

## Microbiological evaluation of industrialized and artisanal Minas fresh cheese commercialized in the Federal District, Brazil

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### Abstract

The aim of this study was to evaluate the microbiological quality of Minas fresh cheese commercialized in Federal District, Brazil. For this, 20 samples of industrialized cheeses were collected in supermarkets and 20 samples of artisanal cheeses were collected in public food markets. The analyzes performed were total count of bacteria, determination of coliforms, count of *S. aureus* and identification of *Salmonella* spp. (*InvA* gene) and *S. aureus* (*SeC* gene). The results showed that 26 samples (65%) were unfit for consumption according to Brazilian legislation. For industrialized cheeses, 6 samples (15%) of were unfit for consumption (3 samples for excess of thermotolerant coliforms and 3 samples for excess of *S. aureus*). For artisanal cheeses, all 20 samples (50%) were unfit for consumption due to the high *S. aureus* counts, including 6 samples that had excess thermotolerant coliforms and 1 sample that was contaminated with *Salmonella*. Comparing the results, industrialized cheeses showed better microbiological quality than artisanal cheeses. Thus, artisanal Minas fresh cheeses commercialized in the public food markets of the Federal District showed the presence of bacterial hazards, and there is an evident need for good hygiene practices in the entire production chain to ensure consumer food safety.

**Keywords:** soft cheese; food hygiene; foodborne pathogens; *S. aureus*.

**Practical Application:** Comparison of the microbiological quality of industrialized and artisanal Minas fresh cheeses.

## 1 Introduction

Cheeses are a source of high-quality proteins, lipids, vitamins (A, B2 and B12) and minerals (calcium and phosphorus). The cheese matrix is a complex assembly of nutrients where protein, especially casein, hydrated with water forms networks (within the cheese matrix) in which fat globules, minerals, bacteria, lactic acid, soluble salts and peptides are all interspersed (Feeney et al., 2021).

Minas fresh cheese is a typical Brazilian cheese, being widely produced and consumed by Brazilians due to its low cost and high acceptance by consumers (Saleh et al., 2019; Souza et al., 2017). This soft white cheese is made from pasteurized cow's milk and Brazilian regulation does not permit the production of Minas fresh cheeses with raw milk, as they do not undergo a ripening process (Brasil, 2004). Because it is a nonripened cheese and has high moisture content (> 55%), Minas fresh cheese must be stored in a refrigerated temperature (not exceeding 8 °C) and has a short shelf life (on average 15 days) (Brasil, 2004; Souza et al., 2017).

The industrialized Minas Fresh cheese is made from pasteurized milk, in a dairy industry, accompanied by the seal of the Federal Inspection Service (Dias et al., 2016). Despite the Brazilian regulation, artisanal Minas fresh cheese, commonly made with raw milk without undergoing quality control, is not inspected, and is regularly sold at Brazilian street markets and

public food markets, presenting unsatisfactory hygienic-sanitary conditions during its production and conservation. It is common for these cheeses to be exhibited without refrigeration (Okura & Marin, 2014; Woluheck et al., 2012).

In Brazil, a large variety of artisanal cheeses exist, and they are appreciated dairy products due to their economic significance and cultural and social values. These artisanal cheeses can be produced using raw milk, and their commercialization is allowed by Brazilian regulation if scientific and technical reports confirm that the ripening time guarantees the food safety of the products (Kamimura et al., 2019; Margalho et al., 2021).

Brazilian artisanal Minas fresh cheeses have shown problems related to microbiological quality (Dias et al., 2016; Garcia et al., 2016). The concern with the quality of artisanal cheese is the health risk resulting from the use of raw milk and inadequate food safety practices during production. These factors, together with inadequate storage and sale practices in the markets, the contamination and growth of pathogens (Gould et al., 2014).

Thus, pathogenic bacteria that cause foodborne diseases such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. may be present in poor quality cheeses (Freitas et al., 2013; Okura & Marin, 2014; Guzman-Hernandez et al., 2016; Viçosa et al., 2019). Then, the aim of the present study was to evaluate the

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microbiological quality of industrial and artisanal Minas fresh cheeses commercialized in the Federal District, Brazil.

## 2 Materials and methods

### 2.1 Sample collection, microbiological and molecular analysis

A total of 40 Minas fresh cheese samples were analyzed (20 industrialized cheeses and 20 artisanal cheeses), which were collected at intervals between March 2018 and February 2020. The 20 samples of industrialized Minas fresh cheeses from different brands were collected in different supermarkets in the Federal District. The industrialized samples had a label containing the mandatory information's, a seal of the Federal Inspection Service, were in their original packaging and within the expiration date. The 20 samples of artisanal Minas fresh cheeses were collected in different public food markets in the Federal District. Samples were transported under refrigeration (4-6 °C) in thermal boxes containing ice packs and were tested immediately after arriving at the laboratory. All samples were analyzed in three repetitions, that is, three aliquots were removed from each package and the results were expressed as mean and standard deviation.

Twenty-five grams of each sample were aseptically homogenized in 225 mL of 0.1% (w/v) peptoned water, thus obtaining the first dilution ( $10^{-1}$ ). The other serial decimal dilutions (up to  $10^{-3}$ ) were obtained from this first dilution. For the total count of mesophilic and psychrotrophic bacteria, each dilution was plated on Plate Count agar (Acumedia, USA) and incubated at 37 °C for 24 h for mesophilic bacteria and at 7 °C  $\pm$  1 °C for 7 days for psychrotrophic bacteria.

Total and thermotolerant coliforms were determined by the Most Probable Number. Each dilution of the samples was inoculated in Lauril Sulfato Triptose broth (HiMedia, USA). After 24 h of incubation at 37 °C, an aliquot from positive cultures (determined by turbidity and gas production) was transferred, simultaneously, to tubes containing Brilliant Green Bile Lactose broth (Kasvi, Brazil) (for the confirmation of total coliforms) and Escherichia coli broth (Kasvi, Brazil) (for the confirmation of thermotolerant coliforms). The tubes were incubated at 37 °C for 24 h for total coliforms and in a water bath at 45 °C for 24 h for thermotolerant coliforms. Tubes with turbidity and gas production were considered positive for both tests.

For the research of *Salmonella* spp., the  $10^{-1}$  dilution of the samples was incubated at 37 °C for 24 h. After incubation, aliquots were transferred to tubes containing 10 mL of tetrathionate broth. A loop-full of enriched broth was streaked onto the Xylose-Lysine-Deoxycholate agar (XLD) (HiMedia,

USA) and Salmonella-Shigella agar (SS) (Kasvi, Brazil) and incubated at 37 °C for 24 h. Presumptive *Salmonella* colonies in XLD and SS agars were confirmed biochemically using triple sugar iron agar (TSI) (Kasvi, Brazil) and lysine iron agar (LIA) (HiMedia, USA) slants, incubated at 37 °C for 24 h. Subsequently, presumptive *Salmonella* isolates were subjected to molecular identification using the PCR technique.

*Staphylococcus aureus* counts were determined by plating each dilution onto the Mannitol Salt agar (Kasvi, Brazil), with incubation at 37 °C for 48 h. The presumptive *S. aureus* isolates were re-isolated in tubes of Mannitol Salt agar to obtain pure cultures and subjected to Gram stain method to confirm Gram-positive cocci. The isolates of *S. aureus* were confirmed by molecular identification using the PCR technique.

Table 1 presents the primer for the enterotoxin C gene (*SeC*) used for identification of *S. aureus* and the primer for the invasion A gene (*InvA*) used for identification of *Salmonella* spp.

For DNA extraction, the isolates were cultivated overnight in Mueller-Hinton broth and had their DNA extracted employing the NucleoSpin Food kit (Macherey-Nagel, Düren, Germany), as per the manufacturer's instructions. The DNA concentrations were then determined using a NanoDrop 2000 (Thermo Scientific, Pittsburgh, USA), and its integrity was confirmed by agarose gel electrophoresis. PCR was performed in a 25  $\mu$ l final volume reaction mixture containing: 2.5  $\mu$ l of PCR buffer; 0.7  $\mu$ l of MgCl<sub>2</sub>; 1.5  $\mu$ l of dNTP (2,5 mM); 0.5  $\mu$ l of Taq DNA polymerase; 1.5  $\mu$ l of each primer forward and reverse; and 18.3  $\mu$ l of Milli-Q water. These thermal cycling reactions were conducted with Techne TC-512 thermal cyler (Bibby Scientific Inc., USA), and each PCR run included negative and reagent controls. The reagent control consisted of all PCR components except for the DNA template. The amplified DNA was separated by electrophoresis at 100 V for 50 min in 1.5% (w/v) agarose gel, stained with ethidium bromide and visualized under UV light. A 100 bp DNA ladder was used as a molecular weight marker.

## 3 Results and discussion

Tables 2 and 3 show the results of the microbiological analyzes of the cheeses analyzed in this study.

For Minas Fresh cheeses, Brazilian legislation (Brasil, 2019) does not establish limits for mesophilic and psychrotrophic bacteria counts. The International Commission on Microbiological Specifications for Foods (ICMSF, 1986) recommends a maximum count of 7.0 log CFU/g of total bacteria. Results showed that of the 40 samples analyzed in this study, 3 samples (4.0%) of industrialized cheeses and 7 samples (17.5%) of artisanal cheeses presented values above 7.0 log CFU/g for mesophilic bacteria count.

**Table 1.** Sequence of primers and size of products amplified in PCR for identification of *SeC* and *invA* genes.

Primer	Sequence 5' - 3'	Amplified product	Bacteria
<i>seC</i> foward	TTTTACACCCAACGTATTAGCAGA	401 bp	<i>S. aureus</i>
<i>seC</i> reverse	TCCCATTATCAAAGTGGTTTCC		
<i>invA</i> foward	CTCGCCTTTGCTGGTTTTAG	298 bp	<i>Salmonella</i> spp.
<i>invA</i> reverse	CTCGCCTTTGCTGGTTTTAG		

**Table 2.** Microbiological results of industrialized Minas fresh cheese samples.

Samples	Mesophilic bacteria (log CFU/g)	Psychrotrophic bacteria (log CFU/g)	Total coliforms (log MPN/g)	Thermotolerant coliforms (log MPN/g)	<i>Salmonella</i> spp.	<i>S. aureus</i> (log CFU/g)
1	5.6 ± 0.60	5.9 ± 0.21	3.1 ± 0.00	<b>3.1 ± 0.00</b>	Negative	ND
2	4.7 ± 0.36	4.0 ± 0.24	0.8 ± 0.26	0.5 ± 0.00	Negative	ND
3	5.1 ± 0.07	6.6 ± 0.13	3.1 ± 0.00	2.1 ± 0.86	Negative	<b>3.6 ± 0.25</b>
4	6.8 ± 0.37	7.5 ± 0.23	3.1 ± 0.00	2.5 ± 0.58	Negative	2.6 ± 0.28
5	6.8 ± 1.54	7.2 ± 0.34	1.1 ± 0.34	0.5 ± 0.00	Negative	ND
6	7.1 ± 0.22	7.9 ± 0.27	3.1 ± 0.00	0.5 ± 0.00	Negative	2.5 ± 0.70
7	6.1 ± 1.16	6.9 ± 0.16	1.0 ± 0.19	0.6 ± 0.18	Negative	ND
8	5.3 ± 0.18	6.7 ± 0.07	0.5 ± 0.05	0.5 ± 0.00	Negative	ND
9	7.3 ± 0.47	7.8 ± 0.10	0.5 ± 0.00	0.5 ± 0.00	Negative	ND
10	5.3 ± 0.55	7.5 ± 0.25	1.9 ± 0.67	0.7 ± 0.23	Negative	ND
11	5.3 ± 1.28	7.1 ± 0.15	3.1 ± 0.00	1.8 ± 1.12	Negative	ND
12	6.9 ± 0.03	4.1 ± 0.21	3.1 ± 0.00	2.2 ± 0.43	Negative	2.1 ± 0.89
13	6.6 ± 0.06	6.1 ± 0.17	3.1 ± 0.00	2.8 ± 0.33	Negative	<b>3.8 ± 0.48</b>
14	7.1 ± 0.40	5.9 ± 0.21	3.1 ± 0.00	<b>3.1 ± 0.00</b>	Negative	ND
15	5.5 ± 0.49	3.3 ± 0.42	3.1 ± 0.00	<b>3.1 ± 0.00</b>	Negative	ND
16	7.8 ± 0.16	6.7 ± 0.05	3.1 ± 0.00	0.5 ± 0.00	Negative	<b>3.0 ± 0.00</b>
17	6.3 ± 0.09	6.7 ± 0.01	3.1 ± 0.00	1.0 ± 1.34	Negative	ND
18	6.6 ± 0.12	7.4 ± 0.30	2.6 ± 0.38	ND	Negative	ND
19	5.5 ± 0.71	4.5 ± 0.12	1.9 ± 0.96	ND	Negative	ND
20	6.9 ± 0.55	6.3 ± 0.44	3.1 ± 0.00	2.9 ± 0.34	Negative	ND

Results are reported as means ± standard deviation of triplicate measurements; ND: not detected.

Studies have reported high counts of mesophilic bacteria in both artisanal and industrialized Minas fresh cheeses from different regions of Brazil. Funck et al. (2015) reported high counts of mesophilic bacteria (7.1-7.3 log CFU/g) in artisanal cheeses from the Northwestern region of Rio Grande do Sul. Garcia et al. (2016) also reported high counts of mesophilic bacteria in 78% of artisanal cheese samples, produced and marketed in Montes Claros region, Minas Gerais. And Woluheck et al. (2012) presented results of 7,5 to 9,0 log CFU/g for the mesophilic aerobic counts in industrialized brands of Minas Fresh cheeses marketed in the city of Curitiba (Paraná). The high count of mesophilic bacteria in dairy products indicates poor hygiene of raw materials and deficiencies in cheese processing and storage (Funck et al., 2015; Woluheck et al., 2012).

For psychrotrophic bacteria, 7 samples (17.5%) of industrialized cheeses and 5 samples (12.5%) of artisanal cheeses presented values above 7.0 log CFU/g. The control of psychrotrophic microorganisms is of great importance for the dairy industry, as this group of microorganisms develops in refrigeration temperatures. High counts of psychrotrophic bacteria are responsible for the deterioration of cheeses, as they produce enzymes that hydrolyze proteins and lipids, reducing their shelf life. In addition, the high count of this group of microorganisms can be attributed to storage at inappropriate temperatures (Oliveira et al., 2015; Santana et al., 2020).

Brazilian legislation (Brasil, 2019) does not establish limits for total coliforms in Minas Fresh cheeses, however, the legislation which approves the Technical Regulations for the Identity and Quality of Dairy Products (Brasil, 1996), establishes a limit of 3.0 log MPN/g. Of the 40 samples analyzed in this study, 12

industrialized cheeses (30%) and 12 artisanal cheeses (30%) had an enumeration of total coliforms higher than 3.0 log MPN/g.

Dias et al. (2016) observed that all samples of industrialized cheeses (5 samples) and artisanal cheeses (5 samples) marketed in the South of Goiás showed total coliforms values higher than 3.0 log MPN/g. Souza et al. (2017) reported an enumeration of total coliforms higher than 3.0 log MPN/g in 77,4% of Minas fresh cheeses samples marketed in Minas Gerais (Zona da Mata Mineira). These microorganisms are important indicators of the hygiene conditions of the production processes, as they are generally environmental contaminants and are easily destroyed by the pasteurization process of milk (Dias et al., 2016; Garcia et al., 2016).

The limit for thermotolerant coliforms in Minas Fresh cheeses is 3.0 log NMP/g (Brasil, 2019). The results showed that 3 industrialized cheeses (7.5%) and 6 artisanal cheeses (15%) presented values above 3.0 log NMP/g, and therefore were unfit for consumption. Thermotolerant coliforms is used as an indicator of direct or indirect fecal contamination of foods (*E. coli* represents 90% of the bacteria in this group), and therefore the possible presence of enteric pathogens. In cheese its presence indicates poor hygiene conditions during processing or post-processing contamination (Okura & Marin, 2014).

Several studies have reported a high frequency of Minas fresh cheeses unfit for consumption due to high number of thermotolerant coliforms. Apolinário et al. (2014) reported that of the 31 samples of industrialized Minas fresh cheeses produced in Minas Gerais, 17 samples (54.8%) showed values above the limit allowed for thermotolerant coliforms. Saleh et al.

**Table 3.** Microbiological results of artisanal Minas fresh cheese samples.

Samples	Mesophilic bacteria (log CFU/g)	Psychrotrophic bacteria (log CFU/g)	Total coliforms (log MPN/g)	Thermotolerant coliforms (log MPN/g)	<i>Salmonella</i> spp.	<i>S. aureus</i> (log CFU/g)
1	7.8 ± 0.08	7.9 ± 0.37	2.5 ± 0.48	0.6 ± 0.15	Negative	5.3 ± 0.02
2	7.5 ± 0.34	7.3 ± 0.01	1.4 ± 0.15	1.1 ± 0.46	Negative	4.9 ± 0.07
3	7.0 ± 0.05	6.4 ± 0.05	0.6 ± 0.14	0.6 ± 0.00	Negative	6.3 ± 0.02
4	6.1 ± 0.04	5.6 ± 0.16	1.1 ± 0.13	0.7 ± 0.14	Negative	3.6 ± 0.18
5	4.9 ± 0.30	5.8 ± 0.17	1.1 ± 0.00	0.5 ± 0.00	Negative	3.0 ± 0.04
6	7.5 ± 0.09	7.6 ± 0.06	0.6 ± 0.18	ND	Negative	5.8 ± 0.49
7	5.6 ± 0.46	7.2 ± 0.04	3.1 ± 0.01	3.1 ± 0.01	Positive	2.9 ± 0.17
8	6.4 ± 0.04	7.5 ± 0.09	2.0 ± 0.93	0.9 ± 0.48	Negative	4.1 ± 0.08
9	8.0 ± 0.14	5.2 ± 0.04	3.1 ± 0.01	1.1 ± 0.29	Negative	3.9 ± 0.01
10	6.2 ± 0.38	5.9 ± 0.50	3.1 ± 0.00	2.8 ± 0.38	Negative	4.9 ± 0.68
11	5.8 ± 0.07	6.0 ± 0.03	3.1 ± 0.00	2.5 ± 0.86	Negative	5.1 ± 0.12
12	6.5 ± 0.60	6.8 ± 0.25	3.1 ± 0.00	1.3 ± 0.07	Negative	3.8 ± 0.86
13	7.1 ± 0.05	5.7 ± 0.13	3.1 ± 0.00	3.1 ± 0.00	Negative	4.8 ± 0.15
14	6.2 ± 0.26	5.0 ± 0.15	3.1 ± 0.00	3.1 ± 0.00	Negative	5.0 ± 0.01
15	6.3 ± 0.39	4.3 ± 0.43	3.1 ± 0.00	1.9 ± 0.05	Negative	4.8 ± 0.13
16	7.2 ± 0.53	4.1 ± 0.20	3.1 ± 0.00	3.1 ± 0.00	Negative	4.9 ± 0.21
17	4.5 ± 0.15	3.1 ± 0.09	3.1 ± 0.00	3.1 ± 0.00	Negative	3.6 ± 0.49
18	6.9 ± 0.07	5.0 ± 0.75	3.1 ± 0.00	2.5 ± 0.59	Negative	3.4 ± 0.17
19	6.1 ± 0.09	6.1 ± 0.06	3.1 ± 0.00	3.1 ± 0.00	Negative	4.0 ± 0.90
20	5.2 ± 0.26	4.1 ± 0.21	2.7 ± 0.00	2.5 ± 0.16	Negative	4.0 ± 0.21

Results are reported as means ± standard deviation of triplicate measurements; ND: not detected.

(2019), reported that of the 19 industrialized samples sold in supermarkets in the city of Duque de Caxias (Rio de Janeiro), 10 samples (52.6%) presented thermotolerant coliforms counts above 3.0 log NMP/g. And Garcia et al. (2016), reported that 17 samples (94.4%) of artisanal cheeses produced and marketed in Montes Claros (Minas Gerais), were unfit for consumption because they exceed limits allowed for thermotolerant coliforms.

According to Perrin et al. (2015), pathogenic strains such as Shiga-toxin producing *Escherichia coli* (STEC) can survive during cheese processing, especially in soft cheeses produced with raw milk. The authors reported that impact of the preharvest interventions (vaccination, use of probiotics, antimicrobials, milk farm sorting) reduced 76-98% the risk of cow carrying STEC and of the STEC concentration in feces. Thus, preharvest treatments need to be applied to reduce the microbial risk in soft cheeses made with raw milk.

Storage temperature is another factor that affects the development of *E. coli* in cheeses. Kim et al. (2014) developed kinetic models to predict the growth of pathogenic *E. coli* in cheeses during storage at changing temperatures. At 4 °C, *E. coli* growth was not observed in any cheeses, however, *E. coli* growth was observed at 10 °C in counts of 6.3 to 8.6 log CFU/g after 13 days of storage. Lima et al. (2015) evaluated the viability of pathogenic *E. coli* in Minas fresh cheeses stored at 8 °C. There was an increase in the counts of *E. coli* during storage at 8 °C. The cheese processing did not eliminate microorganisms and the storage temperature recommended by Brazilian legislation did not eliminate bacterial growth, keeping the risk for the population. These results reinforce the attention to the quality of the milk to ensure the safety of final products.

Brazilian legislation (Brasil, 2019) does not allow the presence of *Salmonella* spp. in cheeses. The results showed that only 1 artisanal cheese (2,5%) presented *Salmonella* spp., and this cheese was already unfit for consumption due to the excess of thermotolerant coliforms. According to Chávez-Martínez et al. (2019), the pasteurization of milk is one of the most effective measures to prevent microbial contamination and the presence of *Salmonella* spp. in cheeses is mainly a result of insufficient pasteurization. Other studies have reported the presence of *Salmonella* in artisanal cheeses or in cheeses made from raw milk. Chávez-Martínez et al. (2019) found *Salmonella* spp. in 8.9% of cheese samples commercialized in Mexico and 5 samples were fresh cheeses made with raw milk. Martínez et al. (2020) reported the presence of *Salmonella* spp. in 17.9% of artisanal fresh cheeses commercialized in Cuba. And Guzman-Hernandez et al. (2016) reported that of the 52 samples of Mexican unpasteurized fresh cheeses, 11 samples (21%) were contaminated with *Salmonella* spp.

The limit for *S. aureus* in Minas fresh cheeses is 3.0 log NMP/g (Brasil, 2019). The results showed that of the 40 samples analyzed, 3 industrialized cheeses (7.5%) and all 20 artisanal cheeses (50%) presented *S. aureus* counts above 3.0 log NMP/g. Although some samples of industrialized cheeses were contaminated with *S. aureus* in counts above the allowed, it is remarkable the results obtained for the samples of artisanal cheeses, where all the samples were unfit for consumption due to the excess of *S. aureus*. Dias et al. (2016) and Garcia et al. (2016) reported similar results and all samples of artisanal Minas fresh cheeses presented counts of *S. aureus* above 3.0 log NMP/g. Martínez et al. (2020) evaluated artisanal fresh cheeses in Cuba and reported that 51.6% of samples presented counts of *S. aureus* above 3.0

log NMP/g. For industrialized Minas fresh cheese samples, Apolinário et al. (2014) and Souza et al. (2017) reported better results with 5 samples (16%) and 16 samples (32%) unfit for consumption due to the high counts of *S. aureus*, respectively.

The high *S. aureus* count in artisanal Minas fresh cheeses suggests the use of raw milk from infected animals (mastitis). *S. aureus* presence in cheeses have been linked to the use of unpasteurized milk or to contamination due to improper handling. Most staphylococcal intoxications are caused by contamination from the food-handler during food processing. However, cows that suffer from subclinical mastitis are accepted as alternative reservoirs of enterotoxin producing *S. aureus*, which contaminate the dairy production chain (Kümmel et al., 2016). The genotype B of *S. aureus* is associated with bovine mastitis and is known for its high prevalence within the herd (Cremonesi et al., 2015), being able to produce staphylococcal enterotoxins and reported as an important source of contamination by *S. aureus* from Swiss cheeses from raw milk (Hummerjohann et al., 2014).

Furthermore, the fact that *S. aureus* strains can adhere to the surface of utensils used during the manufacturing process, besides their ability to be strong biofilm producers, increases their persistence in the dairy production chain (Silva et al., 2017). Ingestion of enterotoxins produced by *S. aureus* in food can result in staphylococcal food intoxication, and some outbreaks linked to consumption of raw milk cheeses and artisanal cheeses have been reported (Johler et al., 2015).

*S. aureus* intoxication is characterized by nausea, vomiting and diarrhea, and it is linked to consumption of food contaminated by one or more preformed heat-resistant staphylococcal enterotoxins (ses) that are produced when the bacterial counts reach 4-5 log CFU/g (Hummerjohann et al., 2014; Kümmel et al., 2016). In our study 13 samples (32,5%) of artisanal cheeses presented *S. aureus* with counts higher than 4 log CFU/g. Martínez et al. (2020), reported that 51.6% of the cheese samples were contaminated with *S. aureus* with concentration higher than 4 log CFU/g.

Staphylococcal enterotoxins (ses) are a group of thermostable toxins that are resistant to gastric pH and gastrointestinal proteases (Hummerjohann et al., 2014). Thus, once ses are formed in food production, these highly stable toxins will not be destroyed by common hygienic measures and pose a health risk for the consumers (Kümmel et al., 2016). Enterotoxins A (seA), B (seB), C (seC), D (seD) and E (seE) are the most common toxins observed in association with staphylococcal food intoxication (Hummerjohann et al., 2014).

*S. aureus* were confirmed in our study by the molecular detection of gene *seC* and all samples tested presented this gene, indicating the potential of these strains to produce enterotoxins. Martínez et al. (2020) also confirmed the occurrence of staphylococcal enterotoxin genes by PCR in samples of fresh cheese marketed in Cuba.

Gallegos-Acevedo et al. (2019) reported a high number of mesophilic and psychrotrophic bacteria, total coliforms, *Staphylococcus* bacteria and molds in artisanal Ranchero cheeses made with raw milk and obtained from local producers in Mexico. Sanitation deficiencies in the production of artisanal

Ranchero cheese were evident, which may translate into being a potential consumers health risk factor.

Silva et al. (2017) reported that Minas fresh cheeses can be handmade on small farms with raw milk, without proper hygiene control, resulting in a product with high levels of bacterial contamination, including *S. aureus* and coliforms. Frau et al. (2021) described that most of the cheese manufactured and sold in the north of Argentina is artisanal fresh cheese and is usually made by farmers on a small scale in their farmhouse, using traditional techniques. The final product has a high moisture content (50 to 65%), a low pH (4.1 to 4.5), and a short shelf life (< 30 d). These products have gained popularity due to the increased interest of consumers in the tradition of cheesemaking. According to Choi et al. (2016) cheese related foodborne illnesses have been generally linked to soft cheese or cheese made from raw or unpasteurized milk, but rarely in hard cheese. Hence, manufacturing and storage process should be controlled appropriately to prevent foodborne illness by these cheeses.

#### 4 Conclusions

The results showed that 65% of Minas fresh cheeses (26/40) were unfit for consumption according to Brazilian legislation. The industrialized cheeses showed better microbiological quality than the artisanal cheeses, highlighting the importance of inspection in the production chain of these cheeses. All 20 samples (50%) of artisanal cheeses were not approved for consumption due to the high *S. aureus* counts. Strains of *S. aureus* isolated from these cheeses had confirmation of the *seC* gene, indicating the potential of these strains to produce enterotoxins. When the bacterial counts reach 4-5 log CFU/g there is a potential risk of toxin production in food and the results showed that most samples of artisanal cheeses (13/20) presented *S. aureus* with counts higher than 4 log CFU/g. Although it is illegal in Brazil, there is production of artisanal Minas fresh cheeses from raw milk without official sanitary inspection. These cheeses may also have been contaminated in processing, in addition to being kept at room temperature when commercialized. Thus, there is a clear need for good manufacturing practices throughout the Minas fresh cheese production chain, to reduce health risks and ensure consumer food safety.

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