Seção Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00382

Colligative property of cryometry in milk

Rafael Alves SANTOMAURO[1], Paula Santolin MANCUSO[1], Aryele Nunes da Cruz Encide SAMPAIO[1], Camila Koutsodontis Cerqueira CÉZAR[1], Evelyn Fernanda Flores CARON[1], Fabio Sossai POSSEBON[1], Juliano Gonçalves PEREIRA[1], Otávio Augusto MARTINS[1]*

Abstract

The aim of the study was to evaluate the colligative property of cryometry and other analytical parameters of various brands of pasteurized whole milk and ultra-high temperature (UHT) whole milk sold in São Paulo State, Brazil. Cryometry measures the freezing point depression caused by non-volatile solutes, providing insights into milk quality and potential adulteration. A total of 40 milk samples from four brands (three pasteurized and one UHT) were analyzed using electronic cryoscopy, density, fat content, lactic acid, and total solids measurements. The cryoscopy method demonstrated high linearity (correlation coefficient of 0.9996), with detection and quantification limits of 0.006 and 0.020 °H, respectively. Significant differences were observed in fat content and density among brands, with the pasteurized brand WMC showing the highest lipid content and density. The lactic acid content in brand WMB was notably lower compared to others. Statistical analysis revealed significant differences in total solids and non-fat solids, with brand WMC having the highest values. The effectiveness of cryometry in assessing milk quality and detecting potential adulterations contributes to improving food safety standards.

Keywords: cryoscopy; density; whole milk; milk quality; non-volatile solutes.

Practical application: This study uses cryometry to assess milk quality, adulteration, and variability.

1 INTRODUCTION

Cryometry involves studying the lowering of the freezing temperature of a solvent when a non-volatile solute is added to it. This means that a pure solvent, such as water, will always freeze at a higher temperature than if a solute is added to form a solution. The dissolved particles will interact with the solvent molecules, hindering their interactions and causing freezing at a lower temperature. Cryometry is one of the four colligative properties (tonometry, ebulliometry, cryometry, and osmometry), which are properties that depend not on the nature of the material but on the concentration of dissolved particles in the solvent (Atkins & Paula, 2014, 2018; Moore, 1976).

In dilute solutions, these colligative properties depend solely on the number of solute particles present, not on their identity. Colligative properties involve studying the physical properties of solutions, specifically of a solvent in the presence of a solute. They are widely used in industrial food processes and various everyday situations. Solidification is the process that occurs when more stable and organized structures are formed due to the reduction of the molecules' kinetic energy, often caused by lowering the temperature (Atkins & Paula, 2018; Moore, 1976).

Cryometry is used by the dairy industry to ensure the physicochemical quality of milk and to prevent fraud in human consumption (Bahramian et al., 2022; Castro et al., 2021; Catunda et al., 2016; Cruz et al., 2018; Sauret et al., 2018).

Cryometry of milk corresponds to the measurement of its freezing point using an electronic cryoscope. This measurement value varies depending on the time of year, geographical region, breed, and cattle feed. The degree of cryometry of milk adulterated with water tends to approach 0°C, which is the freezing point of water. The addition of water to milk not only reduces its quality but can also cause contamination depending on the quality of the added water, posing a health risk to consumers (IAL, 2008).

In this cryometry method, the sample is rapidly cooled a few degrees below its freezing point while being constantly agitated. The resulting vibration causes thermal imbalance within the sample, leading the solution to release latent heat of fusion. The temperature rises until it reaches the freezing point and remains constant for some time. This period is known as the plateau, during which the freezing point is read (Apha, 1992; IAL, 2008).

This study aimed to evaluate the colligative property of cryometry alongside other analytical parameters (non-volatile solutes) of different brands of pasteurized whole milk and ultra-high temperature (UHT) whole milk sold in the São Paulo State, Brazil.

Received: Aug. 2, 2024.
Accepted: Sep. 9, 2024.
[1]*Universidade Estadual Paulista "Júlio de Mesquita Filho", School of Veterinary Medicine and Animal Science, Food Inspection Laboratory, Botucatu, São Paulo, Brazil.*
*Corresponding author: otavio.a.martins@unesp.br
Conflict of interest: nothing to declare.
Funding: Laboratory of Applied Physical Chemistry to Food, Public Nutrition Guidance Service, and Support Foundation for Veterinary Hospitals of Universidade Estadual Paulista "Júlio de Mesquita Filho."

2 MATERIALS AND METHODS

2.1 Samples

Three brands of pasteurized whole milk (whole milk A (WMA), whole milk B (WMB), and whole milk C (WMC)) and one brand of UHT whole milk (whole milk D (WMD)) sold in the city of Botucatu, São Paulo, Brazil, were evaluated. A total of 40 samples were analyzed, including 10 samples from different batches of each brand. The samples were sent to the Laboratory of Food Physicochemistry of the Department of Animal Production and Preventive Veterinary Medicine at the Faculty of Veterinary Medicine and Animal Science of Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp) Campus of Botucatu, São Paulo, Brazil. Each assay was performed in triplicate, and the results were expressed as mean ± standard deviation.

2.2 Validation and cryoscopy determination

The validation of cryometry determination in pasteurized whole milk and UHT milk was conducted to verify if the methodology is suitable for the intended purpose. The parameters used included linearity through the standard curve, detection limit, quantification limit, and repeatability (Brasil, 2011, 2014, 2017; SBM, 2022). The standard curve was determined using sucrose solutions at 20°C with the following concentrations: 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, and 10 g/100 mL. The detection limit (LD) and quantification limit (LQ) parameters for cryometry determination were assessed through seven measurements of pasteurized and UHT whole milk samples. Repeatability was also evaluated with seven measurements of pasteurized and UHT whole milk under three conditions (Brasil, 2011, 2014, 2017; SBM, 2022).

The parameters used were linearity through the standard curve, detection limit, quantification limit, and repeatability (Brasil, 2011, 2014, 2017; SBM, 2022). For linearity, the standard curve determination of sucrose solutions at 20°C was conducted at the following concentrations: 1.0, 2.5, 4.0, 5.5, 7.0, 8.5, and 10 g/100 mL. The parameters for LD and LQ for cryoscopy determination were assessed through seven measurements of pasteurized and UHT whole milk samples. Repeatability was also evaluated with seven pasteurized and UHT whole milk measurements under three distinct conditions (Brasil, 2011, 2014, 2017; SBM, 2022).

The digital electronic cryoscope (ITR—MK 540) was used in Hortvet degrees. The equipment was calibrated with two standard solutions for cryoscopy (0.000 °H and −0.621 °H) using 2.5 mL of sample. A negative control was conducted with milk without added water, yielding values between −0.530 °H and −0.550 °H. For values above −0.530 °H, the following formula was applied to determine the added water content in the milk (%) (Eq. 1) (AOAC, 1995; Brasil, 1981, 2005, 2022; IAL, 2008):

$$\text{Added water (\%)} = [(0.550 - \text{Reading}) \times 100]/0.550 \quad (1)$$

2.3 Lactic acid determination

The samples were homogenized, and 10 mL of the sample was transferred to a 50 mL beaker. Next, two to four drops of 1% phenolphthalein indicator solution were added, and titration was performed with N/9 sodium hydroxide solution or Dornic solution until the endpoint (slightly pink color change). Each 0.1 mL of N/9 sodium hydroxide solution or Dornic solution used in titration corresponds to 1 °D (Dornic degree) or 0.01 g of lactic acid per 100 mL of sample (AOAC, 1995; Brasil, 1981, 2005, 2022; IAL, 2008).

2.4 Density determination

A total of 220 mL of homogenized milk was transferred to a 250 mL polyethylene graduated cylinder. The clean and dry thermolactodensimeter was slowly inserted into the milk sample cylinder, ensuring it did not touch the cylinder walls. The temperature (°C) and density (g/mL) were recorded. Density correction was performed by adding 0.0002 for each degree above 15°C or subtracting 0.0002 for each degree below 15°C. Additionally, 0.0002 was added or subtracted for each group of five degrees in the calculated temperature difference (AOAC, 1995; Brasil, 1981, 2005, 2022; IAL, 2008).

2.5 Fat determination

A total of 10 mL of sulfuric acid with a density of 1.820–1.825 g/mL was transferred into the Gerber butyrometer. Then, 11 mL of homogenized milk was added to the butyrometer using a volumetric pipette, along with 1 mL of isoamyl alcohol with a density of 0.850 g/mL. The mixture was homogenized and centrifuged at 2000 rpm for 2–5 min. The butyrometer was placed in a water bath at approximately 65°C for 5 min. The fat content was then read from the milk butyrometer scale (AOAC, 1995; Brasil, 1981, 2005, 2022; IAL, 2008).

2.6 Total solids

The Ackermann disk, which consists of an inner circle (density), a middle circle (lipid), and an outer circle (total solids), was used. The values for milk fat and density were used. The inner circle (density) and the middle circle (lipid) values coincided. The arrow on the disk indicated the outer circle, which corresponded to the value of total solids (g/100 mL) or total dry extract (g/100 mL) (AOAC, 1995; Brasil, 1981, 2005, 2022; IAL, 2008).

2.7 Non-fat solids

The values obtained from the fat content determination (g/100 mL) and total solids (TS) determination (g/100 mL) were used. These values were applied in the following formula for non-fat solids (NFS) (Eq. 2) (Brasil, 1981, 2005, 2022; IAL, 2008):

$$\text{NFS (g/100 mL)} = \text{TS (g/100 mL)} - \text{lipids (g/100 mL)} \quad (2)$$

2.8 Statistical analysis

The values obtained from the trials of the samples, conducted in triplicate, were statistically analyzed using analysis of variance with a completely randomized design, supplemented

by Tukey's test for mean comparison, considering a significance level of 5% (Montgomery, 2020).

3 RESULTS

Appendices 1, 2, and 3 depict the validation of the cryometry method through the parameters of linearity, LD, LQ, and repeatability. For linearity, the correlation coefficient (r), intercept (a), and slope (b) were 0.9996, −0.0043, and 0.0622, respectively (see Appendix 1). The LD and LQ were 0.006 °H and 0.020 °H, respectively (Appendix 2). In the repeatability study, three coefficients of variation (CVs) were obtained: 0.55%, 0.37%, and 0.37% (see Appendix 3).

The mean values of cryoscopy assay (°H) for the brands WMA (−0.531 °H ± 0.012 °H), WMB (−0.528 °H ± 0.028 °H), WMC (−0.543 °H ± 0.012 °H), and WMD (−0.540 °H ± 0.012 °H) showed no significant differences (p=0.1874). However, samples from brands WMA (−0.531 °H) and WMB (−0.528 °H) exhibited the highest average cryoscopy values (Table 1). The relative frequencies of values greater than −0.530 °H in the brands WMA, WMB, WMC, and WMD were 0.4, 0.3, 0.1, and 0.1, respectively (Tables 2–7).

The brand WMB (0.15 g/100 mL ± 0.01 g/100 mL) showed the lowest significant mean value (p = 0.0142) of lactic acid content compared to the other brands: WMA (0.17 g/100 mL ± 0.02 g/100 mL), WMC (0.17 g/100 mL ± 0.01 g/100 mL), and WMD (0.16 g/100 mL ± 0.01 g/100 mL) (Table 2). In brand WMB, the relative frequency was 0.1 (1/10) for a value lower than 0.14 g of lactic acid/100 mL. Brands WMA and WMD had relative frequencies of 0.1 each for values greater than 0.18 g of lactic acid/100 mL (Table 7).

The brand WMC (1.0327 g/mL ± 0.0013 g/mL) showed the highest significant mean value (p = 0.0358) of density compared to the other brands: WMA (1.0308 g/mL ± 0.0014 g/mL), WMB (1.0314 g/mL ± 0.0018 g/mL), and WMD (1.0310 g/mL ± 0.0013 g/mL) (Table 3). Only brand WMC exhibited a relative frequency of 0.1 for values greater than 1.034 g/mL in density (Table 7).

The brands of whole pasteurized milk, WMA (3.60 g/100 mL ± 0.43 g/100 mL), WMB (3.07 g/100 mL ± 0.76 g/100 mL), WMC (4.68 g/100 mL ± 0.22 g/100 mL), and whole UHT milk,

Table 1. Mean ± standard deviation of cryoscopy (°H) of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Brand of milk	Mean ± standard deviation
WMA	−0.531 °H ± 0.012 °H a[(1)]
WMB	−0.528 °H ± 0.028 °H a
WMC	−0.543 °H ± 0.012 °H a
WMD	−0.540 °H ± 0.012 °H a

[(1)]CV = 3.24% and p = 0.1874. WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk; statistical analysis supplemented with Tukey's test at a 5% significance level. Lowercase letters in the same column indicate that there is no statistically significant difference (p < 0.05).

Table 2. Mean ± standard deviation of lactic acid content (g/100 mL) of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Brand of milk	Mean ± standard deviation
WMA	0.17 g/100 mL ± 0.02 g/100 mL b[(1)]
WMB	0.15 g/100 mL ± 0.01 g/100 mL a
WMC	0.17 g/100 mL ± 0.01 g/100 mL b
WMD	0.16 g/100 mL ± 0.01 g/100 mL ab

[(1)]CV = 9.36% and p = 0.0142. WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk; statistical analysis supplemented with Tukey's test at a 5% significance level. Lowercase letters in the same column indicate that there is no statistically significant difference (p < 0.05).

Table 3. Mean ± standard deviation of density (g/mL) of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Brand of milk	Mean ± standard deviation
WMA	1.0308 g/mL ± 0.0014 g/mL a[(1)]
WMB	1.0314 g/mL ± 0.0018 g/mL ab
WMC	1.0327 g/mL ± 0.0013 g/mL b
WMD	1.0310 g/mL ± 0.0013 g/mL ab

[(1)]CV = 0.15% and p = 0.0358; WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk; statistical analysis supplemented with Tukey's test at a 5% significance level. Lowercase letters in the same column indicate that there is no statistically significant difference (p < 0.05).

Table 4. Mean ± standard deviation of lipid content (g/100 mL) of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Brand of milk	Mean ± standard deviation
WMA	3.60 g/100 mL ± 0.43 g/100 mL a[(1)]
WMB	3.07 g/100 mL ± 0.76 g/100 mL a
WMC	4.68 g/100 mL ± 0.22 g/100 mL b
WMD	3.08 g/100 mL ± 0.16 g/100 mL a

[(1)]CV = 12.63% and p = 0.0001; WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk; statistical analysis supplemented with Tukey's test at a 5% significance level. Lowercase letters in the same column indicate that there is no statistically significant difference (p < 0.05).

Table 5. Mean ± standard deviation of total solids content (g/100 mL) of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Brand of milk	Mean ± standard deviation
WMA	12.19 g/100 mL ± 0.68 g/100 mL a[(1)]
WMB	11.68 g/100 mL ± 1.19 g/100 mL a
WMC	14.11 g/100 mL ± 0.38 g/100 mL b
WMD	11.76 g/100 mL ± 0.45 g/100 mL a

[(1)]CV = 5.99% and p < 0.0001; WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk; statistical analysis supplemented with Tukey's test at a 5% significance level. Lowercase letters in the same column indicate that there is no statistically significant difference (p < 0.05).

WMD (3.08 g/100 mL ± 0.16 g/100 mL), showed extremely significant differences in lipid content (p = 0.0001). Brand WMC exhibited the highest significant mean value in lipid content compared to the other brands (Table 4). Brands WMB and WMD had relative frequencies of 0.3 and 0.1, respectively, for lipid contents below 3 g/100 mL (Table 7).

The p-values for the determinations of TS (Table 5) and NFS (Table 6) were <0.0001 and equal to 0.0001, respectively. These p-values indicate that there are extremely significant differences among the analyzed milk brands for TS and NFS. In the determination of TS, brand WMC (14.11 g/100 mL ± 0.38 g/100 mL) showed the highest significant mean value compared to the other brands: WMA (12.19 g/100 mL ± 0.68 g/100 mL), WMB (11.68 g/100 mL ± 1.19 g/100 mL), and WMD (11.76 g/100 mL ± 0.45 g/100 mL). This statistical result was similarly observed in the determination of NFS, where brand WMC (9.43 g/100 mL ± 0.31 g/100 mL) had the highest significant value compared to brands: WMA (8.59 g/100 mL ± 0.46 g/100 mL), WMB (8.61 g/100 mL ± 0.52 g/100 mL), and WMD (8.68 g/100 mL ± 0.40 g/100 mL). The relative frequencies of NFS values below 8.4 g/100 mL for brands WMA, WMB, WMC, and WMD were 0.2, 0.4, 0.0, and 0.1, respectively (Table 7).

Table 6. Mean ± standard deviation of non-fat solids content (g/100 mL) of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Brand of milk	Mean ± standard deviation
WMA	8.59 g/100 mL ± 0.46 g/100 mL a[(1)]
WMB	8.61 g/100 mL ± 0.52 g/100 mL a
WMC	9.43 g/100 mL ± 0.31 g/100 mL b
WMD	8.68 g/100 mL ± 0.40 g/100 mL a

[(1)]CV = 4.85% and p = 0.0001; WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk; statistical analysis supplemented with Tukey's test at a 5% significance level. Lowercase letters in the same column indicate that there is no statistically significant difference (p < 0.05).

4 DISCUSSION

The correlation coefficient in this study was 0.9996, indicating that the developed method exhibits a perfect linear relationship (SBM, 2022). This is a measure of the degree of linear relationship between two quantitative variables and is one of the criteria for approving linearity, ranging from −1 (perfect inverse linear relationship) to 1 (perfect linear relationship), with 0 indicating no linear relationship. The closer the value is to 1 or −1, the stronger the linear association between the two variables. This study's correlation coefficient was 0.9996, indicating that the developed method exhibits a perfect linear relationship (SBM, 2022).

The LD of an individual analytical procedure is the smallest amount of analyte in the sample that can be detected, but not necessarily quantified, under the established conditions of the assay. The LD for an analytical procedure can vary depending on the type of sample. Therefore, it is necessary to ensure that all stages of the analytical method's processing are included in determining this limit of detection. The lowest acceptable concentration is the lowest concentration for which a degree of uncertainty can be deemed satisfactory. It is essential to conduct independent evaluations on samples with concentrations equal to the determined limit of detection (Perez, 2010; SBM, 2022). The LD of the experiment was 0.006 °H for the determination of the colligative property of cryometry.

Repeatability is defined as the degree of agreement between the results of successive measurements of the same measure carried out under the same measurement conditions (SBM, 2022). The repeatability limit is the maximum acceptable difference between two repetitions—between two independent results of the same test in the same laboratory under the same conditions. Repeatability was satisfactory in the three evaluations, as the CVs were 0.55%, 0.37%, and 0.37%, all below the suggested maximum CV of 10% (SBM, 2022).

The properties of dilute solutions that depend on the number of solute molecules and not the type of solute are called colligative properties (Moore, 1976). Cryoscopy consists of the

Table 7. Relative frequency of cryoscopy, lipids, lactic acid, non-fat solids, and density assays from different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Tests	Parameters [(1)]	WMA	WMB	WMC	WMD
Cryometry (°H)	>−0.530	0.4 (4/10)	0.3 (3/10)	0.1 (1/10)	0.1 (1/10)
	<−0.555	0.0 (0/10)	0.0 (0/10)	0.2 (2/10)	0.1 (1/10)
	−0.555 ≤ × ≤ −0.530	0.6 (6/10)	0.7 (7/10)	0.7 (7/10)	0.8 (8/10)
Lipids (g/100 mL)	≥3.0	1.0 (10/10)	0.7 (7/10)	1.0 (10/10)	0.9 (9/10)
	<3.0	0.0 (0/10)	0.3 (3/10)	0.0 (0/10)	0.1 (1/10)
Lactic acid (g/100 mL)	>0.18	0.1 (1/10)	0.0 (0/10)	0.0 (0/10)	0.1 (1/10)
	<0.14	0.0 (0/10)	0.1 (1/10)	0.0 (0/10)	0.0 (0/10)
	0.14 ≤ × ≤ 0.18	0.9 (9/10)	0.9 (9/10)	1.0 (10/10)	0.9 (9/10)
NFS (g/100 mL)	≥8.4	0.8 (8/10)	0.6 (6/10)	1.0 (10/10)	0.9 (9/10)
	<8.4	0.2 (2/10)	0.4 (4/10)	0.0 (0/10)	0.1 (1/10)
Density (g/mL)	<1.028	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)	0.0 (0/10)
	>1.034	0.0 (0/10)	0.0 (0/10)	0.1 (1/10)	0.0 (0/10)
	1.028 ≤ × ≤ 1.034	1.0 (10/10)	1.0 (10/10)	0.9 (9/10)	1.0 (10/10)

[(1)] Brasil (2018); NFS: non-fat solids; WMA, WMB, and WMC: different brands of pasteurized whole milk; WMD: UHT whole milk.

lowering of the freezing point of milk. A non-volatile solute produces the effect of lowering the freezing point of the milk solution. This effect arises from variations in the disorder of the solvent (Atkins & Paula, 2018). The increase in disorder is independent of the species used to provoke it; for a given solvent, it depends only on the number of solute particles present and not on their chemical identity (Atkins & Paula, 2018; Moore, 1976). The WMB sample showed significantly ($p < 0.05$) lower concentrations of lactic acid (0.15 g/100 mL), lipids (3.07 g/100 mL), total solids (11.68 g/100 mL), and non-fat solids (8.61 g/100 mL), and consequently, it exhibited a less negative cryometry (−0.528 °H) compared to the other evaluated brands (WMA, WMC, and WMD). These results corroborate the definitions of Atkins and Paula (2018) that the lower concentration of solutes in the WMB brand milk solution increases the freezing point through the determination of cryometry.

The WMC brand showed significantly ($p < 0.05$) higher concentrations of lactic acid (0.17 g/100 mL), density (1.0327 g/mL), lipids (4.68 g/100 mL), total solids (14.11 g/100 mL), and non-fat solids (9.43 g/100 mL) and, consequently, had a more negative cryometry (−0.543 °H) compared to the other evaluated brands (WMA, WMB, and WMD). The cryometry data and other non--volatile solutes identified in the analyzed samples of whole milk (pasteurized and UHT) corroborate Moore's (1976) definition of the colligative property of the freezing point. The decrease in the freezing point is proportional to the molality of the solute (Atkins & Paula, 2018). This study demonstrated that non-volatile solutes (lactic acid, density, lipids, total solids, and non-fat solids) in whole milk (pasteurized and UHT) showed an inverse proportionality with the freezing point (cryometry or cryoscopy). Whole milk samples with higher concentrations of non-volatile solutes exhibit a greater decrease in the freezing point or vice versa.

The parameters defined to prevent milk fraud establish a cryoscopy range between −0.555 °H and −0.530 °H (Brasil, 2018). Values above −0.530 °H suggest the addition of water to increase the volume, while values below −0.555 °H may indicate the presence of reconstituted products to mask this fraud. A total of 70% (28/40) of the milk samples complied with the legislation (Table 7), with WMD (8/10) and WMC and WMB (7/10 each) being notable, while WMA had the lowest compliance rate (6/10). Of the samples outside the limit, 22.5% (9/40) had cryoscopic values above −0.530 °H, suggesting water addition. WMA (4/10) had the highest incidence of this fraud, followed by WMB (3/10) and WMC and WMD (1/10 each). In 7.5% (3/40) of the samples, the cryoscopy was below −0.555 °H, possibly indicating the addition of reconstituted products to mask water addition: WMC (2/10) and WMD (1/10).

Milk fat is a high-value byproduct, serving as a raw material for various dairy products such as butter and cream, and it should have a minimum fat content of 3.0% for whole milk (Brasil, 2018). The results showed that 90% (36/40) of the analyzed samples complied with the legislation. The WMB brand had the lowest percentage, at 30% (7/10) (Table 7). Failures in the skimming process or even intentional fraud are possible reasons for this reduced fat content.

Lactic acid is an organic compound naturally present in milk and its derivatives, formed from the fermentation of lactose by lactic acid bacteria. The lactic acid content in milk should be between 0.14 g/100 mL and 0.18 g/100 mL (Brasil, 2018). Levels above the legal limit may indicate failures in hygienic-sanitary conditions during processing and/or storage, leading to the proliferation of lactose-fermenting microorganisms and deterioration of milk quality, while lower levels may be related to dilution of the product. This study showed that 92.5% (37/40) of the samples complied with the law, as shown in Table 7: WMC (10/10), WMA, WMB, and WMD (9/10 each). Only two samples showed deviations in lactic acid content: one from the WMD brand (1/10), with a value above the legal limit (0.18 g/100 mL), and one from the WMB brand (1/10), with a value below the minimum (0.14 g/100 mL).

The NFS in milk are the non-fat, non-volatile components, excluding water and fat. They should represent at least 8.4% of the composition of whole milk (Brasil, 2018). Among the evaluated samples, 82.5% (33/40) had NFS levels of at least 8.4%, as shown in Table 10: WMA (8/10), WMB (6/10), WMC (10/10), and WMD (9/10). Meanwhile, 17.5% (7/40) were non-compliant: WMA (2/10), WMB (4/10), and WMD (1/10). These deviations may occur due to various factors; however, since the samples were not from individual cows, they could be related to milk processing issues, such as water addition or failures in the homogenization process.

Density is an important parameter for assessing milk quality, as it indicates potential changes in total solids content and helps identify adulteration through water addition. The minimum and maximum density values for whole milk are 1.028 g/mL to 1.034 g/mL (Brasil, 2018). The results showed that 97.5% (39/40) of the analyzed samples had densities within the legal limits, demonstrating the overall quality of the evaluated milk: WMA (10/10), WMB (10/10), WMC (9/10), and WMD (10/10). One sample from the WMC brand (1/10) had a density below the legal minimum (1.028 g/mL).

5 CONCLUSION

Cryometry is essential for detecting adulteration and ensuring milk quality. The freezing point values of the samples showed no significant differences between brands but indicated subtle variations that might reflect differences in processing and formulation practices. The analysis of lactic acid, density, lipids, total solids, and non-fat solids complemented the evaluation, showing variations between brands that could impact product quality. The accuracy of the analytical methods used was confirmed, and the results highlighted the ongoing importance of rigorous quality control in the dairy industry to ensure the integrity of the milk consumed.

REFERENCES

American Public Health Association (APHA) (1992). *Standard methods for the examination of dairy products* (16th ed). APHA.

Association of Official Analytical Chemists (AOAC) (1995). *Official methods of analysis of the Association of Official Analytical Chemists*. AOAC.

Atkins, P., & Paula, J. (2014). *Atkins' physical chemistry* (10th ed.). Oxford University Press.

Atkins, P., & Paula, J. (2018). *Físico-química: fundamentos* (6th ed.). LTC.

Bahramian, B., Sani, M. A., Parsa-Kondelaji, M., Hosseini, H., Khaledian, Y., & Rezaie, M. (2022). Antibiotic residues in raw and pasteurized milk in Iran: A systematic review and meta-analysis. *AIMS Agriculture and Food*, *7*(3), 500-519. https://doi.org/10.3934/agrfood.2022031

Brasil (1981). *Métodos analíticos oficiais para controle de produtos de origem animal e seus ingredientes. II – Métodos físicos e químicos*. LANARA.

Brasil (2005). *Métodos físico-químicos para análise de alimentos* (4th ed.). Instituto Adolfo Lutz, Ministério da Saúde.

Brasil (2011). *Guia de validação e controle de qualidade analítica: Fármacos em produtos para alimentação e medicamentos veterinários*. Secretaria de Defesa Agropecuária.

Brasil (2014). *Manual de validação, verificação/confirmação de desempenho, estimativa da incerteza de medição e controle de qualidade intralaboratorial*. Secretaria de Defesa Agropecuária.

Brasil (2017). RDC n. 166, de 24 de julho de 2017. Validação de métodos analíticos. *Diário Oficial da União*, (141).

Brasil (2018). *Portaria nº 38 de 19 de abril de 2018*. Secretaria de Defesa Agropecuária.

Brasil (2022). *Métodos oficiais para análise de produtos de origem animal*. Secretaria de Defesa Agropecuária.

Castro, M. S. M., Oliveira, D. S., Fontenelle, R. O. S., Nascimento, A. P. A., Silveira, R. M. F., Vega, W. H. O., Silva, L. C., Soares, A. T. L., & Vasconcelos, A. M. (2021). Understanding the dairy production systems in rural settlements in the Brazilian semi-arid region: Characterization, typology, and holistic perception. *Tropical Animal Health and Production*, *53*, 417. https://doi.org/10.1007/s11250-021-02840-x

Catunda, K. L. M., Aguiar, E. M., Góes Neto, P. E., Silva, J. G. M., Moreira, J. A., Rangel, A. H. N., & Lima Júnior, D. M. (2016). Gross composition, fatty acid profile and sensory characteristics of Saanen goat milk fed with cacti varieties. *Tropical Animal Health and Production*, *48*, 1253-1259. https://doi.org/10.1007/s11250-016-1085-7

Cruz, G., Díaz, P., & Bonifaz, N. (2018). Milk quality management of small and medium cattle ranchers of collection centers and artisan cheese factories, for continuous improvement: Case study: Carchi, Ecuador. *Granja*, *27*(1), 124-136. https://doi.org/10.17163/lgr.n27.2018.10

Instituto Adolfo Lutz (IAL) (2008). *Métodos físico-químicos para análise de alimentos*. Instituto Adolfo Lutz.

Montgomery, D. C. (2020). *Design and analysis of experiments* (10th ed.). Wiley.

Moore, W. J. (1976). *Físico-química* (2 v.). Edgard Blucher.

Perez, M. A. F. (2010). Validação de métodos analíticos: Como fazer? Por que ela é importante? *Boletim de Tecnologia e Desenvolvimento de Embalagens*, *22*(3), 1-9.

Sauret, A., Andro-Garc, M. C., Chauvel, J., Ligneul, A., Dupas, P., Fressange-Mazda, C., Le Ruyet, P., & Dabadie, A. (2018). Osmolality of a fortified human preterm milk: The effect of fortifier dosage, gestational age, lactation stage, and hospital practices. *Archives de Pédiatrie*, *25*(7), 411-415. https://doi.org/10.1016/j.arcped.2018.08.006

Sociedade Brasileira de Metrologia (SBM) (2022). *Validação de métodos de ensaio*. SBM.

Appendix 1. Linearity of the standard curve of the colligative property of cryometry (°H) for analyses of different brands of pasteurized whole milk and UHT whole milk sold in the São Paulo State, Brazil.

Test	Sucrose (g/100 mL)	Mean ± standard deviation
1	1.0	−0.062 °H ± 0.002 °H
2	2.5	−0.152 °H ± 0.001 °H
3	4.0	−0.243 °H ± 0.003 °H
4	5.5	−0.333 °H ± 0.002 °H
5	7.0	−0.422 °H ± 0.001 °H
6	8.5	−0.531 °H ± 0.002 °H
7	10.0	−0.620 °H ± 0.002 °H
Intercept coefficient (a)		−0.0043
Slope coefficient (b)		0.0622
Correlation coefficient (r)		0.9996
Equation of the line (1)		y = 0.0622x − 0.0043

(1) y = bx + a: b = slope coefficient and a = intercept coefficient. The x-axis = concentration (g/100 mL) of aqueous sucrose solution and the y-axis = cryoscopy (°H) of the solutions.

Appendix 2. Limits of detection (LD) and quantification (LQ) of cryoscopy (°H) determination for analyses of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Repetition	°H
1	−0.539
2	−0.541
3	−0.546
4	−0.544
5	−0.544
6	−0.545
7	−0.542
Mean ± standard deviation	−0.543 °H ± 0.002 °H
t (unilateral with 99% confidence)	3.143
LD (°H)[1]	0.006
LQ (°H)[2]	0.020

[1]$LD = t_{(n-1;\,1-\alpha)} \cdot s$ [2]LQ = 10. s

Appendix 3. Repeatability of cryoscopy (°H) determination for analyses of different brands of pasteurized whole milk and UHT whole milk sold in São Paulo State, Brazil.

Repetition	Analysis 1	Analysis 2	Analysis 3
1	−0.537 °H	−0.540 °H	−0.539 °H
2	−0.541 °H	−0.541 °H	−0.537 °H
3	−0.543 °H	−0.543 °H	−0.536 °H
4	−0.544 °H	−0.544 °H	−0.540 °H
5	−0.542 °H	−0.541 °H	−0.540 °H
6	−0.545 °H	−0.544 °H	−0.541 °H
7	−0.542 °H	−0.545 °H	−0.538 °H
Mean	−0.542 °H	−0.543 °H	−0.539 °H
Standard deviation	±0.003 °H	±0.002 °H	±0.002 °H
CV[1]	0.55[2]	0.37	0.37

[1]CV (%) = [standard deviation/mean] × 100; [2]Ideal = CV < 10%.