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Antibiotic residues, macromolecules and starch adulterant additive in ultra-high temperature milk

José Fernando NARDY[1], Rafael Alves SANTOMAURO[1], Gabriel Henrique Gomes MARIANO[1], Fábio Sossai POSSEBON[1], Juliano Gonçalves PEREIRA[1], Otávio Augusto MARTINS[1]

Abstract

The physicochemical characteristics of ultra-high temperature milk can be altered by fraudulent actions during production, processing, storage, and transportation. This study aimed to evaluate the presence of antibiotic residues, macromolecular composition (protein, lactose, and lipids), presence of adulterating starch additive, and other physicochemical components of five different brands of whole ultra-high temperature milk. The assays performed included screening for antibiotic residues (β-lactams, sulfonamides, tetracyclines, and quinolones), determination of macromolecules (lactose, protein, and lipids), detection of adulterating starch additive, physicochemical tests (cryoscopy, lactic acid, density, total solids, non-fat solids, and thermal stability), and determination of mineral salts using gravimetric and transmittance infrared methods. The results showed a relative frequency of 1.0 for the absence of antibiotic residues and adulterating starch additive in all ultra-high temperature milk samples. The average values for the macromolecules, acidity (lactic acid), density, total solids, and non-fat solids showed no significant differences ($p > .05$) among the analyzed milk brands. Brand M3 (-0.546 °H ± 0.007 °H) exhibited a significantly more negative mean cryoscopy value compared to other brands ($p < .05$). The gravimetric method yielded significantly higher mean RMF values ($p < .05$) than the transmittance infrared method. The primary conclusion is that no antibiotic residues or adulterating starch additives were detected in the whole ultra-high temperature milk samples.

Keywords: fraud; transmittance infrared; contaminants; food safety.

Practical Application: Assessment of UHT milk for contamination by antibiotics and adulterants.

1 INTRODUCTION

The quality of consumed milk concerns not only health authorities, but also dairy industry stakeholders. Throughout the production chain, fraudulent practices constitute a major challenge, causing economic losses, consumer health risks, and industrial issues such as yield reduction (Walstra et al., 2005). Furthermore, milk quality exhibits direct correlation with animal health status, as the product can become a disease transmission vector (Callefe & Langoni, 2015).

Milk composition is influenced by microbiological, physicochemical, and processing factors. Due to its high susceptibility to alterations and contamination, components are frequently added to mask quality defects (Corrêa et al., 2015). The main concerns regarding milk quality are related to its storage conditions, thermal treatment efficiency, and physicochemical integrity, especially concerning the addition or removal of substances, whether naturally occurring or foreign to its composition (Polegato & Rudge, 2003).

Milk constitutes a high-biological-value food due to its complex composition of water, proteins, lipids, carbohydrates (lactose), minerals, and vitamins (Oliveira & Santos, 2012; Walstra et al., 2005). Its sensitive biochemical properties undergo significant alterations under various conditions (Corrêa et al., 2015). The physicochemical properties of bovine milk may vary with physiological conditions, animal husbandry practices, and seasonal factors (Chen et al., 2017). In addition, fraudulent adulteration can compromise milk authenticity and may be investigated using physicochemical analytical methods (Abrantes et al., 2014). Milk is considered adulterated when water or foreign substances are added to its composition, such as acidity neutralizers, density-restoring agents (e.g., starch), and preservatives, or when natural components are removed (Bezerra et al., 2022; Brasil, 2020; Mareze et al., 2015).

Another major concern is the presence of antibiotic residues in milk, which has become one of the main critical control points for chemical contamination in dairy products (Medeiros et al., 2020). Antimicrobial agents pose potential consumer health risks, as unintentional exposure to chemical residues may cause side effects, selection of resistant bacterial strains, hypersensitivity, and intestinal microbiota imbalances. Furthermore, these residues inhibit microbial multiplication in milk, thereby interfering with quality control laboratory analyses (Brasil, 2020). Brazilian regulation explicitly prohibits commercialization of milk containing veterinary drug residues or contaminants.

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[1]*Universidade Estadual Paulista "Júlio de Mesquita Filho", School of Veterinary Medicine and Animal Science, Botucatu, São Paulo, Brazil.*
*Corresponding author: otavio.a.martins@unesp.br
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Ultra-high-temperature (UHT) treatment is designed to destroy microorganisms in vegetative forms and achieve commercial sterility, thereby extending the shelf life of milk while maintaining its quality characteristics. According to Brazilian regulations, UHT milk is subjected to heat treatment at 130–150 °C for 2–4 seconds under continuous-flow conditions, immediately cooled to below 32 °C, and aseptically packaged in sterilized, hermetically sealed containers. According to fat content, UHT milk is classified as whole, semi-skimmed, or skimmed (Brasil, 1997; 2020; 2025).

Based on this information, the present study aimed to evaluate:

- The presence of antibiotic residues;
- Macromolecular composition (protein, lactose, and lipids);
- Occurrence of starch adulterant additives;
- Additional physicochemical components in different brands of commercially available whole UHT milk.

1.1 Relevance of the study

This study investigated fraud and contaminants in UHT milk. Five brands were evaluated for the absence of antibiotics (β-lactams, sulfonamides, tetracyclines, quinolones) and adulterants such as starch, as well as for physicochemical parameters (macromolecules, cryoscopy, minerals). The results confirmed compliance with legal standards, ensuring consumer safety.

2 Materials and methods

2.1 Samples

Five brands of commercially available whole UHT milk were analyzed in this study. Designated as M1–M5, samples were procured from local supermarkets or hypermarkets. All specimens were transferred to the Laboratory of Applied Food Physicochemistry at the Public Food Guidance Service (SOAP), Department of Animal Production and Preventive Veterinary Medicine, School

of Veterinary Medicine and Animal Science (FMVZ), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Botucatu Campus, Rubião Júnior District, Botucatu, SP, Brazil.

A total of thirty-seven samples from different batches, all within their expiration dates, were evaluated. The sample brands were divided into the following treatment groups: M1 (n = 7), M2 (n = 8), M3 (n = 7), M4 (n = 8), and M5 (n = 7). All physicochemical assays were performed in triplicate. Samples were systematically homogenized immediately prior to qualitative and quantitative testing.

2.2 Antibiotic residue analysis

A volume of 200 µL of homogenized milk was added to a microtube and incubated at 40°C for five minutes. A test strip from the 4Sensor BSTQ Unisensor® rapid test was then inserted and incubated at 40°C for the following 5 minutes. The 4Sensor BSTQ Unisensor® test strip simultaneously detects four groups of antibiotics in milk: beta-lactams, sulfonamides, tetracyclines, and quinolones, which were interpreted visually. This procedure followed the manufacturer's protocol (Cl Cap-Lab, 2022). Control tests (positive and negative) were performed using antibiotics from the beta-lactam, sulfonamide, tetracycline, and quinolone groups.

2.3 Macromolecules

2.3.1 Lactose and protein analysis

A volume of 25 mL of homogenized milk was added to a cylindrical polyethylene flask and analyzed using the Master Complete Akso® milk analyzer (Milkotester Ltd., Model LM2, Serial 11007). The analyzer was properly calibrated according to the manufacturer's instructions prior to use. The milk analyzer, which uses the transmittance infrared method, quantified the concentrations of the macromolecules lactose and protein.

2.3.2 Lipid analysis

The Gerber method was performed as follows: 10 mL of sulfuric acid solution (density 1.820–1.825 g/mL) was transferred to a Gerber butyrometer using a volumetric pipette, burette, or micropipette. Carefully avoiding acid mixing, 11 mL of homogenized milk was layered using a volumetric pipette. Subsequently, 1 mL of isoamyl alcohol (density 0.850 g/mL) was added. The butyrometer neck was dried with paper and securely stoppered. After repeated inversion mixing (using protective cloth/gloves), the samples were centrifuged at 2,000 rpm for five minutes, followed by immersion in a water bath at 65 ± 2°C for five minutes. Fat content was determined via the integrated graduated scale (AOAC, 1995; Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008).

2.4 Starch adulterant additive

A volume of five mL of homogenized sample was transferred to a 150 mL glass beaker. Approximately 20 mL of distilled water was added, and the mixture was heated to a boil using either a hot plate or a Bunsen burner, then left to stand for 5 minutes. After cooling, five drops of iodine tincture solution were added (prepared with 6 g of resublimed iodine ground in a mortar and 4 g of potassium iodide in 70 % ethanol, completed to 100 mL). In the presence of starch as an adulterant additive, a blue coloration appeared. This qualitative method is based on the reaction between starch and iodine, forming a blue-colored absorption compound. A positive control test was conducted using a 1% starch solution (Brasil, 2022).

2.5 Physicochemical analyses

2.5.1 Cryoscopy

A digital Hortvet cryoscope (ITR brand, model MK 540) was employed for determining the freezing point of milk. The equipment was calibrated using two standard cryoscopy solutions (0.000 and -0.621°H). After calibration, 2.5 mL of

properly homogenized milk was transferred to a cryoscopy tube. The tube containing the sample was inserted into the device, and the freezing point reading was taken in °H. Milk was considered non-adulterated (not diluted with water) when cryoscopy values ranged between -0.530 and -0.550°H. For values greater than -0.530°H, the following formula was applied to estimate the percentage of added water (AOAC, 1995; Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008) (Eq. 1):

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

2.5.2 Lactic acid determination

The samples were homogenized, and 10 mL was transferred to a 50 mL beaker. Then, two to four drops of 1% phenolphthalein indicator solution were added. Titration was performed using a N/9 sodium hydroxide or Dornic solution until the endpoint (a slight pink coloration) was reached. Each 0.1 mL of N/9 sodium hydroxide or Dornic solution used in the titration corresponded to

1°D or 0.01 g of lactic acid per 100 mL (AOAC, 1995; Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008).

2.5.3 Density measurement

Approximately 220 mL of the homogenized milk was transferred to a 250 mL polyethylene graduated cylinder. A clean and dry thermolactodensimeter was slowly inserted into the cylinder containing the milk sample, ensuring that contact with the walls and bottom of the cylinder was avoided. After a few seconds, the reading was taken at the liquid surface level (density). Temperature (°C) and density (g/mL) readings were recorded. Density correction was applied by adding 0.0002 for each degree above 15°C or subtracting 0.0002 for each degree below 15 C. An adjustment of ± 0.0002 was applied for every 5-degree interval in the temperature difference (AOAC, 1995; Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008).

2.5.4 Total solids determination

The Ackermann disk method was employed, utilizing the concentric circle system: the inner (density), middle (lipid), and outer circle (total solids). The values of milk fat and density were applied by aligning the inner circle (density) with the middle circle (lipid). The pointer on the disk then indicated the value on the outer circle, corresponding to the total solids content (g/100 mL) or total dry extract (g/100 mL) (AOAC, 1995; Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008).

2.5.5 Non-fat solids calculation

The values obtained from the lipid (g/100 mL) and total solids (TS, g/100 mL) determinations were used. The following formula was applied (Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008) (Eq. 2):

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

2.5.6 Total caloric value

A mathematical calculation was used to determine the Total Caloric Value (TCV) in kcal/100 g. The formula applied was (Eq. 3) (Brasil, 2022):

$$\text{TCV (kcal/100 g)} = \text{protein (\%)} \times 4 + \text{carbohydrate (\%)} \times 4 + \text{lipid (\%)} \times 9 \quad (3)$$

2.5.7 Fixed mineral residue 2.5.7.1. gravimetric method

Two grams of the sample were weighed into a porcelain crucible previously heated in a muffle furnace at 550°C for two hours, cooled in a desiccator with silica to room temperature then weighed. The sample was carbonized on a heating plate at low temperature. The carbonized sample was then incinerated in a muffle furnace at 550°C for six hours until a white or slightly gray coloration was obtained. After cooling in a desiccator with silica to room temperature, it was weighed again. The following formula was used to calculate the FMR (Eq. 4):

$$\text{FMR (\%)} = (100 \times N) / M \quad (4)$$

Where:

N: weight of ash (g);

M: weight of sample (g) (Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008).

2.5.7.1 Infrared transmittance method

A volume of 25 mL of homogenized milk was added to a cylindrical polyethylene container and analyzed using the Master Complete Akso® milk analyzer (Milkotester Ltd., Model LM2, Serial 11007). The analyzer was properly calibrated according to the manufacturer's instructions prior to use. Using the infrared transmittance method, the device quantified the mineral content or fixed mineral residue (FMR).

2.5.8 Moisture

Five grams of the sample were placed in a porcelain crucible previously heated in an oven at 105°C for two hours, cooled in a desiccator with silica to room temperature, and then weighed. The crucible containing the sample was then heated in an oven at 105°C for six hours. Subsequently, it was cooled in a desiccator with silica to room temperature, then weighed. The following formula was used to calculate the moisture content (Eq. 5):

$$\text{Moisture (\%)} = (100 \times N) / M \quad (5)$$

Where:

N = moisture (mass loss in grams);

M = sample weight (g) (Brasil, 1981; 2005a; 2005b; 2022; Instituto Adolfo Lutz, 2008).

2.6 Statistical analysis

The values obtained from the assays performed in triplicate were statistically analyzed using analysis of variance (ANOVA) in a completely randomized design, and the means were

compared using Tukey's test at a 5% significance level. However, results from the qualitative assays (presence or absence) were evaluated based on absolute and relative frequencies, as well as percentages (Montgomery, 2020). Statistical analysis was performed using the licensed software Origin® Pro 2015.

2.7 Chemical waste disposal

All chemical waste generated during the execution of this study in the Laboratory of Food Physical-Chemistry of Animal Origin was sent to the Conservation and Maintenance Section of the General Administration at UNESP, Botucatu Campus, São Paulo, Brazil. This section is responsible for managing all chemical waste generated at UNESP – Botucatu Campus and makes the technical and analytical decisions regarding its disposal.

3 RESULTS

3.1 Antibiotic residues

As shown in Table 1, the relative frequency (RF) of absence was 1.0 for all tested antibiotic groups (β-lactams, sulfonamides, tetracyclines, and quinolones) across all UHT whole milk brands (M1-M5). This indicates no detectable residues in any samples. Positive and negative controls were properly implemented for all qualitative antibiotic screening tests.

3.2 Macromolecules

Table 2 shows that there were no significant differences ($p > .05$) among the analyzed brands (M1-M5) for the lactose, protein, and TCV assays. The lowest significant mean lipid value was observed in brand M2 (3.05 ± 0.16%), which was statistically lower ($p < .05$) than the highest value found in brand M5 (3.26 ± 0.17%). The coefficients of variation (CV) for the comparisons among brands (M1 to M5) in the lactose, protein, lipid, and TCV assays were 3.72, 2.95, 4.31, and 2.68%, respectively. The low CV values obtained demonstrated high stability and homogeneity in the experiment.

3.3 Starch adulterant additive

The relative frequency (RF) for absence in the qualitative test for the starch adulterant additive was 1.0 across all brands (M1-M5) of whole UHT milk. It is important to note that both positive and negative controls were performed for the qualitative starch analysis (Table 3).

3.4 Physicochemical assays

Table 4 shows that the coefficients of variation (CV) among brands (M1–M5) in the acidity, density, total solids (TS), non-fat solids (NFS), cryoscopy index (IC), and moisture & volatiles (UV) assays were 5.83, 0.09, 14.49, 2.93, 1.04, and 0.69%, respectively. These low CVs demonstrate experimental stability and homogeneity. No significant differences ($p > .05$) were observed among brands for acidity, TS, and NFS. The lowest significant mean density ($p < .05$) occurred in brand M5 (1.0297 g mL^{-1} ± 0.0004), whereas the highest significant densities were found in brands M2 (1.0319 g mL^{-1} ± 0.0018) and M4 (1.0315 g mL^{-1} ± 0.0006). In cryoscopy, brands M1(-0.534°H ± 0.003) and M2 (0.533°H ± 0.006) exhibited the lowest significantly negative mean values ($p < .05$) compared with the least negative mean for brand M3 (-0.546°H ± 0.007). Regarding moisture, brands M3 (88.83% ± 0.31) and M5 (88.90% ± 0.70) exhibited the highest significant means ($p < .05$), while brand M1 presented the lowest (87.61% ± 1.13) Table 5 indicates CVs of 5.66 and 10.32% for the salt content assay (transmittance-infrared method) and fixed mineral residue (FMR) assay (gravimetric method), respectively, again confirming stability and homogeneity. Using the transmittance-infrared method, brands M1 (0.70 ± 0.00%), M2 (0.66 ± 0.05%), and M4 (0.69 ± 0.04%) had significantly higher average salt concentrations ($p < .05$) than brand M5 (0.60 ± 0.00%). In contrast, gravimetric FMR values for brands M1(0.74 ± 0.04%), M2 (0.75 ± 0.06%), M3 (0.77 ± 0.03%), M4 (0.76 ± 0.05%), and M5 (0.76 ± 0.15%) did not differ significantly ($p > 0.5$). Statistically, the gravimetric method yielded higher mean FMR values ($p < .05$) than the transmittance-infrared method for salt determination (Table 5).

4 DISCUSSION

4.1 Antibiotic residues

Global surveillance consistently monitors β-lactam, sulfonamide, tetracycline, and quinolone residues in milk (Niu et al., 2023; Zheng et al., 2013). β-lactam residues pose significant health risks, including allergic reactions, gastrointestinal microbiota disruption, and antibiotic resistance development. While Chinese studies report detectable β-lactam residues in milk and dairy products (Niu et al., 2023), in the present study, no β-lactam residues were detected in UHT milk samples using the employed method.

Scientific studies have identified sulfonamides such as sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine, sulfacetamide, trimethoprim, sulfisomidine, and sulfadiazine in milk, though concentrations were below the limits established by Chinese and European regulations (Ben et al., 2022; Chen et al., 2014; Jia et al., 2022; Li et al., 2017; Niu et al., 2023; Shi et al., 2020; Wang et al., 2017; Zhang et al., 2020). In contrast, this study did not detect sulfonamide residues in UHT milk samples across various brands using the aforementioned method.

Niu et al. (2023) and Zhang et al. (2014) reported the presence of tetracyclines in milk and dairy products at concentrations around 42.39 μg/kg. Notably, Zhang et al. (2014) found the highest levels of tetracyclines in milk purchased from supermarkets. In this study, no tetracycline residues were detected in UHT milk through the method employed.

Other studies have detected quinolones in milk and dairy products. These studies noted that quinolone residues are not destroyed by thermal treatment and can easily transfer from raw milk to dairy products, including pasteurized and UHT milk (Du et al., 2019; Han et al., 2013; Niu et al., 2023; Zhang et al., 2014). In this experiment, no quinolone residues were detected in the different brands of whole UHT milk evaluated.

4.2 Macromolecules

Macromolecules, broadly classified as proteins, carbohydrates, lipids, and nucleic acids, are of great interest across various industries, including the pharmaceutical, biomedical, cosmetic, and food sectors due to their unique structural and functional characteristics. As such, the effective extraction and analysis of these macromolecules from natural sources has long been a focus of research (Bilal & Iqbal, 2020; Jeevanandam et al., 2022; Ling & Hadinoto, 2022).

In a study by Antunes et al. (2025), the mean lactose content in cow milk was 4.6 ± 0.13%. This aligns with the values obtained in the current study for UHT milk brands M1 through M5 (4.66 ± 0.08%).

Sun et al. (2023) reported a mean protein value of 5.44 ± 0.11% in UHT milk using the Kjeldahl method. In the current study, using the transmittance infrared method, a mean protein value of 3.10 ± 0.05% was obtained for brands M1 to M5, which was numerically lower than that reported by Sun et al. (2023).

Daszkiewicz et al. (2024) used the Soxhlet extraction method to determine lipid content, reporting a mean value of 3% in UHT milk. In the present study, lipid content was determined using the Gerber method, yielding a mean

Table 1. Absolute frequency (AF), relative frequency (RF), and relative frequency percentage (RF %) of qualitative assays (presence or absence) for antibiotic residues from the beta-lactam, sulfonamide, tetracycline, and quinolone groups in different brands (M1, M2, M3, M4, and M5) of whole UHT milk.

Antibiotic	UHT Milk Brand	Result	AF	RF	RF (%)
β-lactam	M1 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M2 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M3 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M4 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M5 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
Sulfonamide	M1 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M2 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M3 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M4 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M5 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
Tetracycline	M1 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M2 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M3 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M4 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M5 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
Quinolone	M1 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M2 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M3 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100
	M4 (n = 8)	Present	0	0.0	0
		Absent	8	1.0	100
	M5 (n = 7)	Present	0	0.0	0
		Absent	7	1.0	100

value of 3.15 ± 0.08% for all UHT brands, which is in agreement with both Daszkiewicz et al. (2024) and Brasil (2025). The Total Caloric Value (TCV, kcal/100 g) reflected the mean macromolecule contents (lactose, protein, and lipid) of the UHT milk brands, and was calculated based on 9 kcal/g for lipids, 4 kcal/g for proteins, and 4 kcal/g for carbohydrates (Brasil, 2003; 2022).

4.3 Starch adulterant additive

The addition of substances that reconstitute density and cryoscopy index is primarily intended to conceal the fraudulent addition of water to milk. Montgomery et al. (2020) identified the main compounds used for this purpose as chlorides, sucrose, starch, and ethanol. These substances can mask both the water adulteration and the analytical methods used to detect it. It is important to emphasize that starch specifically reconstitutes the density of milk adulterated with water (Brasil, 2022; Montgomery et al., 2020). In the present study, no starch was detected. The characteristic interaction between the triiodide ion (I_3^-) and amylose in starch, which produces an intense blue to nearly black coloration depending on reagent concentrations, was not observed in the qualitative assay of UHT milk samples M1, M2, M3, M4, or M5 (AOAC, 2016; Brasil, 2022).

4.4 Physicochemical assays

The mean acidity values of UHT milk brands M1, M3, M4, and M5 were within the range established by Brazilian regulations (Brasil, 2025), whereas the mean value for M2 (0.1638 ± 0.0151 g/100 mL) was slightly above the upper limit. The measured fat and non-fat solids contents met the applicable minimum requirements for the corresponding milk categories

Table 3. Absolute frequency (AF), relative frequency (RF), and relative frequency percentage (RF %) of the qualitative assay (presence or absence) for the starch adulterant additive in different brands (M1, M2, M3, M4, and M5) of whole UHT milk.

Brand	Result	AF	RF	RF %
M1 (n = 7)	Absent	7	1.0	100
	Present	0	0.0	0
M2 (n = 8)	Absent	8	1.0	100
	Present	0	0.0	0
M3 (n = 7)	Absent	7	1.0	100
	Present	0	0.0	0
M4 (n = 8)	Absent	8	1.0	100
	Present	0	0.0	0
M5 (n = 7)	Absent	7	1.0	100
	Present	0	0.0	0

Table 2. Mean ± standard deviation of lactose (%), protein (%), lipid (%), and total caloric value (TCV, kcal/100 g) in different brands (M1, M2, M3, M4, and M5) of whole UHT milk. Statistical analysis (ANOVA) of macronutrients and TCV complemented by Tukey's test at a 5 % significance level.

Macronutrient	UHT Milk Brands				
	M1	M2	M3	M4	M5
Lactose (%)	4.76 ± 0.05 a1	4.63 ± 0.27 a	4.57 ± 0.26 a	4.72 ± 0.07 a	4.60 ± 0.00 a
Protein (%)	3.16 ± 0.05 a	3.10 ± 0.13 a	3.07 ± 0.13 a	3.15 ± 0.05 a	3.04 ± 0.05 a
Lipid (%)	3.11 ± 0.15 ab	3.05 ± 0.16 a	3.14 ± 0.08 ab	3.21 ± 0.10 ab	3.26 ± 0.17 b
TVC (kcal/100 g)	59.69 ± 1.50 a	58.35 ± 1.71 a	58.86 ± 2.16 a	60.41 ± 0.72 a	59.89 ± 1.61 a

[1]One-way ANOVA supplemented by Tukey's HSD post-hoc test (α = 0.05).

Table 4. Mean ± standard deviation of acidity (°D), density (g·mL^{-1}), total solids (TS, %), non-fat solids (NFS, %), cryoscopy index (CI, °H), and moisture and volatiles (MV, %) in different brands (M1, M2, M3, M4, and M5) of whole UHT milk. Statistical analysis (ANOVA) complemented by Tukey's test at a 5% significance level.

Assay	UHT Milk Brands				
	M1	M2	M3	M4	M5
Acidity (°D)	15.57 ± 0.79 a1	16.38 ± 1.51 a	15.57 ± 0.79 a	15.38 ± 0.52 a	15.29 ± 0.49 a
Density (g.mL^{-1})	1.0308 ± 0.0005 ab	1.0319 ± 0.0018 b	1.0306 ± 0.0001 ab	1.0315 ± 0.0006 b	1.0297 ± 0.0004 a
Total solids (TS. %)	11.71 ± 0.24 a	10.18 ± 3.52 a	11.71 ± 0.10 a	11.97 ± 0.18 a	11.61 ± 0.21 a
Non-fat solids (NFS. %	8.57 ± 0.29 a	8.83 ± 0.38 a	8.70 ± 0.09 a	8.84 ± 0.19 a	8.60 ± 0.19 a
Cryoscopy (°H)	- 0.534 ± 0.003 a	- 0.533 ± 0.006 a	- 0.546 ± 0.007 b	- 0.540 ± 0.004 ab	- 0.538 ± 0.007 ab
Moisture (%)	87.61 ± 1.13 a	88.46 ± 0.17 ab	88.83 ± 0.31 b	88.28 ± 0.21 ab	88.90 ± 0.70 b

[1]One-way ANOVA supplemented by Tukey's HSD post-hoc test (α = 0.05).

Table 5. Mean ± standard deviation of salt content (%) determined by the transmittance infrared method and fixed mineral residue (FMR, %) determined by the gravimetric method in different brands (M1, M2, M3, M4, and M5) of whole UHT milk. Statistical analysis (ANOVA) complemented by Tukey's test at a 5 % significance level.

Brand	Salt content (%)	FMR (%)
M1	0.70 ± 0.00 b[1]A[2]	0.74 ± 0.04 aB
M2	0.66 ± 0.05 bA	0.75 ± 0.06 aB
M3	0.64 ± 0.05 abA	0.77 ± 0.03 aB
M4	0.69 ± 0.04 bA	0.76 ± 0.05 aB
M5	0.60 ± 0.00 aA	.76 ± 0.15 aB

[1]Lowercase letters: Comparisons across brands within the same assay (Tukey's test. $\alpha = 0.05$); [2]Uppercase letters: Comparisons across assays within the same brand (Tukey's test. $\alpha = 0.05$).

(Brasil, 2025). Density, total solids, cryoscopic index, and moisture were evaluated against the reference values adopted in this study. No statistically significant differences ($p > .05$) were observed among brands for acidity, density, total solids, non-fat solids, or cryoscopic index.

Benmeziane-Derradji (2021) reports that mineral content, measured as ash in milk, ranges from 0.60 to 1.30%. Mion et al. (2023) note that the mineral salts or fixed mineral residue (FMR) content in milk is around 0.73%. In this experiment, the ash or FMR values ranged from 0.60 to 0.77%, corroborating the findings of Benmeziane-Derradji (2021) and Mion et al. (2023).

Another important aspect regarding the FMR assay is the comparison between the two methods used in this study: transmittance infrared and gravimetric. The results obtained by the transmittance infrared and the gravimetric method showed statistically significant differences ($p < .05$). The gravimetric method yielded higher concentrations of mineral salts or ash in the UHT milk samples and showed no significant differences ($p > .05$) among the brands M1 to M5 when analyzed statistically. Therefore, we consider the gravimetric method to be more precise and robust for determining ash or FMR in whole UHT milk samples.

5 CONCLUSION

Based on the evidence presented in this study, it can be concluded that:

- No antibiotic residues were detected using qualitative methods;
- Macromolecule concentrations (protein, carbohydrate, and lipid) comply with current regulations;
- The adulterating starch additive was not detected;
- Physicochemical assays (acidity, density, TS, SNF, cryoscopy, and moisture) conform to regulatory standards;
- Average mineral salt values (ash/RMF) determined by gravimetry were significantly higher ($p < .05$) than those obtained via transmittance infrared across all whole UHT milk samples (M1–M5).

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