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Hygienic-health quality and microbiological hazard of clandestine Minas Frescal cheese commercialized in north Tocantins, Brazil

Qualidade higiênico-sanitária e perigos microbiológicos de queijos Minas Frescal clandestinos comercializados no norte do Tocantins

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Highlights

Clandestine Minas Frescal cheeses can compromise public health.
Clandestine samples do not meet all microbiological quality standards.
Coagulase-positive *Staphylococcus* at high counts was identified.
The presence of E. coli with pathogenic potential (EPEC, STEC, and EHEC).

Abstract

Cheese is a popular product that integrates the diet of the majority of the population, almost on a daily basis. It is rich in nutrients and, therefore, also an excellent substrate for the multiplication of microorganisms, including pathogens. The microbiological contamination of these products is highly relevant to the industry, resulting in an economic loss, and to public health, due to the risk of causing foodborne diseases. The aim of this study was to evaluate the hygienic-sanitary quality and the presence of bacterial pathogens in the clandestine Minas Frescal cheeses sold in the street open markets of Araguaína, TO, Brazil. Twenty-one samples were collected to evaluate the presence of total (TC) and thermotolerant (TTC) coliforms, *Escherichia coli*, and the pathotypes enteropathogenic *E. coli* (EPEC), and shiga toxin-producing *E. coli*

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(STEC) and enterohemorrhagic *E. coli* (EHEC), coagulase-positive staphylococci (CPS), *Salmonella* spp., and *Listeria monocytogenes.* The results showed that 100% of the cheese samples were in disagreement with the maximum standards of TC and TTC defined in the Brazilian legislation that regulates food quality control. In addition, 85.71% of the samples had values above the standard Brazilian maximum limit. In the study of diarrheagenic E. coli, 52.38%, 66.6%, and 4.76% of the samples were positive for EPEC, STEC, and EHEC, respectively, indicating fecal origin contamination of the samples and a potential consumer risk. No *Salmonella* spp. or *Listeria monocytogenes* were detected in these cheese samples. The high count of total and thermotolerant coliforms found in the samples demonstrates unsatisfactory sanitary conditions in the production, storage, and/or commercialization of this food product. The presence of EPEC, STEC, EHEC, and coagulase-positive staphylococci at high concentrations shows the health risk of the imminent consumption of Minas Frescal cheese.
Key words: EHEC. EPEC. Foodborne diseases. Public Health. STEC.

Resumo

O queijo é um produto popular que compõe quase que diariamente a dieta da população. É rico em nutrientes e por isso também um excelente meio de multiplicação de micro-organismos, inclusive os patogênicos. A contaminação microbiológica desses produtos assume destacada relevância tanto para a indústria, pelas perdas econômicas, como para a saúde pública, pelo risco de causar doenças transmitidas por alimentos, sobretudo quando produzido clandestinamente. Diante do exposto, o objetivo desta pesquisa foi avaliar a qualidade higiênico-sanitária e a presença de patógenos bacterianos nos queijos Minas Frescal clandestinos comercializados nas feiras livres do município de Araguaína - TO. Foram coletadas 21 amostras avaliando-se a presença de coliformes totais (CT) e termotolerantes (CTT), *Escherichia coli* e, por abordagem molecular, caracterizar os patótipos enteropatogênica (EPEC), produtora de toxina shiga (STEC) e enterohemorrágica (EHEC), estafilococos coagulase positiva (ECP), *Salmonella* spp. e *Listeria monocytogenes.* Foi verificado que 100% das amostras de queijo estavam em desacordo com o padrão máximo de CT e CTT previstos na legislação que regulamenta seu controle de qualidade. Além desses grupos, 85,71% das amostras encontravam-se acima do limite máximo previsto para ECP. Na pesquisa de *E. coli* diarreiogênicas 476 isolados foram testados e foi verificado que 52,38%, 66,6% e 4,76% das amostras de queijos foram positivas para EPEC, STEC e EHEC, respectivamente, indicando contaminação de origem fecal nas amostras e potencial presença de outros enteropatógenos. Em nenhuma amostra foi detectada a presença de *Salmonella* spp. e *Listeria monocytogenes.* A alta contagem de coliformes totais e termotolerantes encontrados nas amostras demonstram condição sanitária insatisfatória na produção, armazenamento e/ou comercialização deste alimento, potencialmente produzido com leite cru. A presença de EPEC, STEC e EHEC e estafilococos coagulase positiva em elevadas concentrações, evidenciam o iminente risco à saúde pelo consumo do queijo Minas Frescal clandestinos.
Palavras-chave: DTAs. EHEC. EPEC. Saúde Pública. STEC.

Introduction

Milk is considered one of the most complete foods because of its high nutritional value. It consists of proteins, carbohydrates, fats, minerals, vitamins, and water, in addition to compounds with high digestibility, components that make it essential for the human diet, making it widely marketed and consumed by the population, especially by children and the elderly (Salvador et al., 2012).

Milk producers, predominantly small, find in cheese production a way to add value to the product (Lopes, Carmo, Lima, & Carvalho, 2006; Teider et al., 2019). This process is usually carried out in a rudimentary way on the rural properties themselves, without the hygienic-sanitary control determined by the Brazilian legislation to guarantee that they are safe for consumption. The fact that they are produced with raw, unpasteurized milk, implies a potential risk in their consumption, due to the non-elimination of the pathogenic microbiota that may be present, often resulting from unsatisfactory conditions in obtaining the raw material or infectious processes related to animal health (Amorim, Couto, Santana, Ribeiro, & Ferreira, 2014).

The Collegiate Board Resolution nº 12/2001 of Agência Nacional de Vigilância Sanitária (ANVISA) is the current Brazilian law in force until December 2020, that establishes the maximum allowed parameters of microorganisms present in cheeses for the purposes of registration and inspection, namely: coliforms at 45°C/g (5 × 10^3 most probable number/g), coagulase-positive staphylococci (5 × 10^3 MPN/g), absence of *Salmonella* spp./25 g and *L. monocytogenes*/25 g (ANVISA, 2001), and coliforms at 30°C/g (10^5 MPN/g) Ministério da Agricultura, Pecuária e Abastecimento [MAPA], (1996), evaluating if the products are in satisfactory microbiological conditions or not.

The poor quality of production can be indicated by the presence of *Escherichia coli,* as it is a microorganism that indicates fecal contamination in the product (N. Silva et al., 2017). The main clinical symptoms of diarrheal *E. coli* in humans are watery diarrhea, accompanied by fever, malaise, and vomiting (J. A. Silva & Silva, 2005). Enteropathogenic *E. coli* (EPEC), and shiga toxin-producing *E. coli* (STEC) and enterohemorrhagic *E. coli* (EHEC) have been declared as major causes of diarrhea since 1940 and continue to be related to sporadic cases of outbreaks (C. D. O. Souza et al., 2016; Gomes et al., 2016).

Staphylococcal food poisoning, which is caused by eating dairy products, such as cheese, is common and is continually reported (Carmo et al., 2002; Argudín, Mendoza, & Rodicio, 2010; Johler et al., 2015; Sato'o et al., 2014). The milk heating process used in pasteurization inactivates *Staphylococcus* spp., but not the previously produced enterotoxins, which remain active in the food product for a long period (Carmo et al., 2002; Johler et al., 2015).

Salmonella spp. is the leading global foodborne disease agent, with tens of millions of cases per year worldwide (World Health Organization [WHO], 2019), including in Brazil (N. Silva et al., 2017); being one of four causes of diarrhea worldwide, and although large outbreaks of *Salmonella* spp. attract media attention, 60% to 80% of all cases of salmonellosis are not recognized as part of an outbreak, being classified as sporadic or even not being diagnosed as such (WHO, 2019). The consumption of milk and its derivatives is related to important pathogens

that cause foodborne diseases, mainly due to contamination with *Salmonella* spp. (Elbagory, Eman, & Eman, 2015).

The high humidity, pH, and storage under refrigeration are factors of fresh cheeses that favor the multiplication or viability of *L. monocytogenes.* This microorganism is considered an opportunistic pathogen, since the occurrence of infection is mainly related to the immunological conditions of the affected individuals: pregnant women and the elderly, the latter being increasingly affected by human listeriosis (Cruz, Martinez, & Destro; 2008; Fai et al., 2011).

Between 2009 and 2017 in the municipality of Araguaína, State of Tocantins, Brazil, of the nine outbreaks of foodborne diseases, five were caused by toxins produced by *Staphylococcus aureus,* two by *E. coli,* and one by *Salmonella* spp. (Vigilância Epidemiológica do Município de Araguaína-TO, 2019).

Due to the various reports of foodborne diseases in the world, and in the municipality of Araguaína, and the potential risk to public health due to the consumption of clandestine fresh cheeses, the objective of this work was to evaluate the hygienic-sanitary quality and the presence of bacterial pathogens in the minas frescal cheese clandestines (MFCC), marketed in street open markets in the municipality of Araguaína, Tocantins, Brazil.

Materials and Methods

Sampling

Twenty-one samples of MFCCs sold in street open markets in the municipality of Araguaína-TO, acquired between April and June 2019, were tested. Each sample was purchased from a different trader and had a different origin. The samples were identified, placed in a transparent plastic bag for first use, packed in an isothermal box with ice, and sent to the Laboratory of Hygiene and Public Health, Federal University of Tocantins, Campus Araguaína/TO, Brazil, for immediate processing.

Microorganism count

For the analysis of total coliforms (TC) and thermotolerant coliforms (TTC), the most probable number (MPN) methodology was used, according to the APHA 9:2015 and APHA/AWWA/WEF 9221:2012. For the isolation of isolates suggestive of *E. coli* from each tube of EC broth with growth and gas production, streaks were made on eosin methylene blue agar (EMB), and the plates were incubated at 35°C for 18 to 24 hours. Typical colonies (green with a metallic shine) were purified, multiplied on a plate count agar (PCA), and incubated at 35°C for 18 to 24 h.

Coagulase positive staphylococci

Coagulase-positive staphylococci (CPS) were counted according to the methodology recommended by the International Organization for Standardization (ISO) 6888-1:1999/Amd 1:2003. The detection of coagulase production followed the methodology described by Costa, Pereira, Custódio and Silva (2011), in which equine plasma was used. The bacterial population was calculated according to ISO 7218:2007/Amd 1:2003 and the results were expressed in CFU/g.

Salmonella spp. and Listeria spp.

Qualitative analysis of *Salmonella* spp. and *Listeria monocytogenes* was performed according to the ISO 6579:2002/Amd 1:2007 modified, and ISO 11290-1:1996/Amd 1:2004 modified, respectively. The modification of both consisted of the molecular identification from isolates suspected of differential plating.

Recovery of microorganisms

The isolates suggestive of *E. coli, Salmonella* spp., and *Listeria* spp. were seeded in Brain Hart Infusion (BHI) broth at 37°C for 18 to 24 h. Then, 0.5 mL of cloudy BHI were stored with 0.5 mL of glycerin and frozen at -18°C. These were recovered by inoculating 30 µL in 3 mL of BHI broth and incubating the mixture at 37°C for 24 h. One side of the cloudy BHI broth was streaked on PCA agar and incubated at 37°C for 24 h. An isolated colony was inoculated again in BHI broth and incubated as previously described. Each cloudy BHI isolate was subjected to DNA extraction.

Molecular confirmation of isolates

The isolates were confirmed using the uniplex PCR technique. DNA extraction was carried out according to Ribeiro et al. (2016). In the confirmation of pathogenic *E. coli,* Paton and Paton (1998) searched for pathogenic strains of enteropathogenic *E. coli* (EPEC) containing the *eaeA* gene, and *E. coli* producing Shiga-like toxin (STEC), by detecting the *stx1* or *stx2* genes. For the confirmation of *Salmonella* spp., the methodology recommended by Shanmugasamy, Velayutham and Rajeswar (2011) was used, using gender-specific PCR, targeting the *invA* gene. For the confirmation of *Listeria* spp., the PCR method was used to confirm the genus by searching for the *iap* gene, as described by Chen and Knabel (2007). Table 1 shows the primers and amplification conditions used. Table 2 shows the quantities of each PCR component to which the samples were added for amplification.

Table 1
Primers and amplification conditions

Microorganism	*Primers**	Sequence (5'3')	Size (pb)	Genes	Amplification	Reference
E. coli	*eaeA* F	GACCCGGCACAAGCATAAGC	*384*	*eaeA*	3 min. initial denaturation at 94° 32 cycles of: 30 sec denaturation at 92°C 30 sec annealing at 59°C 1 min. extension at 72°C Final extension for 1 min. 30 sec at 72°C	Paton and Paton (1998)
	eaeA R	CCACCTGCAGCAACAAGAGG				
	stx1 F	ATAAATCGCCATTCGTTGACTAC	*180*	*stx1*	3 min. initial denaturation at 94° 32 cycles of: 30 sec denaturation at 92°C 30 sec annealing at 61°C 1 min. extension at 72°C Final extension for 1 min. 30 sec at 72°C	
	stx1 R	AGAACGCCCACTGAG ATCATC				
	stx2 F	GGCACTGTCTGAAAT GCTCC	*255*	*stx2*		
	stx2 R	TCGCCAGTTATCTGACATTCTG				
***Salmonella* spp.**	*S139* F	GTGAAATTATCGCCACGTTCGGGCAA	*284*	*invA*	1 min. initial denaturation at 94° 35 cycles of: 1 min. denaturation at 94°C 30 sec annealing at 64°C 30 sec extension at 72°C Final extension for 7 min. at 72°C	Shanmu gasamy et al. (2011)
	S141 R	TCATCGCACCGT CAAAGGAACC				
***Listeria* spp.**	*iap* F	CGCAAGAAGAAATTGCCATC	*1450-1600*	*iap*	5 min. initial denaturation at 95°C 40 cycles of: 45 sec denaturation at 94°C 45 sec. annealing at 52°C 2 min. extension at 72°C Final extension for 10 min. at 72°C	Chen and Knabel (2007)
	iap R	TCCGCGTTAGAAAAATTCCA				

Legend: *F = foward, R = reverse.

Table 2
Reagent composition and quantities (per µL) for PCR per isolate tested in a final volume reaction of 25µL

	E. coli *Salmonella* spp.	*Listeria* spp.
Ultrapure water	18,35	18,05
10x buffer	2,5	2,5
dNTP 10mM/each	0,75	0,75
$MgCl_2$ (50 mmol)	0,75	1,0
Primer Foward (10 pmol)	0,75	0,75
Primer Reverse (10 pmol)	0,75	0,75
Taq DNA polymerize (5U/µL)	0,15	0,2
DNA target (≈50 ng/µL)	0,5	0,5

Statistical analysis

The data were tabulated in spreadsheets of the Microsoft Excel® software version 1910, with mean and standard deviation analysis of TC and TTC counts.

Results and Discussion

In assessing the hygienic and sanitary quality of the product, 100% of the samples surveyed were outside of the permitted standards for TC and TTC. Regarding TC, the estimates ranged from 9.3×10^5 to 1.1×1010 MPN/g and an average (standard deviation - SD) of $1.1 (\pm 2.3) \times 10^9$ MPN/g was obtained from all the samples. Regarding TTC, the results ranged from 4.3×10^5 to 2.4×10^9 MPN/g, with an average (SD) of $4.5 (\pm 7.5) \times 10^8$ MPN/g, as shown in table 3. In the quantification of CPS, of the 21 samples analyzed, 18 (85.71%) had counts outside the standards acceptable by law, as shown in Table 4.

Table 3
Most Probable Number (MPN) of total and thermotolerant coliforms obtained from 21 Minas Frescal cheeses marketed in Araguaína, Tocantins, Brazil, in the period from April to June 2019

MPN/g total coliforms (30°C)	n of samples	Maximum limit (MAPA, 1996)
up until 10^5	-	Até 105 MNP/g
> 10^5 a 10^7	08	
> 10^7	13	
MPN/g thermotolerant coliforms (45ºC)	**n of samples**	**Maximum limit (ANVISA, 2001)**
up until 5,0 x 10^3	-	Até 5,0 x 103MNP/g
> 5,0 x 10^3 a 1,0 x 10^6	01	
> 10^6 a 10^8	07	
> 10^8	13	

Table 4
Distribution of coagulase positive staphylococcal count (CPS) in 21 Minas Frescal cheeses marketed in Araguaína, Tocantins, from April to June 2019

CPS CFU/g	n of samples
up until 10^3	03
> 10^3 a 10^4	01
> 10^4 a 10^6	16
> 10^6	01

Of the 21 samples of MFCC analyzed, 17 showed colonies with characteristics suggestive of *E. coli,* totaling 476 isolates. This indicates contamination of fecal origin in 80.95% of the samples evaluated. Of the total *E. coli* isolates, 29 (6.09%) were confirmed as EPEC; 70 (14.70%) as STEC (60 - 12.60% - *stx1* gene and 10 - 2.10% - *stx2* gene), and 1 (0.21%) as EHEC (*eaeA* and *stx* genes simultaneously in the same isolate). As seen in Table 5, it is possible that there was a recovery of clonal isolates in the same cheese sample, since the culture method had pre-enrichment. For example, from cheese sample 1, 20 isolates suggestive of *E. coli* were recovered, of which seven presented the *stx1* gene (possible clones) and another also presented the *stx1* gene; however, it was simultaneously positive for *eaeA* gene, being characterized as EHEC.

Of all MFCC samples, 52.38% (11/21) presented isolates characterized as EPEC. Likewise, 52.38% (11/21) and 14.28% (03/21) presented positive *E. coli* isolates for *stx1* and *stx2* genes, respectively, making them positive for STEC. In addition, another sample (01/21), which represents 4.76%, presented isolates with simultaneous positivity for the and *stx1* genes, making it positive for EHEC. Analyzing only the MFCC samples with *E. coli* isolation (17/21), in 64.71% (11/17) of them, it was possible to recover positive isolates for the *eaeA* gene, and the same percentage of samples was found to be positive for *stx1* gene, while 17.65% (03/17) presented positivity for *stx2* gene. The distribution of isolates according to the MFCC sample evaluated is described in Table 5.

Table 5
PCR results to identify the *eaeA*, *stx1* and *stx2* genes of the 476 *E. coli* isolates from 21 clandestine Minas Frescal cheeses samples marketed in Araguaína, Tocantins, Brazil, from April to June 2019

Cheese sample	n of isolates strains	*eaeA* (positive)	*stx1* (positive)	*stx2* (positive)
1*	20	1	8	0
2	16	0	9	5
3	0	0	0	0
4	21	1	1	0
5	9	0	2	0
6	16	0	0	0
7	0	0	0	0
8	30	1	2	1
9	23	1	4	0
10	30	3	20	0
11	16	1	3	0
12	25	2	1	0
13	57	14	0	0
14	47	2	3	0
15	21	0	0	4
16	0	0	0	0
17	61	2	7	0
18	40	0	0	0
19	30	0	0	0
20	0	0	0	0
21	14	1	0	0
Total	476	29	60	10

Legend: * The positive isolate for the *eaeA* gene was simultaneously for the *stx1* gene.

A total of 314 recovered isolates were suggestive of *Salmonella* spp. and 377, of *Listeria* spp., from the 21 MFCC samples. In the molecular search for gender-specific genes, no isolates were confirmed. Therefore, none of the MFCC samples tested positive for *Salmonella* spp. or *Listeria* spp.

A high average TC was found in the samples, with all estimates above the standard (10^5) and 13 (61.90%) above 10^7 MPN/g, which mainly shows the potential production of cheese using raw milk, in addition to the contamination environmental damage resulting from serious production failures, generally related to poor hygiene habits, handling without observing hygiene criteria, and poor sanitation of equipment and materials. The local productions that are subjected to inspection and registration by the competent public agencies aim to neutralize the critical points that can lead to contamination of the product. This scenario is very different from

environments of homemade production that do not undergo inspection and control and, therefore, are more subjected to production and pasteurization failures, when existent.

This is reinforced by the study by Dias, Ferreira, Carvalho and Soares (2016), who attributed the high contamination by TC to the fact that the milk used in the manufacture of illegal cheeses is not subjected to pasteurization, while the possible contamination of inspected cheeses occurs by recontamination after pasteurization, due to excessive handling and hygiene failures. Barreto de Deus et al. (2017) reported high counts of TC in cheeses marketed informally on the beaches of the state of Bahia, attributing the high levels of deteriorating and pathogenic microorganisms to inadequate handling and marketing habits. The high TTC counts reported in this study may indicate contamination of fecal origin of the product. This is reinforced by the recovery of *E. coli* isolates in 81% (17/21) of the samples. Its detection may suggest the presence of other pathogenic microorganisms of enteric origin. Therefore, they are often used as indicators of potential fecal contamination, direct or indirect, and of the potential risk of the presence of pathogens in zoonotic foods (Franco & Landgraf, 2002).

Several studies have witnessed TTC levels above what is allowed by legislation in cheeses (Amorim et al., 2014; Apolinário, Santos, & Lavorato, 2014; Dias et al., 2016; Barreto de Deus et al., 2017; I. A. Souza et al., 2017). This is probably due to failures in cheese processing, either due to an inadequate hygiene of materials and equipment, or improper hygiene habits of the handlers that work in the dairy plants. It could not even be fully guaranteed that all the cheeses derived from industrial establishments registered with the competent health agency were in accordance with the legislation (Amorim et al., 2014; Dias et al., 2016), although they still showed better microbiological quality than non-inspected cheeses and, potentially, less risk to public health.

According to Murray, Rosenthal, Kobayashi and Pfaller (2014), EPEC is considered an important cause of childhood diarrhea in poor countries, and the disease is rarer in adolescents and adults due to the development of protective immunity. More than half of the evaluated samples (52.38%) were positive for EPEC; thus, the MFCC marketed in the municipality may cause outbreaks of foodborne diseases. Milk pasteurization before cheese production would eliminate the possibility of the presence of this pathogen, that is derived from raw milk.

Other studies demonstrate that not only the MFCC assessed by this work in the municipality of Araguaína has this potential for public health risk. Campos et al. (2018) also detected an isolate of 147 cheese samples from raw EPEC-positive milk. Further Ribeiro et al. (2019) identified two isolates from 10 evaluated samples of clandestine cheeses.

The presence of the *stx1* and *stx2* genes in 52.38% (11/21) and 14.28% (03/21) of the samples, respectively, are reasons for great concern. STEC is characterized by the production of a cytotoxin named Shiga toxin, encoded by the *stx1* and *stx2* genes (Nataro & Kaper, 1998; Paton & Paton 1998; Gomes et al., 2016). STEC strains are found in raw milk and raw milk cheeses (Farrokh et al., 2013) and are an important cause of human outbreaks, causing severe hemolytic uremic syndrome (HUS) and purple thrombocytopenia (Paton & Paton, 1998; Miszczycha et al., 2014).

As reported (Paton & Paton, 1998; Farrokh et al., 2013), the *stx2* virulence gene is the most important type of *stx* gene, because it has been shown that the probability of developing HUS during infection is greater when *stx2* producing STEC is involved. Considering that almost 15% of the samples in the present study had *E. coli* colonies positive for this gene, it is possible that the consumption of illegal MFCC can be related to serious cases of infection and complications in humans.

The number of isolates suggestive of *E. coli* tested (476) compared to the number of samples evaluated (21) was of fundamental importance, in order to detect the presence of the *eaeA* and *stx* genes in an isolate simultaneously, characterizing it as EHEC, which corresponds to 4.76% (01/21) of the evaluated samples. The detection of this type of *E. coli* is very serious, given the severe disease caused by EHEC. On the other hand, Ribeiro et al. (2019) did not detect this simultaneity in 114 isolates from 10 samples of formal and 10 informal cheeses, although, as in this study, the presence of STEC and EPEC strains was also detected in clandestine samples.

Comparing this study with that of Campos et al. (2018), who detected only one isolate (0.68%) positive for the *eaeA* gene from 147 of the tested samples, the results presented here are expressive and can be much higher if it is taken into account that 47.6% (10/21) of the samples showed positive strains for *stx* and *eaeA*, but not simultaneously for both. Independently of the virulence factor characterization, the fact that it is verified that *E. coli* was isolated, by itself, is already a result that may imply a health risk associated to consumption due to the potential occurrence of other enteropathogens.

Staphylococcus coagulase-positive indicates contamination related to failures during milk pasteurization, such as an inadequate weather or temperature, and recontamination during manual handling (Mehli, Hoel, Bjorge, Nordeng, & Karlsen, 2017). When present in high concentration (10^5 - 10^6 CFU/mL or g) and under appropriate conditions of temperature, pH, water activity, and O_2, they produce one or more staphylococcal enterotoxins in food, which after ingestion, can cause poisoning (Hobbs & Roberts, 1999; Borges, Nassu, Pereira, Andrade, & Kuaye, 2008).

Several studies have shown the presence of CPS above the limits allowed by the Brazilian legislation for different types of cheese. Arruda, Nicolau, Reis, Araújo and Mesquita (2007) found the presence of CPS above the permitted values in the analysis of 42 samples of Minas Frescal cheese sold in open street markets in the city of Goiânia-GO, attributing this high contamination to the fact that the product is being sold in street open markets, thus having greater handling, which facilitated contamination. Amorim et al. (2014) found that 100% of informal Minas Frescal cheese samples showed high CPS counts, which varied from 2.5×10^6 to 1.75×10^7 CFU/g.

The clear deficiency of physical structure noted in street open markets, such as the absence of drinking water for hand hygiene, in addition to the production of cheese without the thermal treatment of milk, only aggravates the possibility of product contamination by *Staphylococcus* spp. This generates high counts in food, as the commensal pathogen of the skin could be reduced by having the simple hand hygiene habits and by pasteurizing the raw material. We did not confirm the presence of *Salmonella* spp. in the evaluated

samples. However, it is important to note that microorganisms of this genus are present mainly in the intestines of birds and pigs, and contamination of products ready for consumption may occur if, in the production environment, there are factors and conditions that lead to a direct or indirect contact, mainly, with the feces of these animals. In addition, the isolation of *E. coli* represents a risk of the occurrence of enteropathogens, which were perhaps not detected in this sample unit.

In a United States Food and Drug Administration (FDA) study, only two of 885 samples of cheese produced from raw milk were positive for *Salmonella* spp., which demonstrated an occurrence of 0.22% of positive samples (Correll, 2014). Thus, using this proportionality, if the present study had evaluated a larger number of samples, the detection of *Salmonella* spp. could have been identified. In addition, factors such as high TC and TTC counts favor the inhibition of pathogenic microbiota, such as *Salmonella* spp., due to a reduced pH (Trmcic et al., 2016). The presence of lactic acid bacteria in raw milk, with bacterial antagonism to pathogens and production of acids and peroxides with antimicrobial action (Beloti, 2015) can also justify the absence of this pathogen in the tested samples.

Of the 21 samples analyzed, none were positive for *Listeria* spp. The study by Dailey, Martin and Smiley (2014) also did not identify the presence of *L. monocytogenes* in several samples of food products, including cheese, attributing the intense presence of aerobic mesophilic, coliform, and *Staphylococcus* bacteria to the limiting factor in the multiplication of *Listeria* spp. Considering that microorganisms of this species may be psychrotrophic, raw refrigerated products may pose a greater risk for the presence of *Listeria* spp. However, a low temperature control was observed in the market regarding the storage of MFCC samples evaluated in the present study, often stored in cool boxes, without ice, and exposed to the sun. Although the presence of enteropathogens, such as *E. coli,* in the present study was confirmed as an indicator of fecal contamination, the high counts of TC and TTC may also explain the absence of *L. monocytogenes,* which may be present in the intestines of animals, but not being detected. The intense microbial population results in a dispute over nutrients, oxidative stress, and pH reduction, causing difficulties in multiplication and, consequently, seasonality or bacterial death (Beloti, 2015).

In short, public policies must be implemented in order to train producers to have good manufacturing practices and technologies to produce cheeses from pasteurized milk and in a hygienic way, to reduce the bureaucracy of the registration of products produced by family farming, so that the product reaches the consumer's table with quality and safety. A sanitary inspection agency must carry out training programs with producers and traders in production and sale, legalizing production and marketing. In addition, it is necessary to implement consumer awareness actions to purchase only registered products and to commercialize in adequate conditions of hygiene and storage, minimizing the health risks.

Conclusion

The high count of total and thermotolerant coliforms evidenced in the MFCC samples shed a light on unsatisfactory sanitary conditions in the production, storage,

and/or commercialization of this food product. The manufacturing of these products from raw milk can be the main reason for the presence of pathogens and other indicators in high quantities in the product. The presence of *E. coli,* EPEC, STEC, EHEC, and coagulase-positive staphylococci at high concentrations shows an imminent health risk from the consumption of MFCCs.

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