±0.05cA              | 2.85±0.05bB                 |
| 270            | 16.25±0.05cA              | 2.70±0.10bB                 |
| 300            | 16.67±0.05cA              | 2.70±0.20bB                 |

* Means and deviations followed by the same small case letter in the vertical axis and capital letters on the horizontal axis do not differ statistically between themselves by the Tukey test at the level of probability of 5%.

The analysis of the data in Table 1 reveals that there were variations in the fat levels, both in the samples collected from the top point and those collected from the bottom point. One can see that at the starting time (zero minutes), the observed levels in both points were of 3.85±0.05 and 3.75±0.05 for the top and bottom evaluation points, respectively. As can be seen, the levels don't differ significantly among themselves at the level of 5% in Tukey's Test.

After the 60 minute mark of the milk at rest, one can see that the fat levels increased until the time of 210 minutes, with values of 4.95±0.05, 9.70±0.60, 13.60±0.20 and 15.75±0.15 at the times of 60, 120, 180 and 210 minutes, respectively. As can be seen, the levels found differ significantly among themselves at the level of 5% in Tukey's Test. This behavior can be observed in the curves for the fat separation from the milk, shown in Figure 2.

Fig. 2: Separation of the fat in milk stored in a temporary horizontal tank.

After 210 minutes, a stabilization of the fat levels can be observed, because the values found were 15.85±0.05, 16.25±0.05 and 16.67±0.05 at the times of 240, 270 and 300 minutes, respectively. When these data are analyzed, they show that there is no statistically significant differences between the data at the evaluation times. This behavior suggests that the movement of the fat from the lower to the higher regions of the tank stabilized. One can therefore infer that the time of separation of the fat and milk when stored under the conditions of this study is 210 minutes, or 3.5 hours.

In a study conducted by Wangdi, Vijchulata and Chairatanayuth (2014), in Thailand, the characteristics were investigated of the separation by gravity of the fat globules in chilled milk. Similar to this study, the milk samples collected from the tanker truck remained at rest for a period of 8 hours, with five samples being taken, which remained at rest for the times of 0, 2, 4, 6 and 8 hours, in a place with a temperature of 4 °C. While in the present study samples were collected at the top and
bottom points through a faucet, in Thailand the milk samples were collected by means of a pipette at the top (between 250 and 200 ml), middle (between 150 and 100 ml) and lowest point (between 50 and 0 ml) of the container in which the sample was stored.

In the Thai study, there was a significant influence of time on the fat levels, starting at the top point, where the fat content increased from 3.85 to 5.07%, an increase of about 31.69% after a time interval of 2 hours. The fat content continued to increase in the top sample and at the end of the 8 hours of milk at rest, the fat content increased to 7.07%, consisting in a total increase of 83.63% compared to the initial fat content of 3.85%. This is corroborated in the present study, where in 120 minutes (2 hours), the fat content increased from 3.85±0.05 to 9.70±0.60, an increase of 152% from the initial value of 3.85% and the value continued to increase, but the rate of change decreased with the increase of time intervals.

The behavior of the fat content obtained from the samples collected at the bottom collection point (Pi) is the opposite of the one obtained from the samples collected at the top collection point (Ps), because as the analysis time progresses, the fat content of the collected samples can be seen to decrease after 180 minutes of storage, moving from 3.15±0.05 to 2.75±0.05% at 210 minutes. As such, one can see that there is a statistically significant difference between the two means in these times. It is also clear that from 210 minutes to 300 minutes of rest, there was a stabilization in the fat content, going from 2.75±0.05 at 210 minutes to 2.70±0.20 at 300 minutes.

As shown in Table 1 and Figure 2, there was a gradual reduction of the fat content at the bottom collection point and one can see that the largest value occurred at 120 minutes (2 hours), which was 81.33%, going from 3.75±0.05 to 3.05±0.05. Just as in the work by Wandi, Vijchulata and Chairatatanayuth (2014), who found a gradual reduction in fat content in the middle and lowest points, with an average decrease of around 8.05% and 12.99% for the medium and lower fractions, respectively.

After 8 hours of milk at rest, the decrease in fat content was around 20.8% in the middle and 28.80% at the bottom.

Servello et al., (2004) developed an extensive study to define the optimal time of agitation and, in his work, a fat loss rate of around 30% was observed at the bottom of the tank after three hours of milk at rest. Jackson (1981), on the other hand, observed a fat loss rate of 60%, but in 1.5 hours. This difference may be due to the different methods used in the collection of the samples.

In the evaluation of the quality of chilled raw milk as a function of the transportation and storage conditions in isothermal tanks and industrial silos performed by Brazil et al., (2012), the fat content in the milk stored in a refrigerated tank was 3.53% and in the silo 3.45%, lower than the values found in this work.

The data found here are in agreement with the recommendations of IN no. 62/2011, which established that the fat content in milk should be 3.0 g/100 g of milk (Brazil, 2011), with the composition of milk varying widely between breeds and in lesser intensity between animals of the same breed. It hover around 35g/liter. The fat contributes to a better palatability of the product. It is responsible for a large number of essential fatty acids. Each gram of fat provides 9 calories. The nutritive value of fat is due to the fat-soluble vitamins (A, D, E and K) and the presence of the carotene precursors of vitamin A (Tronco, 2008).

According to Foschiera (2004), the fat is formed by globules of different sizes, suspended in the aqueous phase. Each globule is surrounded by a phospholipid membrane and it is this layer that prevents the union of all the globules. According to Koblitz, (2011), maintaining the milk at rest leads to the separation of this component, forming a top layer. Since it is less dense than water, the fatty matter floats when the milk is at rest, forming the so-called cream layer, the main component of the milk sub-products butter and cream (Floriano, 2013).

Cunha et al., (2013) found no significant differences in the mean levels of lactose, protein, fat and SCC of the milk samples collected before and after the training of transporters. Although no statistical difference was found in the mean levels of fat between the collection times, the coefficient of variation (CV) of the data was 10.09%, which was a greater value than the CV for lactose and protein levels, which were 2.92 and 4.18%, respectively. According to Goodrigge et al., (2004), the collection in the lower or top layers from the milk tank may explain the variations concerning fat contents, since the fat globules tend to concentrate on the top layer of chilled milk.

Regarding the density of the milk measured according to storage time, one can see that the values obtained are similar in all samples, both in samples collected at the top collection point (Ps) and in samples collected at the bottom collection point (Pi). These data can be seen in Table 2.

The values obtained reveal a density of 1.039±0.05 for the collection at Ps at the beginning of the process, time zero, and a density of 1.014±0.35 at the time of 210 minutes. The analysis of these data, although very similar, show they are statistically significantly different by the Tukey test at a probability of 5%. From the time of 210 up to the time of 300 minutes, an equilibrium in the density could be seen, since the values went to 1.012±0.70. These density values, therefore, are no different, when evaluated. The same behavior was observed in the samples taken from the bottom collection point (Pi) for
the density values across the test times. The density can be modified by adding water or prior skimming, because water has a higher density than fat, 1g/cm³ and 0.9301g/cm³, respectively.

The behavior of density in this study is corroborated by the literature, which points out that the higher the fat content, the lower the density, with skimmed milk having a higher density than whole milk, because according to Castro (2005), density is the relationship that exists between the mass and volume of a body. As such, one can see the relationship between the solids and the solvent in milk.

This study revealed that in the 300 minutes of storage, the samples collected in Ps and Pi had a density of 1.012±0.70 and 1.045±0.20, respectively. It should be emphasized that these samples show levels of 16.67±0.05 and 2.70±0.20% for fat in the samples collected at the time of 300 minutes in the Ps and Pi, respectively.

Table 2: Physiochemical parameters of the milk - Non-fatty solids - SNF, Density and Cryoscopic Index (Means followed by Standard Deviation)

| Time* | SNFs¹ | SNFi² | Di³ | ICi⁴ |
|-------|------|------|-----|------|
| 0     | 8.25±0.50aA⁵ | 8.80±0.05aA | 1.039±0.30aA | -0.545±0.002aA |
| 60    | 8.33±0.13aA   | 8.86±0.05aA | 1.039±0.55aA | -0.542±0.001aA |
| 120   | 8.83±0.55aA   | 8.72±0.10aA | 1.028±1.30aA | -0.542±0.004aA |
| 180   | 8.50±0.10aA   | 8.95±0.05aA | 1.020±0.60aAB| -0.544±0.005aA |
| 210   | 8.14±0.10aA   | 8.71±0.10aA | 1.014±0.35bB | -0.553±0.015bA |
| 240   | 8.14±0.00aA   | 8.77±0.05aA | 1.013±0.25bB | -0.554±0.005bA |
| 270   | 8.18±0.10aA   | 8.93±0.16aA | 1.012±0.75bB | -0.552±0.001bA |
| 300   | 8.49±0.25aA   | 8.91±0.18aA | 1.012±0.70bB | -0.553±0.027bA |

* Time in Minutes. ¹Non-fatty solids (SNF) Top Point. ²Non-fatty solids (SNF) Bottom Point. ³Density - Top Point. ⁴Density - Bottom. ⁵Cryoscopic Index - Top Point. ⁶Cryoscopic Index - Bottom.

# Means and deviations followed by the same small case letter in the vertical axis and capital letters on the horizontal axis do not differ statistically between themselves by the Tukey test at the level of probability of 5%.

Table 3: Physiochemical parameters of the milk - Proteins, Lactose and Solids (Means followed by Standard Deviation)

| Time | Ps¹ | Pi² | Ls³ | Li⁴ | SIs⁵ | SII⁶ |
|------|-----|-----|-----|-----|------|------|
| 0    | 3.03±0.05aA⁵ | 3.41±0.05aA | 4.52±0.04aA | 4.69±0.15aA | 0.70±0.00aA | 0.70±0.00aA |
| 60   | 3.12±0.03aA   | 3.42±0.05aA | 4.51±0.11aA | 4.74±0.31aA | 0.70±0.00aA | 0.70±0.00aA |
| 120  | 3.24±0.20aA   | 3.40±0.05aA | 4.89±0.30aA | 4.62±0.11aA | 0.70±0.00aA | 0.70±0.00aA |
| 180  | 3.12±0.05aA   | 3.43±0.06aA | 4.68±0.05aA | 4.82±0.04aA | 0.70±0.00aA | 0.70±0.00aA |
| 210  | 2.98±0.09aA   | 3.42±0.04aA | 4.46±0.20aA | 4.59±0.12aA | 0.70±0.00aA | 0.70±0.00aA |
| 240  | 2.99±0.06aA   | 3.42±0.06aA | 4.45±0.05aA | 4.65±0.04aA | 0.70±0.00aA | 0.70±0.00aA |
| 270  | 3.01±0.10aA   | 3.44±0.03aA | 4.47±0.05aA | 4.79±0.08aA | 0.70±0.00aA | 0.70±0.00aA |
| 300  | 3.10±0.05aA   | 3.43±0.05aA | 4.69±0.15aA | 4.78±0.04aA | 0.70±0.00aA | 0.70±0.00aA |

* Time in Minutes. ¹Proteins - Top Point. ²Proteins - Bottom Point. ³Lactose - Top Point. ⁴Lactose - Bottom. ⁵Solids - Top Point. ⁶Solids - Bottom.

#Means and deviations followed by the same small case letter in the vertical axis and capital letters on the horizontal axis do not differ statistically between themselves by the Tukey test at the level of probability of 5%.

The density of the milk may be associated to the Cryoscopic Index (IC), a test serving to control the volume of water present in the milk, recommending the addition of water or removal of its components, or even the addition of some compound to mask a problem (Tronco, 2008; Botaro and Santos, 2016). The cryoscopic point is defined as the temperature at which the milk passes from a liquid to a solid state. The freezing temperature of milk is lower than water due to the substances contained in it, such as lactose and mineral salts. The freezing point can vary depending on the season of the year, feed, breed, health status, age, among others (Alberton, 2012). The higher the freezing point, therefore, the greater the water content in the milk (Robim et al., 2012). The behavior of this parameter on the data obtained in this study corroborate the fat content found in the samples taken at the established times in each collection point.

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The data show that the higher fat rate, the lower the density and the higher the cryoscopic index. At 300 minutes of storage, therefore, the fat contents off in Ps were 16.67±0.05%, the density was 1.012±0.70 g/mL and Cryoscopic Index was-0.553±0.027H (Degrees Hortvet °H), thus showing a clear correlation between these three parameters. The samples taken at the same time of 300 minutes in the Pi, on the other hand, had the opposite behavior.

Castro and Luz (2015) evaluated the quality of chilled raw milk before and after 30 and 60 days of freezing. As a result of all samples under different conditions, they obtained a mean density ranging from 1029.8 to 1031.0 g/cm3 and a cryoscopic index that remained between -0.530 and -0.544°H, values complying with the standards of current legislation. The fat and protein contents remained between 3.5 and 4.0% and 2.90 and 3.19% and the results obtained for ESD remained within a range of 8.46 and 8.77% for the Degreased Dry Extract per formula and 8.19 to 8.90% for the gravimetric Degreased Dry Extract.

The Non-Fatty Solids (SNF), or Degreased Dry Extract (ESD), comprise all the elements of the milk minus the water and fat. They also include, therefore, the solids (SI), also known as ashes, the lactose (L) and the proteins (P). For the dairy industry, the fat, protein, lactose, total dry extract (EST) and degreased dry extract (ESD) contents are criteria used to pay producers, assign raw material within the processes and to predict industrial yield (Beloti et al., 2008; Costa, 2014).

With respect to these evaluated parameters, one can see that they didn't show variations in relation to storage time, not undergoing sedimentation or flotation and remaining stable, therefore.

It should be emphasized that the lactose content found was 4.52% at time zero and 4.69% at 300 minutes of storage. No significant differences (p<0.05) were therefore observed in the Ps and Pt at the evaluated times. The lactose values of this study were similar to the results obtained by Teixeira (2003), who found 4.66% while studying the lactation of Holstein cows. The results obtained here were similar to those in a study performed by Machado (2000), who obtained a mean lactose level of 4.51% in milk samples from expansion tanks (Oliveira and Santos, 2012).

In the study by Brazil et al., (2012), the lactose levels were 4.52% for the milk stored in a isothermal tank and 4.51% for the milk stored in a silo, very similar to this study, but higher than the result of Silva (2008), who reported a lactose level of 4.41% in the period, while Alberton (2012), studying the % lactose in different seasons of the year, observed higher values (4.47 %) in summer and smaller (4.40 %) in fall.

Lactose is the sugar found only in milk, being a disaccharide composed of glucose and galactose (C_{12}H_{22}O_{11}H_{2}O) that is of technological importance in all milk acidification, lactic fermentation, processes, which is the basis of the manufacture of yogurts, fermented butters, cheeses, and the most stable constituent of milk, with virtually no variation between the bovine breeds (Mendes, 2010). The lactose isolated from milk in powder form serves as a raw material in the pharmaceutical industry. It should be noted that the IN-62 from MAPA (Brazil, 2011) does not determine minimum or maximum limits for the milk.

From a qualitative and quantitative point of view, proteins are the noblest nitrogenous compounds found in milk, which are essential in the formation of tissues and the foundation of animal life. It is also the main component in animal and human nutrition since the beginning of life (Koblitz, 2011; Santos et al., 2011).

The protein content of milk has become valuable and is considered in the payment- by-quality programs because it is a nutrient that promotes the quality of raw materials and the industrial yield of milk derivatives. IN 62/2011 determines a minimum total protein content of 2.9 g/100 g of milk (Brazil, 2011), but there can be variations in protein content and the other nutrients in milk as a function of the season, breed, genetics, nutrition and lactation stage (Glantz et al., 2010; Barbosa et al., 2012; Ye et al., 2013).

The data collected in this study revealed that the quantified protein levels had higher values than the requirements of IN-62 from MAPA, which requires values higher than 2.9%. When analyzing these data, one can see that the protein levels determined in the Ps samples in relation to the levels of the Pt samples did not have significant differences at the level of probability of p<0.05.

Brazil et al., (2012) studied the storage of milk and found protein contents of 3.34% in a isothermal tank and of 3.35% in an industrial silo, while Castro and Luz (2015) found that the protein contents remained between 2.90 to 3.19%, values in accordance with the IN 62 (2011) and considered excellent for chilled raw milk, similar to this work.

Several environmental factors influence the protein composition of milk, especially breed, feed and disease management, followed by season of the year, lactation stage and age of the cow. An unbalanced and nutritionally poor diet can cause changes in the composition of the milk, especially regarding the fat and protein levels and the saline balance, causing, for example, low yields in the production of cheeses and a decrease in the milk's thermal stability (Silva, 2009).
The results obtained in this study can serve as support for the technical departments of dairy industries to monitor and qualify the raw material and consequently improve the industrial yield.

It is also hoped that this work will strengthen the commercial relations system between economic agents acting in the milk production chain. It is also expected that Milk Production Associations can use this information as indicators and to assist in the interface between primary production, industry and the consumers of the product.

IV. CONCLUSION
Through this study analyzing the solid constituents of milk, one can conclude that only the fat separates completely from the other constituents, concentrating on the top of the cold milk.

Milk samples taken improperly or without homogenization from the isothermal tanks awaiting unloading of the milk on reception platforms, may compromise the quality of milk and the physicochemical and microbiological characteristics of the product.

Payment by quality is a motivating factor for the producer, but it is not enough that the milk leaves the property in compliance with standard IN 62 (2011). If the sample taken on the platform does not match the sample from the property, the producer will be penalized.

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