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Comparison of Physicochemical, Textural, Ripening, and Microbiological Properties of Cheeses Produced from Buffalo and Cow Milk

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Abstract

Cheese is one of the most widely consumed dairy products worldwide, appreciated by consumers both as a main dish and as an accompaniment due to its distinctive sensory attributes. Given its high consumption frequency, physicochemical and microbiological assessments are essential to ensure public health safety and product acceptability. This study aimed to evaluate the physicochemical properties, firmness, ripening characteristics, and microbiological profiles of cow's and buffalo's milk cheeses commercially available in the Botucatu region, São Paulo, Brazil. The results demonstrated that the cheese groups—cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella—exhibited firmness characteristics classified as soft, hard, and firm/semi-hard, respectively. In terms of ripening, the cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella groups were categorized as brine-ripened, mold-ripened, and unripened/fresh cheeses, respectively. Microbiological analysis revealed that some cow's milk fresh cheese and buffalo's milk mozzarella samples failed to comply with the microbiological standards for *Listeria monocytogenes* and coagulase-positive *Staphylococcus*, representing a potential public health concern and highlighting the need for stricter sanitary inspection and regulatory oversight.

Keywords: public health; ripening; sensory properties; firmness; contamination.

Practical Application: Physicochemical and microbial quality of cow and buffalo milk cheeses.

1 INTRODUCTION

Cheese is one of the most important dairy products worldwide, classified according to its ripening status (ripened or unripened) and texture (soft, semi-hard, hard, or extra hard). It is obtained through the partial or complete coagulation of proteins from whole milk, skimmed milk, partially skimmed milk, cream, whey cream, buttermilk, or any combination of these materials (Codex Alimentarius Commission, 2024a). Production involves the action of rennet or other suitable coagulating agents, followed by partial draining of the whey formed during coagulation. This process concentrates milk proteins, particularly casein, resulting in a product with a protein content generally higher than other dairy products. Unripened cheeses, such as fresh cheeses, are ready for consumption immediately after production (Codex Alimentarius Commission, 1978).

According to the Codex General Standard for Cheese (Codex Alimentarius Commission, 2024a), the fat content of the milk used for cheese production must be declared in a manner acceptable to the country where the product is sold to the final consumer. Additionally, cheese products may be classified according to their fat in dry matter (FDM) content as follows: (a) high-fat (FDM ≥ 60%); (b) full-fat (45% ≤ FDM < 60%); (c) medium-fat (25% ≤ FDM < 45%); (d) low-fat (10% ≤ FDM < 25%); and (e) skim (FDM < 10%) (Codex Alimentarius Commission, 2022, 2024a).

Buffalo and cow's milk mozzarella cheeses are typically packaged and stored in a liquid medium, such as water, stretching water, whey, or diluted brine, to preserve their flavor, freshness, and elastic texture. This packaging system protects the delicate structure of mozzarella during transportation, stabilizing its composition and physicochemical properties throughout storage (Biegalski & Cais-Sokolińska, 2024; Faccia et al., 2013, 2019; Guidone et al., 2016; Ricciardi et al., 2015). However, this type of packaging also increases the product's perishability, with a typical shelf life ranging from 1 to 2 weeks. Both manufacturing procedures and storage conditions can significantly influence this period, as higher temperatures tend to accelerate microbiological and physicochemical changes, shortening the product's shelf life (Biegalski & Cais-Sokolińska, 2024; Faccia et al., 2019).

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According to Codex Alimentarius Commission (2024a), physicochemical parameters such as firmness and ripening stage serve as indicators of cheese quality in commercialized products. These assessments are based on the analytical values of moisture on a fat-free basis, which depend on the total moisture content, total weight, and fat content of the cheese (Codex Alimentarius Commission, 2022, 2024a, 2024b). Cheeses are widely consumed either as main courses or as ingredients in various culinary preparations. Among the sensory characteristics most valued by consumers are uniform melting, free oil formation, homogeneous fusion of cheese pieces, and desirable browning during cooking (Ah & Tagalpallewar, 2017; Berta et al., 2016; Biegalski & Cais-Sokolińska, 2024; Dai et al., 2019; Sutariya et al., 2022). These properties are largely influenced by physicochemical parameters related to firmness and the degree of ripening in cheeses marketed worldwide (Biegalski & Cais-Sokolińska, 2024; Codex Alimentarius Commission, 2024a).

Based on this context, the present study aimed to (a) evaluate the physicochemical characteristics related to firmness and ripening and (b) assess the microbiological profiles of cow's and buffalo's milk cheeses acquired from commercial establishments in the municipality of Botucatu, São Paulo, Brazil.

1.1 Relevance of the work

This study is relevant as it evaluates the physicochemical, textural, ripening, and microbiological characteristics of commercially available cheeses made from cow and buffalo milk. Given the widespread consumption of cheese, understanding its physicochemical properties and potential microbiological risks is important for food safety and public health. The identification of samples that did not meet sanitary standards highlights the need for regulatory oversight and proper inspection of these foods.

2 MATERIAL AND METHODS

2.1 Reagents and equipments

Sulfuric acid—95–98%—Sigma-Aldrich, catalog number 258105. Sulfuric acid solution—1.605 g/mL. Lugol solution—Sigma-Aldrich, catalog number 32922. Amyl alcohol (iso) (for milk analysis), 0.850 g/mL—Synth, catalog number A1074. Pure water (PW) was obtained using a TE 4007-10 water purification system from Tecnal Scientific Equipment (São Paulo, Brazil). The scale used was an ATX224 model from Shimadzu (São Paulo, Brazil). The pH values were measured using an AK103 pH meter from Akso (Rio Grande do Sul, Brazil). Digital sterilizing and drying incubator from Solidsteel (São Paulo, Brazil). Bacteriological incubators from Solidsteel (São Paulo, Brazil). Digital water bath from Solidsteel (São Paulo, Brazil). Gerber centrifuge—Simplex II model—TR Ltda—ITR (Rio Grande do Sul, Brazil). Gerber milk fat meter, 0 to 8%, with stopper.

2.2 Samples

The cow's milk cheese samples included fresh cheese (CFC) and mozzarella (CMC). The buffalo's milk cheese samples consisted of mozzarella (BMC). A total of 42 samples were analyzed, sourced from different establishments in Botucatu, São Paulo, Brazil: 18 CFC, 12 CMC, and 12 BMC samples, collected between March 2018 and March 2023. Samples were stored and transported under refrigeration (approximately 4°C) and sent to the Physical Chemistry and Microbiology Laboratories at the Laboratory of Food Inspection (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. All physical–chemical and microbiological tests were performed in triplicate.

2.3 pH

Notably, 10 g of each homogenized sample was weighed and placed in a 100-mL beaker. Then, 50 mL of pure water was added and mixed with a glass stirrer for 30 s. Surface fat was removed using a stainless-steel spatula. The pH meter was calibrated with pH 7 and pH 4 reference solutions at 20°C. Measurements were taken at the same temperature (Instituto Adolfo Lutz [IAL], 2008).

2.4 Moisture

Clean porcelain capsules and their respective lids were dried in an oven at 105°C for 2 h, then cooled to room temperature in a desiccator, and weighed. Subsequently, 5.00 g of each homogenized sample was transferred into the pre-weighed capsules. The samples were then dried at 105°C for 4 h, cooled again in a desiccator, and reweighed. The drying and cooling steps were repeated until a constant weight was achieved. Moisture content was calculated using the formula: Moisture (g/100 g) = (P × 100)/Pa; where P is the mass loss (g), and Pa is the initial sample mass (g) (IAL, 2008).

2.5 Dry matter or total solids content

The dry matter (DM) was calculated as follows: DM (g/100 g) = 100 – moisture (g/100 g) (International Organization for Standardization [ISO] & International Dairy Federation [IDF], 2004).

2.6 Lipids

Lipids were determined using the butyrometer method (IAL, 2008). Notably, 1 g of the homogenized sample was placed in a 50-mL glass beaker. A volume of 10 mL of sulfuric acid solution (1.605 g/mL) was added, and the mixture was heated in a water bath at 65°C. The content was mixed with a glass stirrer until the sample dissolved completely. The solution was carefully transferred to a milk butyrometer. The beaker was rinsed three times with 3 mL of sulfuric acid solution. A volume of 1 mL of amyl alcohol reagent (0.850 g/mL) was added. The butyrometer was stoppered, shaken, and placed in a water bath at 65°C for 15 min. It was then centrifuged at 2,000 rpm for 5 min. This process of water bath heating and centrifugation was repeated twice. The lipid layer was measured, and the result was calculated using the formula: Lipids (g/100 g) = (L × 11.33)/Pa, where L is the lipid layer reading on the butyrometer, and Pa is the sample mass (g) (ISO & IDF, 2008).

2.7 Fat in dry matter

Fat in dry matter (FDM) was determined according to Codex Alimentarius Commission (2024b) recommendations. The formula used was: FDM (g/100 g) = (100 × lipids)/DM, where DM is the dry matter.

2.8 Firmness and ripening characteristics

The analysis of these characteristics was based on moisture on a fat-free basis (MFFB) value. The MFFB values were calculated using the weight of moisture in the cheese (WMC), the total weight of the cheese (TWC), and the weight of fat in the cheese (WFC). The formula used to express MFFB was: MFFB (%) = [WMC/(TWC – WFC)] × 100. Based on the MFFB values, the samples were classified as follows: (a) MFFB < 51%: Extra hard; (b) MFFB 49–56%: Hard; (c) MFFB 54–69%: Firm/Semi-hard; (d) MFFB > 67%: Soft. For ripening, the classifications were: (a) MFFB < 51%: Aged; (b) MFFB 49–56%: Mold ripened; (c) MFFB 54–69%: Unripened/fresh; (d) MFFB > 67%: In brine (Codex Alimentarius Commission, 2024a).

2.9 Starch detection

Notably, 10 g of the homogenized sample was weighed and placed in a 250-mL glass beaker. A volume of 50 mL of pure water was added. The mixture was heated with a Bunsen burner for 5 min and then cooled, and five drops of Lugol solution were added. A blue color indicated the presence of starch (IAL, 2008).

2.10 Salmonella spp. detection

For the detection of Salmonella spp., the protocol described by Silva et al. (2017) was used, with adaptations:

The first step involved pre-enrichment. Notably, 25 g of the sample was weighed into a sterile stomacher bag, followed by the addition of 225 mL of buffered peptone water (BPW). The mixture was homogenized for approximately 120 s in a stomacher and incubated at 36 ± 2°C for 24 ± 2 h.

For selective enrichment, 1 mL of the BPW solution containing the sample was transferred into tubes containing Tetrathionate Broth (TT) supplemented with iodide solution (0.2 mL) and 0.1 mL of Rappaport-Vassiliadis (RV) broth. Both culture broths were homogenized in a tube shaker and incubated in a water bath at 41 ± 2°C for 24 ± 2 h.

Following incubation, the tubes were homogenized in a vortex shake,r and the content was streaked onto Bismuth Sulfite (BS) agar and Xylose Lysine Desoxycholate (XLD) agar plates. These plates were incubated at 36 ± 1°C for 24 ± 2 h.

At least two typical colonies from each plate were selected. Using an inoculation needle, the central portion of each colony was transferred with metal sticks and inoculated into a tube containing Triple Sugar Iron (TSI) agar, streaking the slant and stabbing the bottom of the tube. The same needle was then used to inoculate a tube containing Lysine Iron Agar (LIA), following the same technique. These tubes were incubated at 36 ± 1°C for 24 ± 2 h.

The typical colony characteristics in the plating media were: (a) XLD Agar: Dark pink colonies with a black center and a slightly transparent reddish zone around them. Strongly H_2S-positive Salmonella strains can produce colonies with a large, shiny black center, or completely black colonies; (b) BS Agar: Flat, brown, gray, or black colonies, with or without metallic sheen. The medium surrounding the colonies gradually changes to brown or black.

The biochemical profile was as follows: (a) TSI: Alkaline slope (cherry red) and acidic bottom (yellow), with or without H_2S production (darkening of the agar) and gas (bubbles or agar displacement); (b) LIA: Alkaline slope and bottom (purple, no change in medium color), with or without H_2S production (darkening of the agar).

Confirmation of the microorganism was performed through genetic material extraction followed by polymerase chain reaction (PCR) analysis. Colonies grown in the LIA and TSI tubes were initially inoculated into a microtube containing 2 mL of brain heart infusion (BHI) broth and incubated at 36 ± 1°C for 24 ± 2 h. Genetic material was extracted following the protocol described by Pui et al. (2011), with adaptations; a 400 µL aliquot of the culture, following incubation, was transferred to a 1.5-mL microcentrifuge tube and centrifuged at 4,500 rpm for 5 min. The supernatant was discarded, and the resulting pellet was resuspended in 400 µL of phosphate-buffered saline (PBS), followed by centrifugation under the same conditions. After discarding the supernatant, the pellet was resuspended in 200 µL of DNase-free water and incubated at 100 °C in a dry bath for 10 min. The sample was then centrifuged at 4,500 rpm for 10 min, and the supernatant—containing the extracted genetic material—was transferred to a clean microcentrifuge tube and stored at –20 °C until PCR analysis.

For PCR, the mix was prepared. Each 16 µL reaction contained: 8 µL of GoTaq®, 3 µL of the extracted DNA, 0.7 µL of the *invA* forward primer, 0.7 µL of the *invA* reverse primer, and 3.6 µL of DNase-free water. The mix was aliquoted into 200-µL microtubes and subjected to thermal cycling for target gene amplification. A negative control was included, consisting of 2 µL of DNase-free water. Following amplification, the PCR products were analyzed by electrophoresis (Swamy et al., 1996).

2.11 *Listeria monocytogenes* detection

For the detection of *Listeria monocytogenes*, the protocol described by Silva et al. (2017) was used, with adaptations:

Notably, 25 g of the sample was weighed into a sterile plastic bag, to which 225 mL of LEB (Listeria Enrichment Broth) was added. The mixture was homogenized for approximately 120 s and incubated at 30 °C for 20–26 h.

Following, 0.1 mL of the LEB broth containing the sample was inoculated into a tube with Fraser broth. The contents were mixed using a tube shaker and incubated at 35 ± 2 °C for 18–24 h.

The occurrence of medium darkening was observed, indicating hydrolysis of esculin. If positive, differential plating was

performed. If negative, the Fraser broth was reincubated for up to 48 ± 2 h, and the medium was reassessed. Tubes that turned positive were plated, while negative samples were discarded.

Selective differential plating was performed from the Fraser broth. A 0.1 mL inoculum of the culture was streaked onto Modified Oxford Agar (MOX) plates. Plates were incubated at 35 ± 2°C for 26 ± 2 h. The presence of typical *Listeria monocytogenes* colonies—small (~1 mm), surrounded by a black halo due to esculin hydrolysis—was recorded. If typical colonies were not observed, the plates were reincubated for an additional 24 ± 2 h and reassessed.

Confirmation of presumptive colonies proceeded as follows: (a) twenty typical colonies from each MOX plate were streaked onto horse blood agar; (b) the plates were incubated at 35 ± 2 °C for 22 ± 4 h and examined for small, translucent colonies with narrow zones of β-hemolysis; (c) a representative colony was used to inoculate both BHI broth and BHI or TSA+YE (Tryptone Soya Agar with Yeast Extract) plates; (d) the agar plates were incubated at 35°C for 24 h to perform confirmatory biochemical tests.

The biochemical confirmation tests included: (a) The catalase test: A loopful of culture from BHI or TSA+YE plates was transferred onto a microscope slide and covered with a drop of 3% hydrogen peroxide solution. Immediate bubbling indicated a positive result. *Listeria* spp. is catalase-positive. (b) Gram staining: A smear prepared from BHI or TSA+YE agar cultures was Gram-stained. *Listeria* spp. appears as short Gram-positive rods; older cultures may exhibit Gram variability. (c) Motility test: Cultures from BHI or TSA+YE were inoculated by stab into SIM (Sulfur Indole Motility) medium to a depth of ~1 cm from the bottom. Tubes were incubated at 20–25 °C for up to 7 days and monitored daily. *L. monocytogenes* exhibits characteristic umbrella-shaped motility restricted to the upper region of the stab line. (d) Hemolysis verification test: A blood agar no. 2 plate supplemented with sheep blood was marked into 20–25 sectors. Each suspect colony was stab-inoculated near the bottom of the agar in a designated sector, avoiding contact with the plate bottom. Plates were incubated at 35°C for 24–48 h. Hemolytic activity was visualized by backlighting the plates. *L. monocytogenes* and *L. seeligeri* produce narrow zones of hemolysis limited to the stab area. *Listeria ivanovii* forms large, well-defined hemolytic zones, while *Listeria innocua* is non-hemolytic. (e) Sugar fermentation test: Using BHI or TSA+YE cultures, each isolate was inoculated into tubes containing purple base broth supplemented with 0.5% of the carbohydrate of interest. Tubes were incubated at 35 °C for up to 7 days and observed daily for acid production (indicated by a color change from purple to yellow) and gas production (optional, collected in a Durham tube). *Listeria* spp. does not produce gas. All strains ferment maltose. *L. monocytogenes* hydrolyzes rhamnose but does not ferment xylose or mannitol.

2.12 Coagulase-positive *Staphylococcus* count

An aliquot of 0.1 mL was inoculated onto Baird-Parker (BPB) agar plates supplemented with egg yolk emulsion and 1% potassium tellurite. The inoculum was surface-plated, and the plates were incubated at 37 °C for 24–48 h. Colonies with typical characteristics of *Staphylococcus* spp.—black or dark gray, shiny, and surrounded by clear halos—as well as atypical colonies, were counted and subjected to the coagulase test using EDTA-treated plasma. Tubes were incubated at 36 ± 2°C for 24 ± 2 h (Salfinger & Tortorello, 2015). Samples showing clot formation were considered positive, while those that remained fluid were considered negative.

2.13 Statistical analysis

The data obtained from the assays, performed in triplicate, were statistically analyzed using analysis of variance (ANOVA) under a completely randomized design. Tukey's test was applied for mean comparisons at a 5% significance level (Montgomery, 2013). Absolute frequency (AF), relative frequency (RF), and relative frequency expressed as a percentage (RF%) were used to evaluate the results of starch detection, presence of *Salmonella* spp. and *Listeria* spp., and counts of coagulase-positive *Staphylococcus*.

2.14 Chemical waste disposal

All chemical waste generated during this study in the laboratories was forwarded to the Conservation and Maintenance Section of the General Administration (UNESP, Botucatu Campus, São Paulo, Brazil). This section is responsible for managing all chemical waste generated at the UNESP—Botucatu Campus and for making technical and analytical decisions regarding its disposal.

3 RESULTS AND DISCUSSION

3.1 pH

The mean pH values for the CFC, CMC, and BMC sample groups were 6.56 ± 1.09, 7.09 ± 0.63, and 5.26 ± 0.33, respectively (Table 1). The BMC group had a significantly lower pH ($p < .05$) compared to the CFC and CMC groups. Bansal and Veena (2024) reported that pH monitoring during

Table 1. Mean ± standard deviation values of pH, moisture (g/100 g), dry matter (g/100 g), lipid content (g/100 g), and fat in dry matter (g/100 g) in cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella samples.

Assay	Cheese Type		
	CFC	CMC	BMC
pH	6.56 ± 1.09 b[1]	7.09 ± 0.63 b	5.26 ± 0.33 a
Moisture (g/100 g)	55.16 ± 12.01 b[1]	36.25 ± 7.52 a	44.96 ± 0.51 a
DM (g/100 g)	44.84 ± 12.01 a[1]	63.75 ± 7.52 b	55.04 ± 0,51 b
Lipids (g/100 g)	19.06 ± 7.22 a[1]	26.24 ± 5.41 b	23.68 ± 0.91 ab
FDM (g/100 g)	43.33 ± 14.32 a[2]	41.18 ± 6.37 a	43.03 ± 1.66 a

[1]$p < .05$. [2]$p > .05$. [ab]Different lowercase letters in the same row indicate statistically significant differences between groups ($p < .05$). Statistical analysis was performed using ANOVA, followed by Tukey's test at a 5% significance level. DM: dry matter; FDM: fat in dry matter; CFC: cow's milk fresh cheese; CMC: cow's milk mozzarella; BMC: buffalo's milk mozzarella.

cheese production is critical to ensuring both the quality and safety of the final product. Cheese pH affects curd texture and flavor by influencing the hydration of casein and the overall metabolic activity—such as enzymatic action during ripening, amino acid decarboxylase activity, and bacteriocin production (Bansal & Veena, 2024).

In general, a very low pH produces a grainy texture and may favor mold growth during storage. Conversely, cheeses with high pH values (> 5.6) may not knit properly, leading to a brittle body and a curd-like texture, which in turn impairs functional attributes such as meltability and stretchability. Additionally, high pH may also raise food safety concerns (Bansal & Veena, 2024; Johnson & Sommer, 2020).

In the present experiment, fresh cheese (CFC: pH 6.56) showed a pH value consistent with findings from Gobbetti and Cagno (2017) and Trmčić et al. (2017), who suggested that a pH > 6.2 is ideal. Those same authors also proposed an optimal pH range of 5.01–5.4 for mozzarella cheese (Gobbetti & Cagno, 2017; Trmčić et al., 2017). Our findings showed that buffalo milk mozzarella (BMC: pH 5.26) corroborated with the values suggested by Bansal and Veena (2024). However, this was not the case for cow milk mozzarella (CMC: pH 7.09), which had an elevated pH value, raising potential food safety concerns for the commercial product (Bansal & Veena, 2024; Gobbetti & Cagno, 2017; Trmčić et al., 2017).

3.2 Moisture

Regarding the mean moisture content, the CFC, CMC, and BMC sample groups had values of 55.16 g/100 g ± 12.01 g/100 g, 36.25 g/100 g ± 7.52 g/100 g, and 44.96 g/100 g ± 0.51 g/100 g, respectively (Table 1). The CMC and BMC groups showed significantly lower moisture values ($p < .05$) compared to the CFC group.

Commercially available types of mozzarella are classified according to their moisture content and the type of milk used in production. "Fiordilatte mozzarella" is produced from cow's milk, whereas "Mozzarella di Bufala" is derived from buffalo milk (Piscopo et al., 2024). According to Piscopo et al. (2024), both types are considered fresh mozzarella due to their short shelf life and must have a moisture content ≥ 52%. Akhtar et al. (2023) noted that most pizza manufacturers prefer to use low--moisture mozzarella, which typically has a moisture content between 42 and 45% and provides extended shelf life (Akhtar et al., 2023; Piscopo et al., 2024).

In our study, the BMC group samples (44.96%) exhibited a moisture level consistent with the range recommended by Akhtar et al. (2023) and Piscopo et al. (2024) for pizza production. However, this was not observed in the CMC group (36.25%), which showed an excessively low moisture content. Only the CFC group samples (55.16%) had a moisture content exceeding 52%, indicating a fresh cheese with a short shelf life, although not classified as mozzarella but rather as a fresh cow's milk cheese (Akhtar et al., 2023; Piscopo et al., 2024).

Furthermore, the moisture value observed in CFC is similar to that reported by Caldeira et al. (2024) for fresh cow's milk cheeses (52.25 g/100 g). Regardless of the cheese classification, all moisture values obtained in this study complied with the standards set by the Brazilian Ministry of Agriculture (Brasil, 1997), which establishes a maximum moisture content of 60 g/100 g.

3.3 Dry matter

As expected, Table 1 shows that the DM value for the CFC group (44.84 g/100 g ± 12.01 g/100 g) was significantly lower ($p < .05$) compared to the CMC (63.75 g/100 g ± 7.52 g/100 g) and BMC (55.04 g/100 g ± 0.51 g/100 g) groups.

Von Oesen et al. (2024) evaluated DM and firmness to characterize cheese on an industrial scale. They found that the DM values for the cheeses ranged from 49.83 to 58.03%. In our experiment, the DM value for the BMC group samples (55.04%) corroborates with the data obtained by Von Oesen et al. (2024), while the DM data were incompatible for the other groups. According to Piscopo et al. (2024) and Akhtar et al. (2023), the CFC samples are considered fresh cheese with a short shelf life. In contrast, the CMC samples are considered mozzarella with a longer shelf life.

The Brazilian Ministry of Agriculture (MAPA) (Brasil, 1997) stipulates that the DM in mozzarella cheese must be at least 35 g/100 g, while MAPA (Brasil, 2004) sets the minimum DM for fresh cheese at 32 g/100 g. The DM values for our CFC (44.84 g/100 g), CMC (63.75 g/100 g), and BMC (55.04 g/100 g) samples are in accordance with the recommendations of MAPA (Brasil, 1997, 2004).

3.4 Lipids

The average lipid values of the cheese samples from the CFC, CMC, and BMC groups were 19.06 g/100 g ± 7.22 g/100 g, 26.24 g/100 g ± 5.41 g/100 g, and 23.68 g/100 g ± 0.91 g/100 g, respectively (Table 1). The CMC samples have significantly higher lipid values ($p < .05$) compared to the CFC and BMC groups. Levak et al. (2023) found a fat content ranging from 30.27 to 33.06% and classified such samples as full-fat cheeses in terms of fat content. Therefore, the CFC, CMC, and BMC cheeses analyzed in the present study do not fall under the full-fat cheese category according to Levak et al. (2023). Additionally, according to MAPA (Brasil, 1997), a minimum lipid content of 35 g/100 g is recommended for mozzarella cheese; thus, the evaluated samples in this study do not meet this requirement. However, Ali et al. (2024) reported a mean lipid content of 13.5 g/100 g in cow's milk cheeses marketed in the United Arab Emirates.

3.5 Fat in dry matter

Table 1 presents the mean values of FDM for the cheese samples from the CFC (43.33 g/100 g ± 14.32 g/100 g), CMC (41.18 g/100 g ± 6.37 g/100 g), and BMC (43.03 g/100 g ± 1.66 g/100 g) groups. Statistical analysis revealed no significant differences ($p > .05$) in FDM values among the evaluated groups.

According to Levak et al. (2023), samples are classified as full-fat cheeses when ≥ 45 % ≤ FDM < 60%. Based on this statement, the CFC, CMC, and BMC samples from our study had values below 45%, and, therefore, they are not considered

full-fat cheese. Similarly, Ali et al. (2024) also obtained FDM values below 45% in bovine milk cheeses, reporting a mean value of 23.2 g/100 g ± 1.3 g/100 g.

Nevertheless, Mapa recommends that fresh cheese and mozzarella should have a minimum FDM of 32 g/100 g and 35 g/100 g, respectively. The FDM values obtained in our study are in accordance with these recommendations (Brasil, 1997, 2004).

3.6 Firmness and ripening characteristics

Table 2 presents the physicochemical characteristics of firmness and ripening of cheese samples marketed for final consumer use. These firmness and ripening characteristics are based on the values obtained for moisture on a fat-free basis (MFFB). The MFFB values for the samples from the CFC, CMC, and BMC groups were 68.15, 49.15, and 58.91%, respectively.

It is important to highlight that the MFFB values directly depend on the physicochemical results of moisture and lipid content. Therefore, the firmness and ripening characteristics were classified according to the classical recommendations of Codex Alimentarius Commission (2024a).

Based on Codex Alimentarius Commission (2024a) principles, Sabatelli et al. (2023) used MFFB values to experimentally categorize cheese samples into soft, semi-soft, semi-hard, and hard/extra-hard groups. In our study, we applied this concept to determine the physicochemical firmness and ripening characteristics of the cheeses. Accordingly, the CFC, CMC, and BMC samples were classified as soft, hard, and firm/semi-hard, respectively. Regarding ripening, the CFC, CMC, and BMC samples were categorized as brine, mold-ripened, and unripened/fresh, respectively.

Therefore, concerning physicochemical firmness characteristics, buffalo milk mozzarella cheese is not as hard as cow milk mozzarella cheese.

3.7 Starch detection

Table 3 shows that no starch was detected in the cheese samples from the CFC, CMC, and BMC groups. MAPA (Brasil, 1997, 2004) does not allow the addition of starch in the production of cow or buffalo milk cheeses. Our findings indicate that the evaluated samples comply with current regulatory standards.

Table 2. Physicochemical characteristics of firmness and ripening according to the Codex Alimentarius Comission (2024a) classification for cheese samples (cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella), based on moisture on a fat-free basisvalue.

Sample	MFFB (%)	Characteristics	
		Firmness	Ripening
CFC	68.15	Macio	Brine
CMC	49.15	Duro	Mold ripened
BMC	58.91	Firme/Semiduro	Unripened/Fresh

CFC: cow's milk fresh cheese; CMC: cow's milk mozzarella; BMC: buffalo's milk mozzarella; MFFB: moisture on a fat-free basis.

Palyvou-Gianna et al. (2021) studied the development of a novel fresh cheese incorporating cured cheese dispersed in a starch-based paste. In that specific case, the authors evaluated the type of cured cheese and the type of starch (corn or rice starch), aiming to develop an innovative product for consumer markets.

3.8 Detection of *Salmonella* spp.

The RFs and RF% of absence of *Salmonella spp.* in the cheese samples from groups CFC (n = 18), CMC (n = 12), and BMC (n = 12) were 0.43 (43%), 0.28 (28%), and 0.29 (29%), respectively. *Salmonella* spp. was not detected in any of the samples analyzed in this study (Table 4).

These results confirm that the CFC, CMC, and BMC samples evaluated in this study comply with the requirements of MAPA (Brasil, 2018), which mandate the absence of *Salmonella spp.* in 25 g of cheese samples, as well as with the guidelines established by Agência Nacional de Vigilância Sanitária (ANVISA) (Brasil, 2022), which also emphasize the necessity of *Salmonella spp.* absence in cheeses sold in Brazil.

Table 3. Absolute frequency, relative frequency, and relative frequency percentage (%) of the qualitative starch test (detected or not detected) in cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella cheese samples.

Sample	Detection	AF	RF	RF (%)
CFC (n = 18)	Not detected	18	0.43	43
	Detected	0	0.00	0
CMC (n = 12)	Not detected	12	0.28	28
	Detected	0	0.00	0
BMC (n = 12)	Not detected	12	0.29	29
	Detected	0	0.00	0
Total		**42**	**1.00**	**100**

CFC: cow's milk fresh cheese; CMC: cow's milk mozzarella; BMC: buffalo's milk mozzarella; AF: absolute frequency; RF: relative frequency.

Table 4. Absolute frequency, relative frequency, and relative frequency percentage (%) of the detection (detected or not detected) of *Salmonella spp.* and *Listeria monocytogenes* in cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella cheese samples.

Cheese	Pathogen	Detection	AF	RF	RF (%)
CFC (n = 18)	*Salmonella* spp.	Detected	0	0.00	0
		Not detected	18	0.43	43
	Listeria spp.	Detected	1	0.02	2
		Not detected	17	0.40	40
CMC (n = 12)	*Salmonella* spp.	Detected	0	0.00	0
		Not detected	12	0.28	28
	Listeria spp.	Detected	0	0.00	0
		Not detected	12	0.29	29
BMC (n = 12)	*Salmonella* spp.	Detected	0	0.00	0
		Not detected	12	0.29	29
	Listeria spp.	Detected	2	0.05	5
		Not detected	10	0.24	24
Total			**42**	**1.00**	**100**

CFC: cow's milk fresh cheese; CMC: cow's milk mozzarella; BMC: buffalo's milk mozzarella; AF: absolute frequency; RF: relative frequency.

Degenhardt et al. (2023) published a study on the microbiological characterization of artisanal cheeses in Brazil and reported that the evaluated samples met the criteria established by Brazilian legislation regarding *Salmonella* spp. detection. The authors also evaluated the survival of *Salmonella* spp. in cheese samples categorized by high, medium, and low moisture content in relation to fat levels. The *Salmonella* spp. challenge test demonstrated survival for up to 28 days, depending on the initial contamination levels.

Albuquerque et al. (2024) stated that many artisanal cheeses are produced from raw milk and undergo a relatively short maturation period. The authors argued that the typical maturation conditions used for raw milk cheeses may be insufficient to reduce levels of certain pathogenic bacteria, including *Salmonella* spp., *L. monocytogenes*, and coagulase-positive *Staphylococcus*, to legally acceptable limits. The same study proposed a standardized protocol for assessing the effectiveness of cheese maturation in preventing contamination by *Salmonella* spp. and other pathogens.

3.9 Detection of *L. monocytogenes*

The RFs and RF% of *L. monocytogenes* detection in cheese samples from the CFC ($n = 18$), CMC ($n = 12$), and BMC ($n = 12$) groups were 0.02 (2%), 0 (0%), and 0.05 (5%), respectively (Table 4).

The detection of *L. monocytogenes* in samples from the CFC and BMC groups is highly concerning from a public health standpoint, as it violates the recommendations of MAPA (Brasil, 2018) and ANVISA (Brasil, 2022), both of which emphasize the mandatory absence of *L. monocytogenes* in 25 g of the product intended for consumption. These findings underscore the need for stricter surveillance and control measures by the competent authorities throughout the cheese production chain in Brazil (Brasil, 2018, 2022).

Sakaridis et al. (2022) stressed the importance of monitoring the microbiological quality of cheeses produced from unpasteurized cow's milk. Microbiological assessments enable the evaluation of cheese safety and the tracking of microbial dynamics during ripening and storage. In their study, although *L. monocytogenes* was not isolated, *Salmonella spp.* and *Clostridium perfringens* were detected in two cheese production trials. The authors concluded that the use of high-quality milk produced under hygienic conditions, the implementation of good manufacturing practices, and a controlled environment during the maturation period are critical prerequisites for producing microbiologically safe cheese products.

3.10 Count of coagulase-positive *Staphylococcus*

Table 5 presents the data on the RF and percentages of *Staphylococcus* coagulase-positive counts exceeding 10^2 CFU/g in cheese samples from the CFC ($n = 18$), CMC ($n = 12$), and BMC ($n = 12$) groups, which were 0.02 (2%), 0 (0%), and 0 (0%), respectively.

The presence of coagulase-positive *Staphylococcus* in one CFC sample raises a public health concern. This sample does not comply with the microbiological criteria established by MAPA (Brasil, 2018) and ANVISA (Brasil, 2022), both of which require levels below 10^2 CFU/g in cheese. These findings are consistent with those of Aguiar et al. (2024), who reported *Staphylococcus aureus* as one of the main pathogenic microorganisms detected in cheeses produced from raw milk. Some *S. aureus* strains are enterotoxigenic and capable of producing toxins that cause staphylococcal food poisoning when present in contaminated cheeses. The authors further emphasized that the occurrence of this pathogen in the dairy production chain highlights the importance of quality management practices aimed at preventing contamination and the spread of this microorganism (Aguiar et al., 2024; Albuquerque et al., 2024).

Table 5. Absolute frequency, relative frequency, and relative frequency percentage (%) of coagulase-positive *Staphylococcus* counts (CFU/g) in cow's milk fresh cheese, cow's milk mozzarella, and buffalo's milk mozzarella cheese samples.

Cheese	N	CFU/g[1]	AF	RF	RF (%)
CFC	18	$> 10^2$	1	0.02	2
		$\leq 10^2$	17	0.40	40
CMC	12	$> 10^2$	0	0.00	0
		$\leq 10^2$	12	0.29	29
BMC	12	$> 10^2$	0	0.00	0
		$\leq 10^2$	12	0.29	29
Total			**42**	**1.00**	**100**

CFC: cow's milk fresh cheese; CMC: cow's milk mozzarella; BMC: buffalo's milk mozzarella; N: number of samples evaluated; AF: absolute frequency; RF: relative frequency.

4 CONCLUSIONS

Based on the findings of this study, it can be concluded that: (a) The evaluation of physicochemical properties related to cheese firmness and ripening was feasible through the analysis of moisture and lipid contents; (b) the firmness classification for the CFC, CMC, and BMC cheese groups was determined as soft, hard, and firm/semi-hard, respectively; (c) the ripening characteristics identified for the CFC, CMC, and BMC cheese groups were brine, mold-ripened, and unripened/fresh, respectively; (d) the microbiological profiles of cow's milk (CFC) and buffalo's milk (BMC) cheeses purchased by consumers from retail establishments were highly concerning from a public health standpoint, due to the detection of *L. monocytogenes* and coagulase-positive *Staphylococcus*; and (e) These results underscore the urgent need for Brazilian health authorities to implement more frequent and effective sanitary surveillance in commercial establishments to prevent contamination by these and other foodborne pathogens that pose serious risks to public health.

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REFERENCES

Aguiar, R. A. C., Ferreira, F. A., Cieza, M. Y. R., Silva, N. C. C., Miotto, M., Carvalho, M. M., Bazzo, B. R., Botelho, L. A. B., Dias, R. S., & Lindner, J. D. (2024). Staphylococcus aureus Isolated From Traditional Artisanal Raw Milk Cheese from Southern Brazil: Diversity, Virulence, and Antimicrobial Resistance Profile. *Journal of Food Protection*, *87*(6), Article 100285. https://doi.org/10.1016/j.jfp.2024.100285

Ah, J., & Tagalpallewar, G.P. (2017). Functional properties of Mozzarella cheese for its end use application. *Journal of Food Science and Technology*, *54*(12), 3766–3778. https://doi.org/10.1007/s13197-017-2886-z

Akhtar, A., Nasim, I., Din, M. S., Araki, T., & Khalid, N. (2023). Effects of different fat replacers on functional and rheological properties of low-fat mozzarella cheeses: A review. *Trends in Food Science & Technology*, *139*, Article 104136. https://doi.org/10.1016/j.tifs.2023.104136

Albuquerque, T. M. N. C., Campos, G. Z., d'Ovidio, L., Pinto, U. M., Sobral, P. J. A., & Galvão, J. A. (2024). Unveiling Safety Concerns in Brazilian Artisanal Cheeses: A Call for Enhanced Ripening Protocols and Microbiological Assessments. *Foods*, *13*(11), Article 1644. https://doi.org/10.3390/foods13111644

Ali, A. H., Abu-Jdayil, B., Bamigbade, G., Kamal-Eldin, A., Hamed, F., Huppertz, T., Liu, S.-Q., & Ayyash, M. (2024). Properties of low-fat Cheddar cheese prepared from bovine–camel milk blends: Chemical composition, microstructure, rheology, and volatile compounds. *Journal of Dairy Science*, *107*(5), 2706–2720. https://doi.org/10.3168/jds.2023-23795

Bansal, V., & Veena, N. (2024). Understanding the role of pH in cheese manufacturing: general aspects of cheese quality and safety. *Journal of Food Science Technology*, *61*(1), 16–26. https://doi.org/10.1007/s13197-022-05631-w

Berta, M., Muskens, E., Schuster, E., & Stading, M. (2016). Rheology of natural and imitation mozzarella cheese at conditions relevant to pizza baking. *International Dairy Journal*, *57*, 34–38. https://doi.org/10.1016/j.idairyj.2016.02.038

Biegalski, J., & Cais-Sokolińska, D. (2024). Innovative Covering Liquids Stabilising Water–Fat Leachate from Fresh Mozzarella Cheese Used as Pizza Topping. *Foods*, *13*(4), Article 581. https://doi.org/10.3390/foods13040581

Brasil. (1997). *Portaria MAA Nº 364, de 04 de setembro de 1997*. Aprova o Regulamento Técnico para fixação de Identidade e Qualidade de Queijo Mozzarela (Muzzarela ou Mussarela). Diário Oficial da União. https://wikisda.agricultura.gov.br/dipoa_baselegal/port_364-1997_rt_queijo_mussarela.pdf

Brasil. (2004). *Instrução Normativa Nº 4, de 1 de março de 2004*. Fixa o Regulamento Técnico para fixação de Identidade e Qualidade do Queijo Minas Frescal. Diário Oficial da União. https://www.cidasc.sc.gov.br/inspecao/files/2020/09/IN-MAPA-n%C2%BA--4-de-01-de-mar%C3%A7o-de-2004.pdf

Brasil (2018). *Portaria Nº 837, de 18 de junho de 2018*. Altera os Critérios Microbiológicos para Qualidade de Queijo Mozzarela (Muzzarela ou Mussarela). Diário Oficial da União. https://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/26572301/do1-2018-06-20-portaria-n-837-de-18-de-junho-de-2018-26572271

Brasil. (2022). *Instrução Normativa Nº 161, de 1º de julho de 2022. Estabelece os padrões microbiológicos dos alimentos*. Diário Oficial da União. https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-161-de-1-de-julho-de-2022-413366880

Caldeira, L. A., Valente, G. L. C., Barbosa, C. D., Braga, D. E., Monção, F. P., Fonseca, L. M., Souza, M. R., & Gloria, M. B. A. (2024). Profile of lactic acid bacteria (MALDI-TOF-MS) and physico-chemical and microbiological characteristics of the raw milk and fresh artisanal cheese from Serra Geral, Minas Gerais, Brazil. *Food Research International*, *176*, Article 113831. https://doi.org/10.1016/j.foodres.2023.113831

Codex Alimentarius Commission. (2022). *Group standard for unripened cheese including fresh cheese*: *CXS 221-2001*. FAO & WHO. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B221-2001%252FCXS_221e.pdf

Codex Alimentarius Commission. (2024a). *General standard for cheese: CXS 283-1978*. FAO & WHO. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B283-1978%252FCXS_283e.pdf

Codex Alimentarius Commission. (2024b). *Recommended methods of analysis and sampling: CXS 234-1999*. FAO & WHO. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B234-1999%252FCXS_234e.pdf

Dai, S., Jiang, F., Shah, N. P., & Corke, H. (2019). Functional and pizza bake properties of Mozzarella cheese made with konjac glucomannan as a fat replacer. *Food Hydrocolloids*, *92*, 125–134. https://doi.org/10.1016/j.foodhyd.2019.01.045

Degenhardt, R., Carvalho, M. M., Voidaleski, M. F., Daros, G. F., Guaragni, A., Pereira, G. V. M., & Lindner, J. D. (2023). Brazilian artisanal Colonial cheese: characterization, microbiological safety, and survival of Salmonella enterica serovar Enteritidis during ripening. *Brazilian Journal of Microbiology*, *54*(3), 2129–2135. https://doi.org/10.1007/s42770-023-01022-1

Faccia, M., Angiolillo, L., Mastromatteo, M., Conte, A., Del Nobile, M. A. (2013). The effect of incorporating calcium lactate in the saline solution on improving the shelf life of Fiordilatte cheese. *International Journal of Dairy Technology*, *66*(3), 373–381. https://doi.org/10.1111/1471-0307.12046

Faccia, M., Gambacorta, G., Natrella, G., & Caponio, F. (2019). Shelf life extension of Italian mozzarella by use of calcium lactate buffered brine. *Food Control*, *100*, 287–291. https://doi.org/10.1016/j.foodcont.2019.02.002

Gobbetti, M., & Cagno, R. (2017). Extra-hard Varieties. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.) *Cheese: Chemistry, Physics and Microbiology* (4th ed., pp. 809–828). Academic Press. https://doi.org/10.1016/B978-0-12-417012-4.00032-6

Guidone, A., Zotta, T., Matera, A., Ricciardi, A., De Filippis, F., Ercolini, D., & Parente, E. (2016). The microbiota of high-moisture mozzarella cheese produced with different acidification methods. *International Journal of Food Microbiology*, 216, 9–17. https://doi.org/10.1016/j.ijfoodmicro.2015.09.002

International Organization for Standardization & International Dairy Federation. (2004). *ISO 5534, IDF 4:2004: Cheese and processed cheese – Determination of the total solids content (Reference method)*. ISO. https://www.iso.org/standard/35249.html

International Organization for Standardization & International Dairy Federation. (2008). *ISO 3432, IDF 221: Cheese: Determination of fat content: Butyrometer for Van Gulik method*. ISO & IDF. https://cdn.standards.iteh.ai/samples/46335/3353189af8fa4308a25658ee72ef6c04/ISO-3432-2008.pdf

Instituto Adolfo Lutz. (2008). *Métodos físico-químicos para análise de alimentos* (4th ed.). IAL.

Johnson, A., & Sommer, D. (2020). Essential principles of cheese making (part 2): measuring and controlling acidity and moisture. *Dairy Pipeline*, *31*(4), 1–12. https://www.cdr.wisc.edu/assets/pipeline-pdfs/pipeline_2020_vol31_04.pdf

Levak, S., Kalit, S., Špehar, I. D., Radeljević, B., Rako, A., & Kalit, M. T. (2023). The influence of ripening of semi-hard goat cheese in oil on its physicochemical composition and sensory Properties. *Journal of Dairy Science*, *106*(12), 8493–8503. https://doi.org/10.3168/jds.2023-23533

Montgomery, D. C. (2013). *Design and analysis of experiments* (8th ed). Wiley.

Palyvou-Gianna, E., Vilela, T. P., Gomes, A. M., & Ferreira, J. P. (2021). A Starch-Milk Paste Enables the Incorporation of Ripened Cheese in Novel Fresh Cheese. *Food Technology and Biotechnol*ogy, *59*(4), 507–518. https://doi.org/10.17113/ftb.59.04.21.7262

Piscopo, A., Mincione, A., Summo, C., Silletti, R., Giacondino, C., Rocco, I., & Pasqualone, A. (2024). Influence of the Mozzarella Type on Chemical and Sensory Properties of "Pizza Margherita". *Foods*, *13*(2), Article 209. https://doi.org/10.3390/foods13020209

Pui, C. F., Wong, W. C., Chai, L. C., Nillian, E., Ghazali, F. M., Cheah, Y. K., Nakaguchi, Y., Nishibuchi, M., & Radu, S. (2011). Simultaneous detection of Salmonella spp., Salmonella Typhi and Salmonella Typhimurium in sliced fruits using multiplex PCR. *Food Control*, *22*(2), 337–342. https://doi.org/10.1016/j.foodcont.2010.05.021

Ricciardi, A., Guidone, A., Zotta, T., Matera, A., Claps, S., & Parente, E. (2015). Evolution of microbial counts and chemical and physico-chemical parameters in high-moisture Mozzarella cheese during refrigerated storage. *LWT – Food Science and Technology*, *63*(2), 821–827. https://doi.org/10.1016/j.lwt.2015.04.008

Sabatelli, S., Gambi, L., Baiguera, C., Paterlini, F., Mami, F. L., Uboldi, L., Daminelli, P., & Biancardi, A. (2023). Assessment of aflatoxin M1 enrichment factor in cheese produced with naturally contaminated milk. *Italian Journal of Food Safety*, *12*(2), Article 11123. https://doi.org/10.4081/ijfs.2023.11123

Salfinger, Y., & Tortorello, M. L. (Eds.). (2015). *Compendium of Methods for the Microbiological Examination of Foods* (5th ed.). American Public Health Association.

Sakaridis, I., Psomas, E., Karatzia, M.-A., & Samouris, G. (2022). Hygiene and Safety of Hard Cheese Made from Raw Cows' Milk. *Veterinary Sciences*, *9*(10), Article 569. https://doi.org/10.3390/vetsci9100569

Silva, N., Junqueira, V. C. A., Silveira, N. F. A., Taniwaki, M. H., Gomes, R. A. R., & Okazaki, M. M. (2017). *Manual de Métodos de Análise Microbiológica de Alimentos e Água* (5ª ed.). Blucher.

Sutariya, S. G., Metzger, L. E., & Meletharayil, G. H. (2022). An approach to improve the baking properties and determine the onset of browning in fat-free mozzarella cheese. *Journal of Dairy Science*, *105*(3), 2153–2165. https://doi.org/10.3168/jds.2021-21188

Swamy, S. C., Barnhart, H. M., Lee, M. D., & Dreesen, D. W. (1996). Virulence determinants invA and spvC in salmonellae isolated from poultry products, wastewater, and human sources. *Applied and Environmental Microbiology*, *62*(10), 3768–3771. https://doi.org/10.1128/aem.62.10.3768-3771.1996

Trmčić, A., Ralyea, R., Meunier-Goddik, L., Donnelly, C., Glass, K., D'Amico, D., Meredith, E., Kehler, M., Tranchina, N., McCue, C., & Wiedmann, M. (2017). Consensus categorization of cheese based on water activity and pH—A rational approach to systemizing cheese diversity. *Journal of Dairy Science*, *100*(1), 841–847. https://doi.org/10.3168/jds.2016-11621

Von Oesen, T., Schrader, K., Clawin-Rädecker, I., Martin, D., Treblin, M., Hoffmann, W., Bode, K., Zink, R., Rohn, S., & Fritsche, J. (2024). Physicochemical and Sensory Characterization of Whey Protein-Enriched Semihard Cheese. *Journal of Agricultural and Food Chemistry*, *72*(11), 5898–5911. https://doi.org/10.1021/acs.jafc.3c08731