









## Effect of lactose-free UHT milk macromolecules on the colligative property of cryometry

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### Abstract

Ultra-high-temperature milk in Brazil is marketed in categories based on fat content: whole, semi-skimmed, and skimmed. Additionally, there are options for consumers with some degree of lactose intolerance, classified as “lactose-free” or “low-lactose” according to lactose concentration. Cryometry is a colligative property for detecting milk fraud through the addition of water and/or reconstituted components. This study aimed to evaluate the effect of macromolecules in lactose-free ultra-high-temperature milk on the colligative property of cryometry. Physicochemical characteristics of ultra-high-temperature milk (whole, semi-skimmed, skimmed, and lactose-free) were analyzed, including protein, carbohydrate, and lipid contents; density; total and non-fat solids; minerals; and cryometry (or cryoscopy). The lactose-free milk presented higher protein ( $3.30 \text{ g}/100 \text{ g} \pm 0.13 \text{ g}/100 \text{ g}$ ), carbohydrate ( $4.97 \text{ g}/100 \text{ g} \pm 0.20 \text{ g}/100 \text{ g}$ ), and mineral content ( $0.72 \text{ g}/100 \text{ g} \pm 0.04 \text{ g}/100 \text{ g}$ ) and a lower freezing point ( $-0.569^\circ\text{H} \pm 0.021^\circ\text{H}$ ). Lactose hydrolysis by lactase increases the number of dissolved solutes, reducing the freezing point. Therefore, it plays a significant role in increasing the number of dissolved solutes contributing to the reduction of the cryoscopic point compared to other categories.

**Keywords:** physical chemical analysis; cryoscopy; minerals; milk quality; non-volatile solutes.

**Practical Application:** This study sought to assess the impact of macromolecules in lactose-free ultra-high-temperature milk on the colligative property of cryoscopy.

## 1 INTRODUCTION

Lactose is a disaccharide composed of the monosaccharides glucose and galactose. It is a sugar naturally present in the milk of most mammals and serves as an important source of energy, particularly for newborns (Hodges et al., 2019). In addition to its energetic value, lactose enhances the absorption of minerals such as calcium, magnesium, and zinc by increasing their solubility, lowering intestinal pH, and creating a more favorable environment for their transport. Another advantage is its low glycemic index, which means it provides energy without causing sharp increases in blood sugar levels. Lactose also exhibits a prebiotic effect, selectively stimulating the growth of beneficial intestinal microbiota and contributing to the regulation of the immune system (Pontonio et al., 2020).

Some consumers of dairy products may exhibit varying degrees of lactose intolerance, a condition that, in most cases, results from primary malabsorption of this sugar. The activity of the enzyme lactase, which is responsible for breaking down lactose in the small intestine, is highest during the neonatal period

but tends to decrease progressively after weaning. When lactose is not properly digested, it is fermented by the intestinal microbiota and converted into short-chain fatty acids such as acetate, propionate, butyrate, lactate, and formate, along with gases like hydrogen, methane, and carbon dioxide. This fermentation process can lead to gastrointestinal symptoms, including abdominal distension, diarrhea, flatulence, and discomfort (Li et al., 2023).

For these individuals, the need to hydrolyze the lactose present in milk using the enzyme  $\beta$ -galactosidase (lactase) arose. The hydrolysis of lactose catalyzed by  $\beta$ -galactosidase occurs in two main steps: first, an enzyme-galactose complex is formed, with the simultaneous release of glucose. In the subsequent step, galactose is transferred to a hydroxylated acceptor. Under low lactose concentration conditions, water serves as the primary acceptor, leading to the release of galactose. However, at higher lactose concentrations, galactose may react with other sugars, leading to transglycosylation reactions and the formation of oligosaccharides. (Dantas et al., 2019).

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The Brazilian Health Regulatory Agency (ANVISA) requires the validation of methods used to measure the lactose content in food products. According to current regulations, milk is classified as “lactose-free” when its lactose content is below 100 mg per 100 mL. Products with lactose levels between 100 mg and 1 g per 100 mL are labeled as “low-lactose” (Brasil, 2017b). Furthermore, in the composition of “lactose-free” milk, in addition to glucose and galactose, which are products of lactose hydrolysis, there is also residual lactase, the enzyme used in the process of breaking down the disaccharide (Brasil, 2022).

Ultra-high temperature (UHT) milk undergoes a specific technological process that includes homogenization and thermal treatment between 130 °C and 150 °C for a period of 2 to 4 seconds, followed by immediate cooling to temperatures below 32 °C. After this process, the milk must be aseptically packaged and hermetically sealed to ensure its microbiological stability (Brasil, 2017a). The widespread acceptance of UHT milk in Brazil is associated with its long shelf life, as this product does not require refrigeration for storage before opening, providing greater convenience for the consumer (Martins et al., 1999).

Milk must exhibit a freezing point within the established standard, between  $-0.530^{\circ}\text{H}$  and  $-0.555^{\circ}\text{H}$ . Deviations from this parameter may indicate adulteration, such as the addition of water and/or reconstitutes, which bring the freezing point closer to 0 °C (Brasil, 2018). In samples of whole milk, both pasteurized and UHT, that contain higher concentrations of non-volatile solutes, a greater depression of the freezing point is observed. In contrast, samples with lower solute concentrations exhibit a less negative freezing point. This behavior is explained by the colligative property of cryometry, which relates solute concentration to the lowering of the freezing point (Santomauro et al., 2024).

Therefore, this study aimed to evaluate the effect of macromolecules in UHT milk, after lactose hydrolysis, on the colligative property of cryometry in lactose-free UHT milk.

### 1.1 Relevance of the work

This study holds significance by offering a comprehensive understanding of the influence of macromolecules in lactose-free UHT milk on colligative properties, particularly cryoscopy. Analysis of macromolecules in lactose-free UHT milk revealed that lactose hydrolysis increases solute concentration (proteins, carbohydrates, and minerals), significantly lowering the cryoscopic point ( $-0.569^{\circ}\text{H}$ ). This finding reinforces cryometry as an essential tool for identifying adulterations, such as water addition, in dairy products. The study provides technical insights to enhance quality monitoring, ensuring compliance with regulatory standards.

## 2 MATERIALS AND METHODS

### 2.1 Samples

The samples analyzed in this study represent commercially available products in Brazil, sourced from nine leading dairy

brands commonly found in supermarkets in Botucatu, São Paulo, thereby reflecting the typical composition and processing practices of Brazilian dairy products. The samples were grouped into four treatments: (a) whole UHT milk, (b) skimmed UHT milk, (c) semi-skimmed UHT milk, and (d) lactose-free UHT milk. A total of 20 samples were analyzed: four whole milk samples, five skimmed, five semi-skimmed, and six lactose-free UHT milk samples. Among the lactose-free samples, five were semi-skimmed and one was skimmed. All samples were sent to the Laboratory of Applied Food Physical Chemistry of the Public Food Guidance Service (SOAP), Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animal Science, São Paulo State University “Júlio de Mesquita Filho” (Unesp), Botucatu campus, São Paulo, Brazil. Physicochemical analyses were performed in triplicate, totaling 60 determinations.

### 2.2 Density at 15 °C

Approximately 220 mL of the homogenized milk sample was transferred to a 250 mL graduated cylinder, ensuring no foam formation. The thermolactodensimeter was carefully inserted into the sample, avoiding contact with the walls of the container. After stabilization, the density and temperature of the sample were recorded. For values different from 15 °C, the corresponding correction was applied by adding 0.0002 for each degree above or subtracting the same value for each degree below the reference. For values with a difference of more than five units, the correction for five-degree groups was applied, with each five-degree change being accounted for. The density unit was expressed in g/mL (Association of Official Analytical Chemists [AOAC], 1995; Brasil, 2022; Instituto Adolfo Lutz [IAL], 2005).

### 2.3 Lipids

Ten milliliters of sulfuric acid (1.820–1.825 g/mL) was added to the Gerber butyrometer. Eleven milliliters of homogenized milk were carefully transferred into the butyrometer. One to three milliliters of isoamyl alcohol (0.850 g/mL) was added. The inner mouth of the butyrometer was cleaned with absorbent paper and sealed. The sample was homogenized by successive inversions and centrifuged at 2,000 rpm for 5 minutes. When the ambient temperature was below 20 °C, the butyrometers were preheated in a water bath at 65 °C for 5 minutes. The reading was performed directly on the scale of the glassware used (AOAC, 1995; Brasil, 2022; IAL, 2005).

### 2.4 Total solids

The analyses were performed using the Ackermann method for whole and semi-skimmed UHT milk samples and the Fleischmann method for skimmed milk samples due to the impossibility of using the Ackermann disk because of the low fat content. Ackermann Method: The Ackermann disk was used, where the density and fat values of the sample were aligned with the inner and middle circles, respectively. The total solids (TS) content was read from the outer circle. Fleischmann Method: The TS were calculated using the Equation 1:

$$TS (\%) = [(1.2 \times F) + 2.655 \times (100 \times D) - 100/D] \quad (1)$$

Where: F = fat (%); D = density (g/mL), considering only the decimal values.

The results were expressed as a percentage (AOAC, 1995; Brasil, 2022; IAL, 2005).

### 2.5 Nonfat Milk Solids

The calculation of nonfat milk solids (NFMS) was performed by subtracting the fat content of the milk from the total solids (TS) value, according to the Equation 2:

$$NFMS (\%) = TS (\%) - F (\%) \quad (2)$$

Where F represents fat content, NFMS represents nonfat milk solids and TS, total solids. The values were expressed as a percentage (Brasil, 2022; IAL, 2005).

### 2.6 Electronic cryoscopy, carbohydrate, and fixed residue analyzer

The cryoscopy, carbohydrate, and fixed residue (FR) or ashes analyses were carried out using the electronic milk analyzer (Master Classic, Akso®, São Leopoldo, Rio Grande do Sul, Brazil) according to the manufacturer's recommendations. Approximately 25 mL of homogenized sample was transferred to the measurement cuvette, which was then positioned at the base of the device, ensuring the proper fitting of the suction nozzle. The corresponding analysis mode was selected, and the procedure was initiated. The determination of the freezing point, carbohydrates, and FR (ashes) was based on the values presented by the analyzer, with the corrections indicated in the technical manual being applied. After each measurement, the system was cleaned following the manufacturer's instructions. To avoid interference with the results, precautions were taken, such as checking for the absence of air bubbles and fat clumps in the sample. The equipment was kept calibrated according to the recommended procedures.

### 2.7 Statistical analysis

The values of the trials obtained from the samples were statistically analyzed using analysis of variance (ANOVA) through

a completely randomized design or random trial and complemented with the Tukey test for mean comparison, considering a 5% significance level (Montgomery, 2020).

## 3 RESULTS

The protein content of the lactose-free UHT milk ( $3.30 \text{ g}/100 \text{ g} \pm 0.13 \text{ g}/100 \text{ g}$ ) was significantly higher ( $p = .0002$ ) compared to the whole UHT milk ( $3.05 \text{ g}/100 \text{ g} \pm 0.08 \text{ g}/100 \text{ g}$ ), skimmed milk ( $2.88 \text{ g}/100 \text{ g} \pm 0.13 \text{ g}/100 \text{ g}$ ), and semi-skimmed milk ( $3.02 \text{ g}/100 \text{ g} \pm 0.16 \text{ g}/100 \text{ g}$ ). The coefficient of variation (CV) was 4.205%, demonstrating that the data analyzed were homogeneous and stable in the protein analyses for the different UHT milks (Table 1).

The carbohydrate content of the lactose-free UHT milk ( $4.97 \text{ g}/100 \text{ g} \pm 0.20 \text{ g}/100 \text{ g}$ ) was significantly higher ( $p = .0001$ ) compared to the whole UHT milk ( $4.53 \text{ g}/100 \text{ g} \pm 0.05 \text{ g}/100 \text{ g}$ ), skimmed milk ( $4.35 \text{ g}/100 \text{ g} \pm 0.19 \text{ g}/100 \text{ g}$ ), and semi-skimmed milk ( $4.58 \text{ g}/100 \text{ g} \pm 0.24 \text{ g}/100 \text{ g}$ ). The CV was 3.972%, demonstrating that the data analyzed were homogeneous and stable in the carbohydrate analyses for the different UHT milks (Table 1).

The lipid content of whole UHT milk ( $3.23 \text{ g}/100 \text{ g} \pm 0.18 \text{ g}/100 \text{ g}$ ) was significantly higher ( $p = .0000$ ) compared to lactose-free UHT milk ( $1.00 \text{ g}/100 \text{ g} \pm 0.44 \text{ g}/100 \text{ g}$ ), skimmed milk ( $0.16 \text{ g}/100 \text{ g} \pm 0.15 \text{ g}/100 \text{ g}$ ), and semi-skimmed milk ( $1.32 \text{ g}/100 \text{ g} \pm 0.18 \text{ g}/100 \text{ g}$ ). The Tukey test at 5% significance showed that lactose-free UHT milk ( $1.00 \text{ g}/100 \text{ g} \pm 0.44 \text{ g}/100 \text{ g}$ ) was similar to semi-skimmed UHT milk ( $1.32 \text{ g}/100 \text{ g} \pm 0.18 \text{ g}/100 \text{ g}$ ) in lipid content. The CV was 21.34%, demonstrating that the data analyzed were homogeneous and stable in the lipid analyses for the different UHT milks (Table 1).

The density of lactose-free UHT milk ( $1.0355 \pm 0.0014 \text{ g/mL}$ ) and skimmed UHT milk ( $1.0354 \pm 0.0018 \text{ g/mL}$ ) were significantly higher ( $p = .0000$ ) compared to whole UHT milk ( $1.0313 \pm 0.0005 \text{ g/mL}$ ) and semi-skimmed UHT milk ( $1.0334 \pm 0.0011 \text{ g/mL}$ ). The CV was 0.129%, demonstrating that the data analyzed were extremely homogeneous and stable in the density analyses for the different UHT milks (Table 2).

The values of TS and NFMS in skimmed UHT milk were significantly higher ( $p = .0000$ ) compared to lactose-free, whole, and semi-skimmed UHT milks. The coefficients of variation (CVs) were 22.612% for TS and 30.59% for NFMS assays, respectively. The analyzed data were homogeneous and stable for

**Table 1.** Mean  $\pm$  standard deviation of macromolecules of protein, lipids, and carbohydrates in lactose-free ultra-high temperature milk, whole ultra-high temperature milk, skimmed ultra-high temperature milk, and semi-skimmed ultra-high temperature milk.

Analysis (g/100 g)	LF	W	S	SS	p-value
Protein	$3.30 \pm 0.13 \text{ b}^1$	$3.05 \pm 0.08 \text{ a}$	$2.88 \pm 0.13 \text{ a}$	$3.02 \pm 0.16 \text{ a}$	.0002
Carbohydrate	$4.97 \pm 0.20 \text{ b}^2$	$4.53 \pm 0.05 \text{ a}$	$4.35 \pm 0.19 \text{ a}$	$4.58 \pm 0.24 \text{ a}$	.0001
Lipids	$1.00 \pm 0.44 \text{ b}^3$	$3.23 \pm 0.18 \text{ c}$	$0.16 \pm 0.15 \text{ a}$	$1.32 \pm 0.18 \text{ b}$	.0000

UHT: ultra-high temperature; LF: lactose-free UHT milk; W: whole UHT milk; S: skimmed UHT milk; SS: semi-skimmed UHT milk.

Statistical analysis by ANOVA complemented with the Tukey test at 5% significance.

The lowercase letters "a", "b", and "c" in the tables indicate that there was a statistically significant difference between the results ( $p < .05$ ).

<sup>1</sup>CV = 4.205% and  $p = .0002$ .

<sup>2</sup>CV = 3.972% and  $p = .0001$ .

<sup>3</sup>CV = 21.34% and  $p = .0000$ .

**Table 2.** Mean  $\pm$  standard deviation of density, total solids, nonfat milk solids, and fixed residue in lactose-free ultra-high temperature milk, whole ultra-high temperature milk, skimmed ultra-high temperature milk, and semi-skimmed ultra-high temperature milk.

Analysis	LF	W	S	SS	p-value
Density (g/mL)	1.0355 $\pm$ 0.0014 c <sup>1</sup>	1.0313 $\pm$ 0.0005 a	1.0354 $\pm$ 0.0018 c	1.0334 $\pm$ 0.0011 b	.0000
TS (g/100 g)	13.10 $\pm$ 6.20 a <sup>2</sup>	11.88 $\pm$ 0.20 a	25.84 $\pm$ 0.98 b	10.37 $\pm$ 0.53 a	.0000
NFMS (g/100 g)	12.28 $\pm$ 6.60 a <sup>3</sup>	8.71 $\pm$ 0.13 a	23.78 $\pm$ 3.99 b	9.05 $\pm$ 0.66 a	.0000
FR (g/100 g)	0.72 $\pm$ 0.04 b <sup>4</sup>	0.62 $\pm$ 0.04 a	0.62 $\pm$ 0.04 a	0.63 $\pm$ 0.05 a	.0019

TS: total solids; NFMS: nonfat milk solids; FR: fixed residue; UHT: ultra-high temperature; LF: lactose-free UHT milk; W: whole UHT milk; S: skimmed UHT milk; SS: semi-skimmed UHT milk.

Statistical analysis by ANOVA was complemented with the Tukey test at 5% significance.

The lowercase letters "a", "b", and "c" in the tables indicate that there was a statistically significant difference between the results ( $p < .05$ ).

<sup>1</sup>CV = 0.129% and  $p = .0000$ .

<sup>2</sup>CV = 22.612% and  $p = .0000$ .

<sup>3</sup>CV = 30.59% and  $p = .0000$ .

<sup>4</sup>CV = 6.779% and  $p = .0019$ .

the TS analyses and heterogeneous and unstable for the NFMS analyses for the different UHT milks (Table 2).

The FR of the lactose-free UHT milk (0.72 g/100 g  $\pm$  0.04 g/100 g) was significantly higher ( $p = .0019$ ) compared to whole (0.62 g/100 g  $\pm$  0.04 g/100 g), skimmed (0.62 g/100 g  $\pm$  0.04 g/100 g), and semi-skimmed (0.63 g/100 g  $\pm$  0.05 g/100 g) UHT milks. The CV was 6.779%, indicating that the data analyzed were homogeneous and stable for the FR analyses of the different UHT milks (Table 2). The cryometry value of lactose-free milk ( $-0.569$  °H  $\pm$  0.021 °H) was significantly more negative ( $p = .00003$ ) compared to whole UHT milk ( $-0.537$  °H  $\pm$  0.001 °H), skimmed milk ( $-0.494$  °H  $\pm$  0.021 °H), and semi-skimmed milk ( $-0.527$  °H  $\pm$  0.026 °H). The CV was 3.718%, indicating that the data analyzed were homogeneous and stable for cryometry analyses of the different UHT milks (Table 3). Figures 1, 2, 3 and 4 show the relationship between the macromolecules of protein, carbohydrate, and lipid with cryometry in the different types of UHT milk. Figure 1 shows that the high levels of protein and carbohydrate in lactose-free UHT milk resulted in a more negative cryometry value. However, in whole UHT milk, the higher lipid content (Figure 2 and Table 1) likely contributed to a considerable negative cryometry value (Table 3), as demonstrated by the Tukey test with 5% significance. Statistical analyses (ANOVA) with 5% significance showed that high levels of macromolecules and FR significantly decreased the negative cryometry values.

## 4 DISCUSSION

Cryoscopy is defined as the freezing point of milk. It is influenced by the concentration of non-volatile solutes, meaning the number of dissolved particles regardless of their chemical nature, and is therefore considered a colligative property (Atkins & Paula, 2017; Moore, 1976). The higher the concentration of these solutes, the lower (more negative) the freezing temperature will be (Hernández et al., 2009). In the case of UHT milk, thermal processing compromises the structure of casein micelles, promoting their sedimentation and gelation—factors that reduce the product's shelf life (Cunha, 2011; Datta & Deeth, 2001).

To minimize these effects, the addition of protein stabilizers such as sodium citrate and sodium phosphate is allowed in concentrations up to 0.10% (Brasil, 1997). These compounds act by chelating free ionic calcium, thereby preventing casein

**Table 3.** Mean  $\pm$  standard deviation of cryometry (°H and °C) in lactose-free ultra-high temperature milk, whole ultra-high temperature milk, skimmed ultra-high temperature milk, and semi-skimmed ultra-high temperature milk.

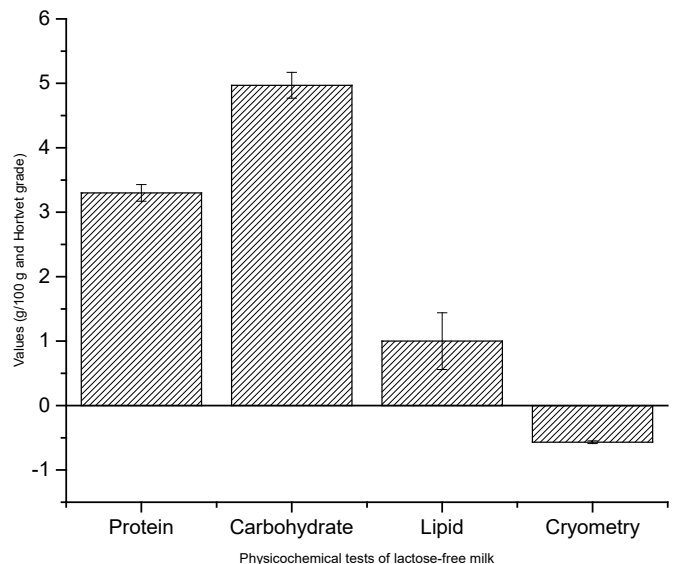
UHT	Cryometry	
	°H	°C
LF	$-0.569 \pm 0.021$ c <sup>1</sup>	$-0.549 \pm 0.021$ c <sup>1</sup>
W	$-0.537 \pm 0.001$ bc	$-0.519 \pm 0.001$ bc
S	$-0.494 \pm 0.021$ a	$-0.477 \pm 0.021$ a
SS	$-0.527 \pm 0.026$ b	$-0.509 \pm 0.026$ b
p-value	.0003	.0003

UHT: ultra-high temperature; LF: lactose-free UHT milk; W: whole UHT milk; S: skimmed UHT milk; SS: semi-skimmed UHT milk.

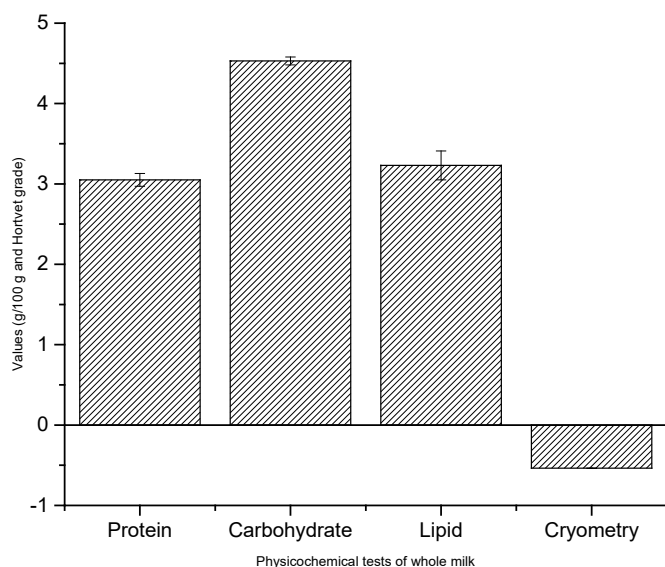
$1^\circ\text{C} = 0.96418 \times ^\circ\text{H} + 0.00085$  (International Dairy Federation, 1982).

Statistical analysis by ANOVA complemented with the Tukey test at 5% significance.

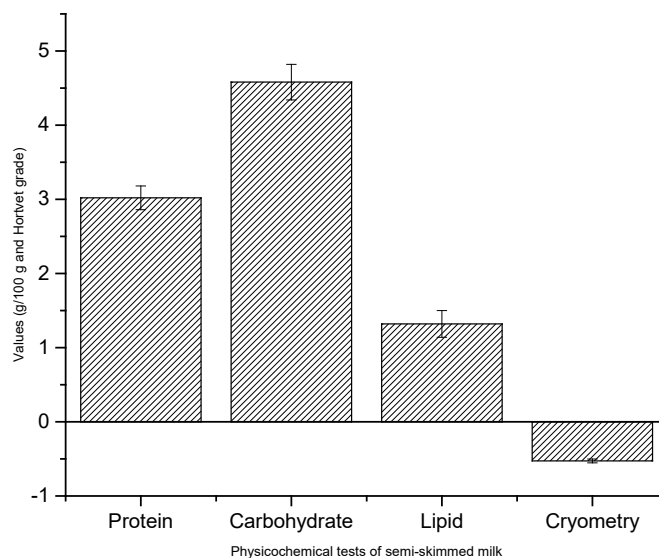
The lowercase letters "a", "b", and "c" in the tables indicate that there was a statistically significant difference between the results ( $p < .05$ ).

**Figure 1.** Mean  $\pm$  standard deviation of macromolecules (protein, carbohydrate, and lipid), in g/100 g, and cryometry, in °H, of lactose-free ultra-high temperature milk.

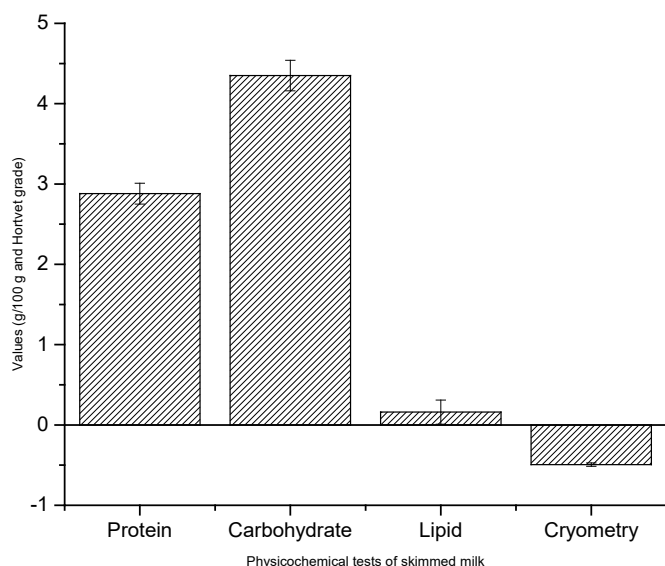
micelle aggregation (Datta & Deeth, 2001; Silva, 2003). However, the addition of these mineral stabilizers increases the number of dissolved solutes in the milk, affecting the cryoscopic



**Figure 2.** Mean  $\pm$  standard deviation of macromolecules (protein, carbohydrate, and lipid), in g/100 g, and cryometry, in  $^{\circ}\text{H}$ , of whole ultra-high temperature milk.



**Figure 4.** Mean  $\pm$  standard deviation of macromolecules (protein, carbohydrate, and lipid), in g/100 g, and cryometry, in  $^{\circ}\text{H}$ , of semi-skimmed ultra-high temperature milk.



**Figure 3.** Mean  $\pm$  standard deviation of macromolecules (protein, carbohydrate, and lipid), in g/100 g, and cryometry, in  $^{\circ}\text{H}$ , of skimmed ultra-high temperature milk.

index. For this reason, cryoscopy is not used as a parameter for detecting water adulteration in UHT milk (Beloti et al., 2015).

Although current legislation does not establish a specific standard for UHT milk, a freezing point range between  $-0.545^{\circ}\text{H}$  and  $-0.572^{\circ}\text{H}$  has been proposed (Beloti et al., 2015). Furthermore, lactose content also influences cryometry: milks with varying lactose concentrations exhibit different freezing points (Lide, 1998). In the present study, lactose-free UHT milk showed the lowest cryoscopic index ( $-0.569^{\circ}\text{H} \pm 0.021^{\circ}\text{H}$ ), as shown in Table 3. This can be attributed to the higher concentration of dissolved particles resulting from the hydrolysis of lactose into glucose and galactose, monosaccharides (Churakova

et al., 2019). This finding is consistent with previous studies reporting lower freezing points in lactose-free milk compared to those containing the disaccharide (Dantas et al., 2021; Neves & Oliveira, 2021). These same samples also showed the highest levels of dissolved minerals ( $0.72 \text{ g/100 g} \pm 0.04 \text{ g/100 g}$ ), reinforcing the influence of colligative properties on cryometry and further lowering the freezing point of the milk.

Milk proteins are important macromolecules from both nutritional and technological perspectives. Nutritionally, they provide essential amino acids, which cannot be synthesized by the human body and must be obtained through the diet. Technologically, milk proteins also play sensory and structural roles: caseins contribute to the characteristic white color of milk and are crucial in the coagulation process, which is essential for cheese production. However, these proteins are sensitive to heat treatment and may undergo denaturation, compromising both their functional properties and nutritional value (Fox et al., 2015).

The protein content in milk must be at least 2.90% (Brasil, 2017a). In the present study, lactose-free UHT milk showed the highest protein content ( $3.30 \text{ g/100 g} \pm 0.13 \text{ g/100 g}$ ) when compared to the other categories (whole, skimmed, and semi-skimmed). It was observed that, except for the skimmed milk, which presented the lowest value ( $2.88 \text{ g/100 g} \pm 0.13 \text{ g/100 g}$ ), all samples met the minimum required threshold. Nevertheless, skimmed milk did not show a statistically significant difference from whole and semi-skimmed milk samples (Table 1). A possible explanation for the slightly reduced protein content in skimmed milk is the loss of proteins associated with the fat globule membranes, which are removed during the skimming process (El-LoLy, 2011).

Lactose is the main carbohydrate found in the milk of mammals, accompanied only by trace amounts of other sugars such as glucose, fructose, glucosamine, galactosamine, and

N-acetylneuraminic acid (Fox et al., 2015). This molecule is composed of two monosaccharides, galactose and glucose, linked by a  $\beta$ -1,4 glycosidic bond (Berg et al., 2002). Its primary function is to provide energy to infants (Hodges et al., 2019). In the present study, the average values indicated that lactose-free UHT milk had the highest carbohydrate content ( $4.97 \text{ g}/100 \text{ g} \pm 0.20 \text{ g}/100 \text{ g}$ ) compared to the other types analyzed (whole, skimmed, and semi-skimmed) (Table 1).

It is noteworthy that the total carbohydrate content in milk is not usually measured; rather, the focus is typically on lactose content, which must be at least 4.30% (Brasil, 2017a). The production of lactose-free milk involves the addition of the enzyme lactase under specific technological conditions, promoting the hydrolysis of lactose into its two constituent monosaccharides. This process increases the number of dissolved molecules in the milk, thereby raising the total carbohydrate content (Churakova et al., 2019), which supports the results observed in this study.

Lipids in milk, like proteins and carbohydrates, are macromolecules that provide energy to animals consuming them. Their content varies depending on the producing species (Fox et al., 2015). Furthermore, milk lipids represent a high-value byproduct, as they are essential raw materials for the production of dairy derivatives such as cream and butter. The commercial classification of milk is directly related to its fat content: for whole milk, the minimum required is 3.00%; for semi-skimmed milk, the values should range from 0.60 to 2.90%; while skimmed milk should contain a maximum of 0.50% fat (Brasil, 2017a). The relatively high CV observed for the lipid content in lactose-free UHT milk (21.34%) can be attributed to the heterogeneity within this group, which comprised both semi-skimmed ( $n = 5$ ) and skimmed ( $n = 1$ ) products. The inherent differences in fat content between these two categories contributed to greater variability in lipid measurements, resulting in a higher CV compared to more homogeneous sample groups. Additionally, the observed similarity in lipid content between lactose-free and semi-skimmed UHT milk is likely due to the fact that the majority of lactose-free samples were derived from semi-skimmed milk bases (5/6). Furthermore, the results obtained in this study demonstrated that all milk categories evaluated complied with the legal parameters: whole milk ( $3.23 \text{ g}/100 \text{ g} \pm 0.18$ ), semi-skimmed ( $1.32 \text{ g}/100 \text{ g} \pm 0.18$ ), lactose-free ( $1.00 \text{ g}/100 \text{ g} \pm 0.44$ ), and skimmed ( $0.16 \text{ g}/100 \text{ g} \pm 0.15$ ) (Table 1). Therefore, no failures in industrial standardization or evidence of intentional fat removal fraud were found.

Density is an important parameter in assessing the quality of milk, as it can indicate changes in the TS content and potential fraud, such as water addition or fat removal. The density of whole milk at  $15^\circ\text{C}$  should be between 1.028 and  $1.034 \text{ g}/\text{mL}$  (Brasil, 2017a), while the density of semi-skimmed or skim milk should range from 1.028 to  $1.036 \text{ g}/\text{mL}$  (Brasil, 2018). Values below 1.028 suggest water addition, while those above 1.034 indicate fat removal (Polegato & Rudge, 2003). The results obtained in this study showed that all evaluated categories were in accordance with the standards: whole milk ( $1.0313 \pm 0.0005 \text{ g}/\text{mL}$ ), skimmed milk ( $1.0354 \pm 0.0018 \text{ g}/\text{mL}$ ), semi-skimmed milk ( $1.0334 \pm 0.0011 \text{ g}/\text{mL}$ ), and lactose-free milk ( $1.0355 \pm 0.0014 \text{ g}/\text{mL}$ ) (Table 2).

The TS represent the non-aqueous portion of milk, all components such as lipids, carbohydrates, proteins, and minerals, excluding water. They must account for at least 11.40% of milk samples (Brazil, 2017a). The categories of UHT whole milk, skim milk, and lactose-free milk assessed were within the established parameters. Semi-skimmed milk was outside the parameter ( $10.37 \pm 0.53\%$ ) (Table 2). It is possible that, during the processing of UHT semi-skimmed milk, a failure occurred in the industrial standardization.

The NFMS, in turn, are the solid components of milk, excluding fat. They must correspond to at least 8.40% of whole milk (Brasil, 2018). All the UHT milk categories analyzed were within the established standard (whole, skimmed, semi-skimmed, and lactose-free). Skimmed milk had the highest content ( $23.78 \pm 3.99\%$ ), likely due to the skimming process, which causes minerals to represent a higher percentage of the total milk composition. The other categories did not differ statistically: lactose-free ( $12.28 \pm 6.60\%$ ), semi-skimmed ( $9.05 \pm 0.66\%$ ), and whole ( $8.71 \pm 0.13\%$ ), as shown in Table 2.

Regarding minerals, there is no legislation that determines minimum and maximum values in milk. Their concentration can vary depending on factors such as the genetics or breed of the cow, the management or environment to which the animals are subjected, as well as their health and nutritional conditions (National Research Council, 1988). The present study found higher levels in the lactose-free UHT milk category ( $0.72 \text{ g}/100 \text{ g} \pm 0.04 \text{ g}/100 \text{ g}$ ), and the other categories showed no significant statistical differences: whole and skimmed ( $0.62 \text{ g}/100 \text{ g} \pm 0.04 \text{ g}/100 \text{ g}$  each) and semi-skimmed ( $0.63 \text{ g}/100 \text{ g} \pm 0.05 \text{ g}/100 \text{ g}$ ). One possibility is that this difference may have occurred by chance and not due to technological procedures; however, it is suggested that further studies be conducted on the levels of dissolved minerals in lactose-free milk.

## 5 CONCLUSIONS

Based on the results obtained, it can be inferred that the increase in the number of dissolved solutes in lactose-free UHT milk, resulting from the hydrolysis of lactose into glucose and galactose, along with the higher mineral content, are determining factors for the reduction in the freezing point (cryoscopic point) of the milk when compared to the UHT whole, semi-skimmed, and skimmed milk categories.

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