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Physicochemical evaluation of sodium hydroxide solutions for lactic acid determinations in milk

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Abstract

The determination of lactic acid in milk requires an aqueous solution of sodium hydroxide with a specific concentration. The most commonly used solutions are Dornic and 0.1 M NaOH. This study aimed to evaluate the physicochemical properties of two solutions with nominally different concentrations of sodium hydroxide to determine the concentration of lactic acid in bovine milk. The samples used were whole ultrahigh temperature (UHT) milk ($n = 21$) and semi-skimmed UHT milk ($n = 12$). Lactic acid determinations were carried out with Dornic and 0.1 M NaOH solutions. The results showed significant differences ($p < 0.05$) in the use of Dornic versus 0.1 M NaOH solutions. Accurate determination of lactic acid is important for assessing milk quality and detecting possible alterations or adulterations. An inappropriate choice of solution can lead to erroneous results, affecting the interpretation of milk quality.

Keywords: Dornic; NaOH; physical chemistry; UHT.

Practical Application: Two titration methods with NaOH were compared to determine lactic acid in milk. Both methods showed high precision and similarity in results, with differences in coefficients and detection and quantification limits.

1 INTRODUCTION

A solution is defined as a homogeneous mixture comprising a solute and a solvent. The solute is the substance that dissolves within the solvent, and the solubility represents the maximum amount of solute that can dissolve in the solvent. In solutions where the components are in the same phase, the substance present in lower concentrations is referred to as the solute, while the substance in the highest concentrations is the solvent (Atkins; Paula, 2014, 2018; Moore, 1976; Skoog et al., 2024).

In an aqueous sodium hydroxide solution, regardless of its concentration, the solute is the base (NaOH) and the solvent is distilled water. In the context of physical–chemical analysis applied to animal-origin foods, determining acidity requires an aqueous sodium hydroxide solution with a specific concentration (AOAC, 1995; Brasil, 1981, 2006, 2022).

Determining milk acidity is one of the most important physicochemical parameters in the dairy industry routine as it provides intrinsic information for milk quality control (Brasil, 2018). Acidification, primarily due to the increase in lactic acid concentration, reflects the physical and chemical changes occurring in the milk (Fabro et al., 2006; Slyke & Bosworth, 1914).

Milk samples with high total bacterial count undergo lactose fermentation by bacteria such as those of the genera *Lactobacillus*, *Streptococcus*, and *Lactococcus*. This fermentation leads to elevated lactic acid levels, which can adversely affect casein stability and the sensory characteristics of the milk (Aydogdu & Mahony, 2023; Huang et al., 2022). Milk samples with an acidity level exceeding 0.18 g of lactic acid per 100 mL are already considered outside the quality parameters of milk. Therefore, a physicochemical change in a product can impact the dairy production chain (Aydogdu & Mahony, 2023; Beggs et al., 2018; Brasil, 2006; Huang et al., 2022; Karlsson et al., 2019; Wang et al., 2015).

Fraudulent practices, such as the addition of sodium bicarbonate, an acidity neutralizer, aim to mask the true concentration of lactic acid in milk (Gondim et al., 2021). However, lactose degradation persists, leading to alterations in other routine quality control tests, including density, fat content, total solids, nonfat solids, and cryoscopy index (Aydogdu & Mahony, 2023; Gondim et al., 2021; Karlsson et al., 2019).

The internationally accepted method for lactic acid determination in milk involves an acid–base titration. This titration quantifies the concentration of lactic acid (g/100 mL) in the analyzed milk, using two different sodium hydroxide solutions. One method uses

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a titrant known as Dornic solution (NaOH at N/9), while the other uses a 0.1 M sodium hydroxide solution as the titrant. Titrants are solutions of known concentration, and milk is the sample with an unknown lactic acid concentration (Aydogdu & Mahony, 2023; Brasil, 2006, 2022; Karlsson et al., 2019; Santoso, 2020).

Therefore, both methodologies utilize an alkaline solution, specifically sodium hydroxide, but differ in their concentrations. One method employs a normality-based solution (Dornic solution), while the other uses a molarity-based solution (0.1 M NaOH). These solutions serve as titrants for a milk volume, which is titrated to the endpoint of acidity neutralization in the presence of an indicator, such as phenolphthalein (Brasil, 1981, 2006, 2022).

Despite the methodological similarities, there are numerous analytical divergences in laboratory routines and interpretations among veterinarians responsible for dairy quality control. Brasil (2006) described two methodologies that use sodium hydroxide solutions with different nominal concentrations to quantify lactic acid in milk through acidity analysis. As previously mentioned, the study of acidity indicates the conservation status of the milk, and a high value typically suggests lactose acidification caused by the proliferation of microorganisms, leading to increased acidity as milk ages. (Brasil, 2006, 2022).

One titration methodology to quantify lactic acid in milk uses 1/9 N sodium hydroxide solution as the titrant, while the other uses 0.1 M sodium hydroxide solution (Brasil, 2006). Unfortunately, these analytical methodologies still create uncertainties for dairy professionals regarding the acidity content in milk. Brasil (2006) established criteria for milk's physical–chemical analysis, suggesting no methodological differences. Furthermore, several dairies in the inland of Sao Paulo state (Brazil) have unofficially reported that these methodologies may be leading to interpretative divergences among analysts.

Based on this information, the present work aimed to conduct a physical–chemical evaluation of two sodium hydroxide solutions with nominally different concentrations to determine the concentration of lactic acid in bovine milk.

2 MATERIALS AND METHODS

2.1 Samples

Two types of cow's milk processed at ultrahigh temperature (UHT) were used, 21 samples from Whole milk and 12 from semi-skimmed milk, totaling 33 samples. The samples were acquired from commercial establishments in Botucatu, São Paulo, Brazil. 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 Paulsita "Júlio de Mesquita Filho" (Unesp) Campus of Botucatu, São Paulo, Brazil. All assays were performed in triplicate.

2.2 Materials and reagents

The materials used included 50-mL and 1,000-mL polypropylene beakers, a 250-mL polypropylene beaker, and volumetric pipettes of 1, 10, and 11 mL. A 10-mL burette (Pyrex®) was also used, along with a Gerber® butyrometer, a Dornic acidimeter (Nalgon®), and a Gerber® centrifuge. Additionally, a thermo-lactodensimeter, an Ackermann® disc, and various reagents were employed: lactic acid ($C_3H_6O_3$, Sigma-Aldrich®), phenolphthalein ($C_{20}H_{14}O_4$, Merck®), sodium hydroxide (NaOH, Merck®), ethyl alcohol (C_2H_6O, Sigma-Aldrich®), sulfuric acid (H_2SO_4, density = 1.820–1.825 g/mL, Merck®), and isoamyl alcohol ($C_5H_{12}O$, Supelco®).

2.3 Sodium hydroxide solution preparation

2.3.1 Dornic solution

Notably, 4.7 g of NaOH was dissolved in a 1,000-mL volumetric flask, and then it was filled to the mark with distilled water. For the titration, 4.5382 g of potassium biphthalate was accurately weighed, which was dried in an oven at 105°C for 1 h, and cooled to room temperature in a desiccator. It was transferred to a 200-mL volumetric flask and filled to the mark with distilled water. The solution was then covered and homogenized. Using a burette, 20 mL of this standard solution was transferred to a 250-mL Erlenmeyer flask. A 20 mL aliquot of this standard solution was titrated with the Dornic solution using phenolphthalein as an indicator until a persistent pink color was observed. This confirmed the neutralization of the standard solution with 20 mL of Dornic solution (Brasil, 1981; IAL, 2008).

2.3.2 0.1M sodium hydroxide solution

Notably, 4.5 g of NaOH was dissolved in a 1,000-mL volumetric flask, using carbon dioxide-free water. The solution was completed to the mark with distilled water, covered, homogenized, and stored in a polyethylene bottle for calibration; 0.5 g of potassium biphthalate, previously dried and cooled, was dissolved in 75 mL of distilled water and titrated with the 0.1 M NaOH solution using phenolphthalein as an indicator until a persistent pink color appeared. Two drops of 1% phenolphthalein solution were added and titrated with NaOH until a persistent pink color appeared. The formula for correction factor calculation (F) (Equation 1) is

$$F = P/(0.2042 \times V \times M), \quad (1)$$

where

P: g of potassium biphthalate used in the titration,

V: mL of NaOH solution spent, and

M: molarity of the solution (Brasil, 2022; IAL, 2008).

2.3.3 Lactic acid solutions

Various concentrations of lactic acid solutions were prepared by weighing 0.005 g, 0.01 g, 0.025 g, 0.05 g, 0.1 g, 0.15 g, and 0.20 g of lactic acid ($C_3H_6O_3$) and dissolving each in a 100-mL volumetric flask, filling it to the mark with distilled water. These solutions were stored in clean, dry amber glass bottles.

2.3.4 1% alcoholic phenolphthalein solution

The pH range of phenolphthalein is from 8.2 to 9.8, changing from colorless to red-purple. Beyond pH 9.8, the color becomes intense red due to modifications in the indicator molecule. A quantity of 1 g of phenolphthalein was weighed, and 95% ethyl alcohol to make up 100 mL was added. It was filtered if necessary. The solution was stored in a clean, dry amber glass bottle with a glass stopper (Brasil, 2022; IAL, 2008).

2.4 Lactic acid determination

Milk samples were homogenized, and 10-mL aliquots were transferred to a 50-mL beaker with 5 drops of 1% alcoholic phenolphthalein solution added. For (a) Dornic acidimeter titrations, the sample was titrated with N/9 sodium hydroxide solution (Dornic solution) until a faint pink endpoint was achieved. Each 0.1 mL of N/9 sodium hydroxide solution corresponds to 1 Dornic degree (°D) or 0.01 g of lactic acid per 100 mL. For (b) titrations with 0.1 M NaOH, the sample was titrated with a 10-mL burette until a pink color appeared. The volume used from the burette was applied in the formula (Equation 2):

$$\text{Lactic acid (g/100 mL)} = (V \times F \times 0.9) / A,$$

where

V: volume in mL of the 0.1 M NaOH solution used in the titration,

F: correction factor of the 0.1 M NaOH solution,

A: sample volume in mL, and

0.9: conversion factor to lactic acid (AOAC, 1995; Brasil, 1981, 2022; IAL, 2008).

2.4.1 Validation

Validation ensured the suitability of the two sodium hydroxide solutions (0.1 M and N/9) for lactic acid determination in UHT milk. The parameters used for validation included linearity (through the standard curve), limit of detection (LOD), limit of quantification (LOQ), and repeatability (Brasil, 2011, 2014; SBM, 2022).

In the study of linearity, a standard curve was determined using lactic acid solutions at the following concentrations: 0.005 g/100 mL, 0.010 g/100 mL, 0.025 g/100 mL, 0.050 g/100 mL, 0.100 g/100 mL, 0.150 g/100 mL, and 0.200 g/100 mL. The parameters for the LOD and LOQ for lactic acid determination using 0.1 M NaOH, and Dornic solutions were determined through seven measurements of UHT whole and semi-skimmed milk. Repeatability was also assessed through seven measurements of UHT milk (whole and semi-skimmed) under two different conditions (Brasil, 2011, 2014; SBM, 2022).

2.4.2 Fractions of solution concentrations

To calculate fractions, 10 mL of lactic acid solutions with different concentrations (0.005 g/100 mL, 0.010 g/100 mL, 0.025 g/100 mL, 0.050 g/100 mL, 0.100 g/100 mL, 0.150 g/100 mL, and 0.200 g/100 mL) were transferred into 100-mL beakers. Five drops of 1% alcoholic phenolphthalein solution were added. (a) In the Dornic acidimeter, it was titrated with the N/9 sodium hydroxide solution (Dornic solution) to the endpoint (lightly pink). The volume (mL) of Dornic solution used in the titration was recorded. (b) It was titrated with the 0.1 M sodium hydroxide solution using a 10-mL burette until a pink color appeared. The volume (mL) of 0.1 M NaOH used was recorded. The fractions (r1 and r2) of the volumes used for each solution (Dornic and 0.1 M NaOH) were calculated. In fraction r1, the numerator was the volume (mL) of the Dornic solution used in the titration, and the denominator was the volume of 0.1 M NaOH used in the corresponding concentration of lactic acid solution. In fraction r2, the numerator was the volume (mL) of 0.1 M NaOH used in the titration, and the denominator was the volume of Dornic solution used in the titration of each corresponding concentration of lactic acid solution. The mean values of r1 and r2 were calculated and approximated to natural numbers N = {0, 1, 2, 3, 4, 5, ...}, which are positive integers (nonnegative) grouped in a set called N, consisting of an unlimited number of elements (Bhupendra, 2022). The average values of r1 and r2 were evaluated as integer and positive numbers, that is, natural numbers, to compare Dornic and 0.1 M NaOH solutions.

2.5 Density determination

Approximately 220 mL of homogenized milk was transferred to a 250-mL polyethylene graduated cylinder. A clean and dry lactodensimeter was then introduced carefully into the cylinder with the milk sample, ensuring it did not touch the sides of the cylinder. After waiting a few seconds, the reading was taken at the liquid level (density). 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. An additional adjustment of ± 0.0002 was made for every five-degree difference from the calculated temperature (AOAC, 1995; APHA, 1992; Brasil, 1981, 2022; IAL, 2008).

2.6 Fat determination

Fat content was determined using the Gerber method. A volume of 10 mL of sulfuric acid was added to a Gerber butyrometer, followed by 11 mL of homogenized milk and 1 mL of isoamyl alcohol. The butyrometer was sealed, mixed, centrifuged, and then heated in a water bath at 65°C for 5 minutes. The fat content was read using the butyrometer scale (AOAC, 1995; Brasil, 1981, 2022; IAL, 2008).

2.7 Total milk solids

The Ackermann® disc was used, which features an inner circle (density), a middle circle (fat), and an outer circle (total solids). The values for fat and density of the milk were applied. The values from the inner circle (density) and the middle circle (fat) were matched. The pointer on the disc indicated the outer circle, corresponding to the total solids (g/100 mL) or total dry extract (g/100 mL) value (AOAC, 1995; Brasil, 1981, 2022; IAL, 2008).

2.8 Nonfat milk solids

The values obtained from the fat determination (g/100 mL) and total solids (g/100 mL) were used. These values were applied to the following formula for nonfat milk solids (NFMS) (Equation 3) (Brasil, 1981, 2013, 2022; IAL, 2008):

$$\text{NFMS (g/100 mL)} = \text{Total Solids (g/100 mL)} - \text{Fat (g/100 mL)}. \quad (3)$$

2.9 Cryometry determination

The Hortvet cryoscope used was the ITR model MK 540 digital cryoscope for determining the freezing point index of milk. The device was calibrated with two standard cryoscopy solutions (0.000 °H and –0.621 °H). After calibration, 2.5 mL of properly homogenized milk was transferred to a cryometry tube. The tube with the sample was placed in the device, and the reading in °H was taken. Milk was considered not to have been adulterated with water if the cryometry values were between –0.530 °H and –0.550 °H. For values greater than –0.530 °H, the following formula was applied to indicate the addition of water to the milk (Equation 4) (AOAC, 1995; APHA, 1992; Brasil, 1981, 2022; IAL, 2008):

$$\text{(\%): Added water (\%)} = [(0.550 - \text{Reading}) \times 100]/0.550. \quad (4)$$

2.10 Statistical analysis

The statistical method was based on an entirely randomized experiment or randomized essay. Analysis of variance (ANOVA) supplemented with the Tukey test for comparison of means was performed. Statistical analysis considered the significance level of 5% (Montgomery, 2020).

3 RESULTS

Tables 1–4 provide a comprehensive validation of the methodologies used to determine lactic acid concentrations (g/100 mL) with Dornic and 0.1 M NaOH solutions through the parameters of linearity, LOD, LOQ, and repeatability. In the linearity study for the determination of lactic acid (g/100 mL) with Dornic solution, the correlation (r), linear (a), and angular (b) coefficients were 0.9995, 0.0356, and 10.8057, respectively (Table 1). Regarding the determination of lactic acid (g/100 mL) with 0.1 M NaOH solution, the correlation (r), linear (a), and angular (b) coefficients were 0.9998, 0.0544, and 12.0101, respectively (Table 2). The LOD and LOQ for the determination of lactic acid (g/100 mL) with Dornic solution were 0.006 g/100 mL and 0.015 g/100 mL, respectively (Table 3). In the determination of lactic acid (g/100 mL) with 0.1 M NaOH solution, the LOD and LOQ were 0.008 g/100 mL and 0.02 g/100 mL, respectively (Table 3). In the repeatability study (Table 4), two coefficients of variation (CVs) were obtained using Dornic (1.91% and 2.56%) and NaOH solutions (1.78% and 2.33%).

The averages of the ratios of the volumes (mL) of the Dornic (r1) and 0.1 M NaOH (r2) solutions spent in titrations of different concentrations of the lactic acid solution was 0.005 g/100 mL, 0.01 g/100 mL, 0.025 g/100 mL, 853901 (± 1), and r2 = 0.980857 (± 1), respectively. The average values of r1 and r2 were approximated in natural numbers greater than zero $N = \{1\}$ for each solution evaluated (Table 5).

The ANOVA results (Table 6) revealed a highly significant difference ($p < 0.01$) in lactic acid content (g/100 mL) between whole and semi-skimmed UHT milk when analyzed with Dornic and 0.1 M NaOH solutions. The CV was 4.10%, indicating that the evaluated data were homogeneous and stable.

The lactic acid content in whole UHT milk was significantly higher ($p < 0.05$) using the 0.1 M NaOH solution compared to the Dornic solution. This was also shown with semi-skimmed UHT milk, where the lactic acid content was significantly higher ($p < 0.05$) with the use of 0.1 M NaOH solution (Table 7).

A comparison of the evaluated UHT milk demonstrated that semi-skimmed UHT milk presented significantly higher

Table 1. Linear regression analysis of the standard curve of the standard lactic acid solution (g/100 mL) titrated with the Dornic solution (mL).

Lactic acid (g/100 mL)	Titration with Dornic solution (mL)
	Averages ± standard deviation
0.005	0.067 ± 0.029
0.010	0.150 ± 0.000
0.025	0.300 ± 0.000
0.050	0.600 ± 0.000
0.100	1.100 ± 0.000
0.150	1.700 ± 0.000
0.200	2.167 ± 0.029
Linear coefficient (a)	0.0356
Angular coefficient (b)	10.8057
Correlation coefficient (r)	0.9995
Line equation	$Y = 0.0356 + 10.8057X$
Standard curve	Lactic acid (g/100 mL) = $(V_{Dornic} - 0.0356)/10.8057$ Where: V_{Dornic} = mL Dornic solution

Table 2. Linear regression analysis of the standard curve of the standard lactic acid solution (g/100 mL) titrated with 0.1 M sodium hydroxide solution (mL).

Lactic acid (g/100 mL)	Titration with NaOH solution 0.1 M (mL)
	Average ± standard deviation
0.005	0.100 ± 0.000
0.010	0.167 ± 0.029
0.025	0.350 ± 0.000
0.050	0.683 ± 0.029
0.100	1.250 ± 0.000
0.150	1.883 ± 0.029
0.200	2.433 ± 0.029
Linear coefficient (a)	0.0544
Angular coefficient (b)	12.0101
Correlation coefficient (r)	0.9998
Line equation	Y = 0.0544 + 12.0101X
Standard curve	Lactic acid (g/100 mL) = $(V_{NaOH} - 0.0544)/12.0101$ where V_{NaOH} = mL NaOH a 0.1 M solution

Table 3. Limits of detection (LOD) and quantification (LOQ) for the determination of lactic acid (g/100 mL) with Dornic and 0.1 M NaOH solutions.

Methods	Aliquot	Lactic acid (g/100 mL)
Dornic	1	0.16
	2	0.155
	3	0.16
	4	0.155
	5	0.155
	6	0.155
	7	0.16
	Average	0.157
	Standard deviation	0.003
	t[1]	1.943
	LOD	0.006
	LOQ	0.015[(2)]
NaOH 0.1 M	1	0.17
	2	0.175
	3	0.175
	4	0.175
	5	0.175
	6	0.165
	7	0.17
	Average	0.172
	Standard deviation	0.004
	t[1]	1.943
	LOD	0.008
	LOQ	0.02[2]

[1]t: unilateral for 95% confidence in the LOD (LOD = ts); [2]LOQ: 5 s.

Table 4. Repeatability for the determination of lactic acid (g/100 mL) with Dornic and 0.1 M NaOH solutions.

Methods	Aliquot	Analysis 1	Analysis 2
Dornic	1	0.16	0.155
	2	0.155	0.16
	3	0.16	0.15
	4	0.155	0.155
	5	0.155	0.16
	6	0.155	0.155
	7	0.16	0.16
	Average	0.157	0.156
	Standard deviation	0.003	0.004
	CV[1]	1.91%	2.56%
NaOH 0.1 M	1	0.175	0.17
	2	0.17	0.175
	3	0.165	0.175
	4	0.17	0.175
	5	0.17	0.175
	6	0.165	0.165
	7	0.17	0.17
	Average	0.169	0.172
	Standard deviation	0.003	0.004
	CV[1]	1.78%	2.33%

[1]CV (%) = [Standard deviation/Average] x 100; r1: Numerator (mL Dornic)/Denominator (mL NaOH 0.1 M); r2: Numerator (mL NaOH 0.1 M)/Denominator (mL Dornic).

Table 5. Proportionality of volume fractions (mL) of Dornic and 0.1 M NaOH solutions spent in titrating the lactic acid solution (g/100 mL).

Lactic acid (g/100 mL)	Dornic (mL)	r_1	NaOH 0.1 M (mL)	r_2
0.005	0.067 ± 0.029	0.670000	0.100 ± 0.000	1.492537
0.010	0.150 ± 0.000	0.898204	0.167 ± 0.029	1.113333
0.025	0.300 ± 0.000	0.857143	0.350 ± 0.000	1.166667
0.050	0.600 ± 0.000	0.878477	0.683 ± 0.029	1.138333
0.100	1.100 ± 0.000	0.880000	1.250 ± 0.000	1.136364
0.150	1.700 ± 0.000	0.902815	1.883 ± 0.029	1.107647
0.200	2.167 ± 0.029	0.890670	2.433 ± 0.029	1.12275
Average of r_1	0.853901 ≅ 1		Respond: {$N > 0$ / $r_1 = 1$}	
Average of r_2	0.980857 ≅ 1		Respond: {$N > 0$ / $r_2 = 1$}	

Table 6. Analysis of variance (ANOVA) with 5% significance in the determination of lactic acid (g/100 mL) in whole and semi-skimmed UHT milk with Dornic and 0.1 M NaOH solutions.

Causes of variations	Degrees of freedom	Sum of squares	Average of the squares	F
Treatments	3	0.00477	0.00159	34.78
Combings	62	0.00283	0.000046	
$p < 0.01$	CV = 4.10%	HSD = 0.005		

CV = coefficient of variation; HSD = honestly significant difference.

Table 7. Average ± Standard deviation of lactic acid determination (g/100 mL) in whole and semi-skimmed UHT milk with Dornic and 0.1 M NaOH solutions. Statistical analysis (ANOVA) complemented with the Tukey test at 5% meaningfulness.

UHT milk	n	Solution	Lactic acid (g/100 mL)
Whole	21	Dornic	0.16 ± 0.00 a
		NaOH 0.1 M solution	0.17 ± 0.01 b
Semi-skimmed	12	Dornic	0.16 ± 0.01 a
		NaOH 0.1 M solution	0.17 ± 0.00 b

CV: 4.10%; HSD: 0.005; $p < 0.01$; CV: coefficient of variation; HSD: honestly significant difference. The lowercase letters indicate that there is a statistically significant difference between the results obtained ($p < 0.05$).

values ($p < 0.05$) of density (1.0336 g/mL ± 0.0005 g/mL) and NFMS (8.91 g/100 mL ± 0.11 g/100 mL) compared to whole UHT milk (1.0313 g/mL ± 0.0008 g/mL and 8.71 g/100 mL ± 0.20 g/100 mL). Whole UHT milk presented significantly higher values ($p < 0.05$) of fat (3.15 g/100 mL ± 0.11 g/100 mL) and total solids (TS) (11.81 g/100 mL ± 0.28 g/100 mL) compared with semi-skimmed milk (1.20 g/100 mL ± 0.24 g/100 mL and 10.11 g/100 mL ± 0.24 g/100 mL). The colligative property of cryometry did not show significant differences ($p > 0.05$) between whole and semi-skimmed UHT milk (Table 8).

4 DISCUSSION

Pearson's correlation coefficient (r) quantifies the degree of linear relationship between two quantitative variables and is one of the criteria for assessing linearity. This coefficient ranges from –1 to 1, with 0 indicating no linear relationship and values of 1 and –1 representing perfect positive and negative linear relationships, respectively. The closer r is to 1 or –1, the stronger

Table 8. Average ± Standard deviation of density determination at 15 oC (g/mL), fat (g/100 mL), TMS (g/100 mL), NFS (g/100 mL), and cryometry (oH) in whole UHT and semi-skimmed milk. Statistical analysis (ANOVA) complemented with the Tukey test at 5% significance.

Analysis	CV (%)	HSD	UHT milk	Average ± Standard deviation
Density[1] (g/mL)	0.06	0.0001	Whole	1.0313 ± 0.0008 a
			Semi-skimmed	1.0336 ± 0.0005 b
Fat[1] (g/100 mL)	7.48	0.13	Whole	3.15 ± 0.11 b
			Semi-skimmed	1.20 ± 0.24 a
TMS[1] (g/100 mL)	2.39	0.20	Whole	11.81 ± 0.28 b
			Semi-skimmed	10.11 ± 0.24 a
NFMS[1] (g/100 mL)	1.90	0.13	Whole	8.71 ± 0.20 a
			Semi-skimmed	8.91 ± 0.11 b
Cryoscopy[2] (°H)	4.19	0.018	Whole	−0.544 ± 0.006 a
			Semi-skimmed	−0.557 ± 0.035 a

[1]$p < 0.05$; [2]$p > 0.05$; CV: coefficient of variation; HSD: honestly significant difference.
The lowercase letters indicate that there is a statistically significant difference between the results obtained ($p < 0.05$).

the linear association between the two variables (SBM, 2022). The correlation coefficients were 0.9995 and 0.9998 for the Dornic and 0.1 M NaOH solutions, respectively, in the determination of lactic acid. These results indicate a strong linear relationship between lactic acid concentration and analytical response for both methods.

The LOD of an individual analytical procedure is the smallest amount of analyte in a sample that can be detected but not necessarily quantified under the stated conditions of the test. The LOD values may vary depending on the sample type. Therefore, it is essential to ensure that all steps of the analytical method are included in the determination of this LOD. The lowest acceptable concentration is considered the lowest concentration for which a degree of uncertainty can be considered satisfactory. Fundamentally, independent assessments are carried out on samples with concentrations equal to the determined LOD (Perez, 2010; SBM, 2022). The LODs of the experiments were 0.006 g of lactic acid/100 mL using the Dornic solution and 0.008 g of lactic acid/100 mL using the 0.1 M NaOH solution.

The LOQ of an individual analytical procedure is the smallest amount of the analyte in the sample that can be quantitatively determined with acceptable precision and accuracy under the established experimental conditions. LOQ is important for quantitative methods. The International Union of Distilled and Applied Chemistry (IUPAC) proposes a value of 10 as the standard value of the equation LOQ = 10 s. However, values of 5 or 6 can also be adopted depending on the required analytical rigor (Perez, 2010; SBM, 2022). The value of 5 was used to calculate the LOQs. The LOQs of the experiments were 0.015 g of lactic acid/100 mL using the Dornic solution and 0.02 g of lactic acid/100 g using the 0.1 M NaOH solution.

Repeatability, defined as the consistency of measurements under the same conditions, is crucial for reliable results. It reflects the maximum acceptable difference between two independent results from the same test conducted under identical conditions (SBM, 2022). Our results had satisfactory repeatability for both Dornic and 0.1 M NaOH solutions, with CVs of 1.91% and 2.56% for Dornic, and 1.78% and 2.33% for NaOH, all well below the 10% threshold.

Bhupendra (2022) described fractions as representations of division where the numerator indicates the number of parts and the denominator denotes the total number of parts. In our study, fractions of volumes used for Dornic and 0.1 M NaOH solutions were compared based on proportionality. These fractions were considered equivalent when rounded to natural numbers, reflecting that there are no significant differences between the two solutions for lactic acid determination in UHT milk. However, the ANOVA demonstrated that there are significant differences ($p < 0.01$) in the use of Dornic and 0.1 M NaOH solutions in determining lactic acid in whole and semi-skimmed UHT milk. In the statistical analysis (Montgomery, 2020), the volume (mL) of 0.1 M NaOH solution used in determining lactic acid (g/100 mL) was significantly higher ($p < 0.01$) than the volume (mL) of Dornic solution used for UHT milk (whole and semi-skimmed). In the present study, the numerical difference was 0.01 g of lactic acid/100 mL higher with the use of 0.1 M NaOH solution compared to the use of Dornic solution in UHT milk samples. Therefore, the use of 0.1 M NaOH solution increases the acidity of whole and semi-skimmed UHT milk by 1 °D. Contrary to our results obtained in the present experiment, Brasil (2006) recommended the use of either Dornic solution or 0.1 M or 0.1 N NaOH solution for the determination of lactic acid in fluid milk (Slyke & Bosworth, 1914). Thus, Brasil (2006) made it explicit that the choice of methodology should be based on the particular interests of those performing such physicochemical analysis as there is no difference in the measurement of lactic acid in milk between methods that use Dornic solution (N/9) and 0.1 M NaOH solution.

The values for density (g/mL), fat content (g/100 mL), total solids (TMS, g/100 mL), nonfat solids (NFMS, g/100 mL), and cryoscopy (°H) obtained in our experiment are in accordance with the parameters established by Brasil (1996) for whole and semi-skimmed UHT milk.

5 CONCLUSION

Dornic and 0.1 M NaOH solutions resulted in different lactic acid values in UHT milk (both whole and semi-skimmed), and the use of the 0.1 M NaOH solution increased the Dornic degree by one in UHT milk samples (whole and semi-skimmed).

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