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Occurrence of *Escherichia coli* and Coliforms in Minas Cheese Plants from São Paulo, Brazil

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Abstract

The aim of this study was to evaluate the occurrence of total coliforms and *Escherichia coli* in Minas cheese throughout the manufacturing process in 3 medium-scale cheese plants from São Paulo, Brazil. Each cheese plant was visited 4 times to collect samples at approximately 2 month intervals, totalizing 12 visits. Raw milk samples had high total coliforms and E. coli counts in all cheese plants, and at the 4 sampling times, with a mean value of 4.7 log most probable number (MPN) mL^{-1} for total coliforms. E. coli was not detected in pasteurized milk samples in any cheese plant. However, all samples of packaged Minas cheese had high levels of total coliforms. The percentages of samples of packaged Minas cheeses with E. coli counts above the tolerance limit for fecal coliforms (2.7 log MPN g-1) varied from 25% to 80% in the 3 cheese plants. Given the potential involvement of E. coli in human diseases and its frequent occurrence in Minas cheese, there is a need for improvement of hygiene practices for its prevention in medium-scale cheese plants in Brazil.

Keywords: Cheese; Microbiological quality; Coliforms; *E. coli*

Introduction

The production of cheese is one of the most important activities of dairy plants worldwide, especially in Brazil, where nearly 35% of the total milk produced in the country is used for cheese production [1]. The Minas cheese is one of most popular Brazilian cheeses [2], being a non-ripened, white color and soft texture product obtained by enzymatic coagulation of milk and complemented or not by the action of specific lactic acid bacteria [3]. Minas cheeses usually have pH values of 5.0 - 6.3, salt concentration between 1.4 and 1.6%, high water activity and moisture content above 55% [3,4]. These characteristics provide ideal conditions for the multiplication of pathogenic and spoilage microorganisms [5-7].

The manufacture of Minas cheese is relatively simple, with steps of milk coagulation, curd transfer to molds and packaging often done manually, hence requiring intense manipulation and facilitating the bacterial contamination [8]. During the process, milk usually remains at 35-40°C for nearly 1 h, which eventually contributes for increasing the mesophilic bacteria in the final product [2,4,8]. The Minas cheese is largely produced in medium-scale cheese plants, which usually have a mean daily output of up to 100 kg of cheese [6]. The microbiological quality of the Minas cheese from those plants is rather variable, mainly because of economical restrictions for investment, the absence of educational programs addressing the hygienic conditions during processing, or unsuccessful attempts to train workers on better hygiene practices [2,4,6].

Coliform bacteria are main contaminants of raw milk and dairy products, including fresh cheeses. The coliforms are easily destroyed by heat treatments usually employed for milk, being an indicator of process failures or post-processing contamination in pasteurized foods [9]. They are also responsible for deterioration of milk, undesired fermentation [7], yield losses and defects in cheeses [2]. *Escherichia coli* is the main indicator of fecal contamination in foods. The *E. coli* alone may represent a health risk because certain strains are pathogenic to humans [9,10]. Regulations in Brazil adopted tolerance limits of 3.0 log most probable number (MPN) g^{-1} for total coliforms, and 2.7 log MPN g^{-1} for fecal coliforms in Minas cheese [6].

Previous studies have shown that Minas cheese samples collected in the market often do not comply with microbiological regulations, especially for fecal coliforms counts [4,6,8,9]. However, there is no available information on the main factors involved in cheese contamination by coliforms and E. coli in cheese plants. The purpose of this study was to evaluate the occurrence of total coliforms and *E. coli* in Minas cheese throughout the manufacturing process in medium-scale cheese plants from São Paulo, Brazil.

Materials and Methods

Sampling procedures

Sampling procedures were performed in 3 cheese plants located in São Paulo state, Brazil, from October 2008 to September 2009. The plants received approximately 7,000 liters of raw milk per day and produced nearly 3,000 kg of Minas cheese per month. The 3 plants were not connected, and did not have suppliers of raw milk in common. Two cheese factories (plants 1 and 3) were supervised by the Brazilian Federal Inspection Service (SIF), while one (plant 2) was supervised by the Inspection Service of the São Paulo State (SISP). Each cheese plant was visited 4 times to collect samples, at approximately 2 month intervals, totalizing 12 visits. During each visit in each cheese plant, the following samples were collected in sterilized containers: bulked raw milk (n=2), pasteurized milk (n=1), curd (n=1), semi-finished cheese inside the molds (n=5) and packaged Minas

cheese (n=5). In one of the plants, brine samples were collected in each visit (n=1).

In all dairy plants, the Minas cheeses were manufactured following the same basic procedures [2,4], by enzymatic coagulation process without lactic acid bacteria. Briefly, the pasteurized and cooled milk was transferred to the coagulation tank through the pipeline system and added with sodium chloride (plants 1 and 2), calcium chloride and lactic acid. The milk was heated to 40°C (plant 1), to 38°C (plant 2) and 42°C (plant 3). The rennet was added and the temperature maintained for coagulation for 30-40 min. The coagulum was cut with metal lyres, homogenized and the curd obtained was distributed in molds, placed in plastic boxes and stored at 4°C for about 16 h, until the time of packaging. In the plant 3, where no sodium chloride was added before coagulation, the salting of cheeses was performed in a brine tank. The Minas cheeses were packaged in plastic bags, sealed with metal clip and kept in cold storage until shipment. The expiry date was 30 days from the date of packaging.

Microbiological analysis

Analyses of total coliforms and *E. coli* analyses were performed in samples of raw and pasteurized milk, curd, Minas cheeses and brine (only from plant 3). The cheese packages were washed and disinfected with alcohol (70%, v/v) before opening. Aliquots of 25 mL of milk or brine, or 25 g of cheese or curds were removed, aseptically diluted in 225 ml of peptone water and homogenized in a stomacher [11]. From the 10-1 dilution, decimal dilutions were prepared, and aliquots of selected dilutions were transferred onto SimPlate CEc (BioControl Systems, Bellevue, WA). Media and sample were gently mixed in the SimPlate device, the excess removed as per the manufacturer's instructions and incubated at 35°C for 24 h [12]. The coliform bacteria produce color changes in the medium due to acid production, while *E. coli* produces a blue fluorescent color due to the reaction of β-glucuronidase with reagent in the media. Thus colored wells without fluorescence under ultraviolet (UV) light indicate the presence of coliforms, while colored wells with fluorescence indicate the presence of *E. coli* [12]. Therefore the wells showing purple color were considered positive for total coliforms, whereas those showing fluorescence under ultraviolet light at 366 nm were considered positive for *E. coli*. Total coliform and *E. coli* counts were determined by using the SimPlate conversion table provided by the manufacturer. The results were expressed in log MPN per gram or milliliter of product, according to the table provided by the manufacturer.

Results and Discussion

The total coliforms counts in samples collected in the cheese plants evaluated are shown in Table 1. Raw milk samples had high total coliforms counts in all cheese plants, and at the 4 sampling times, with a mean value of 4.7 log MPN mL^{-1}. This highly contaminated milk, besides serving as a source of contamination to the processing environment, may decrease the overall quality of the resulting dairy products. The total coliform count is considered a good predictor of the hygienic and sanitary practices during food production [13]. Hence the high levels found in raw milk are a consequence of failures in the milk collection, such as improper cleaning and sanitizing of equipment, during transport of milk from farms to the dairy industries. In general, raw milk containing coliform counts above 2.0 log MPN mL^{-1} indicate unsatisfactory hygienic conditions during milk collection or transport [7].

	n	Sampling order [a]			
		1	2	3	4
		(Log MNP g or mL^{-1}) [b]			
Cheese plant 1					
Raw milk	2	6.3	5.7	6.6	4.9
Pasteurized milk	1	ND	ND	1.2	ND
Curd	1	ND	2.5	3.9	1.3
Minas cheese (in molds)	5	ND	4.0±0.5	3.8±0.3	1.3±0.7
Minas cheese (packaged)	5	3.1 ± 1.2 [c]	7.5 ± 0,1 [c]	5.5 ± 0.2 c	3.5 ± 0.3 [c]
Cheese plant 2					
Raw milk	2	6.4	5.8	4.7	6.2
Pasteurized milk	1	ND	ND	ND	ND
Curd	1	1.3	2.5	1.6	2.8
Minas cheese (in molds)	5	3.4 ± 0.2	3.3 ± 0.6	3.0 ± 0.2	2.8 ± 1.6
Minas cheese (packaged)	5	6.1 ± 0.1 [c]	4.3 ± 0.4 [c]	4.8 ± 0.2 [c]	3.6 ± 0.7 [c]
Cheese plant 3					
Raw milk	2	5.2	6.1	4.8	5.9
Pasteurized milk	1	ND	ND	1.2	1.9
Curd	1	1.6	ND	2.2	ND
Minas cheese (in molds)	5	1.5 ± 0,7	2.8 ± 0.2	2.6 ± 0.5	ND
Minas cheese (packaged)	5	2.7 ± 0.3	5.3 ± 0.2 [c]	4.2 ± 0.6 [c]	5.5 ± 0.3 [c]
Brine	1	4.8	2.2	2.7	1

Table 1: Total coliforms counts in samples collected in 3 cheese processing plants from São Paulo, Brazil; a Sampling dates: 1. Nov/2008, 2. Feb/2009, 3. May/2009, and 4. Aug/2009; b Results are reported as mean + standard deviation; c Samples above the tolerance limit adopted in Brazil for total coliforms in Minas cheese (3.0 log MPN g^{-1}); n Number of samples collected in each sampling date; ND: Not detected (detection limit of the method: 1.0 MPN mL or g^{-1})

The pasteurization of milk reduced the total coliforms in milk samples, except in 3 samples, two from plant 3 and one from plant 1. The presence of coliforms in milk after the pasteurization process indicates failures in the heat treatment or recontamination of the pasteurized milk during transfer to the coagulation tank. However, the total coliform counts in pasteurized milk did not influence the counts in the curd or in the resulting Minas cheese. Several samples of curd had total coliforms that range averaged 1.3 – 3.9 log MPN g^{-1}, indicating recontamination and / or bacterial growth during coagulation. Moreover, the total coliforms counts were usually higher in Minas cheese samples collected from the molds, as a consequence of the intense manipulation of curds in the molding procedure [8].

All samples of packaged Minas cheese had high levels of total coliforms, with variations in each sampling periods. In plants 1 and 3,

80% of packaged cheeses had total coliform counts greater than the Brazilian tolerance limit (3.0 log MPN g^{-1}) [14]. All the packaged Minas cheese collected in plant 2 were above the limit, which confirms that packaging is the most important step for coliform contamination during manufacture of Minas cheese. High coliforms counts may contribute for deterioration of the product, besides being indicative of poor hygienic conditions of production [6,9]. The occurrence of 1.0 – 4.8 log MPN mL^{-1} of total coliforms in brine samples in the plant 3 suggests a possible contribution of the salting procedure for increasing the bacterial load of cheeses. The counts observed in brine in the present study were consistent with values reported in a previous study [15], in which brine contained up to 12.9 log MPN 100 mL^{-1} of total coliforms after 21 days of constant use for salting Minas cheese. The data reported stress the need for a renewal of brine used for salting of Minas cheese at short intervals, or the replacement of brine by direct salting of milk to reduce the contamination of cheeses.

		Sampling order [a]			
		1	2	3	4
n		**(Log MNP g or mL^{-1})** [b]			
Cheese plant 1					
Raw milk	2	3.7	3.6	2.2	ND
Pasteurized milk	1	ND	ND	ND	ND
Curd	1	ND	ND	ND	ND
Minas cheese (in molds)	5	ND	3.0 ± 0.4	1.6 ± 0.6	ND
Minas cheese (packaged)	5	ND	6.4 ± 0.1 [c]	ND	1.9 ± 0.9
Cheese plant 2					
Raw milk	2	5.2	4.8	1.3	ND
Pasteurized milk	1	ND	ND	ND	ND
Curd	1	ND	2.1	ND	ND
Minas cheese (in molds)	5	1.9 ± 0.5	2.5 ± 0.5	ND	1.3 ± 0.6
Minas cheese (packaged)	5	4.4 ± 0.4 [c]	3.6 ± 0.5 [c]	1.4 ± 0.1	2.5 ± 1.3
Cheese plant 3					
Raw milk	2	1.3	3.2	ND	ND
Pasteurized milk	1	ND	ND	ND	ND
Curd	1	ND	ND	ND	ND
Minas cheese (in molds)	5	1.2 ± 0.4	2.4 ± 0.3	ND	ND
Minas cheese (packaged)	5	2.6 ± 0.3	5.1 ± 0.2 [c]	3.2 ± 0,5 [c]	5.1 ± 0.3 [c]
Brine	1	ND	1.3	ND	ND

Table 2: *Escherichia coli* counts in samples collected in 3 cheese processing plants from São Paulo, Brazil; a Sampling dates: 1. Nov/2008, 2. Feb/2009, 3. May/2009, and 4. Aug/2009; b Results are reported as mean ± standard deviation; c Samples above the tolerance limit adopted in Brazil for fecal coliforms in Minas cheese (2.7 log MPN g^{-1}); n Number of samples collected in each sampling date; ND: Not detected (detection limit of the method: 1.0 MPN mL or g^{-1})

Table 2 presents the counts for *E. coli* in samples collected in the cheese plants. Raw milk samples had high *E. coli* counts, as expected considering the total coliforms counts in raw milk (Table 1). However, *E. coli* was not detected in pasteurized milk or in curd samples, except for one sample of curd from cheese plant 2. *E. coli* was detected more frequently in cheese samples, particularly in the packaged Minas cheeses, which reinforces the fact that contamination mainly occurred during manipulation of curd as well as during packaging [8].

The percentages of samples of packaged Minas cheeses with *E. coli* counts above the tolerance limit for fecal coliforms (2.7 log MPN g^{-1}) [3,14] were 25%, 55% and 80%, respectively in cheese plants 1, 2 and 3. The higher percentages found in cheese plants 2 and 3 are indicative of lower hygienic conditions in the final steps of cheese manufacture, including molding, packaging. Thus the detection of highly contaminated cheese samples in the 3 plants immediately after production is a matter of concern, since the products are intended for marketing. Taking into account the shelf life of Minas cheeses (30 days) indicated by the plants evaluated, the high *E. coli* counts certainly undermines the microbiological quality of cheeses as a result of microbial growth. The *E. coli* counts found in the present study are similar to those previously reported [9,16-18], showing up to 93% of Minas cheese or other soft cheeses commercially available at sales points with fecal coliforms above the tolerance limit established by legislation.

The non-compliance of coliforms and *E. coli* results of Minas cheese with microbiological regulations in the 4 sampling periods indicate an overall poor hygienic conditions and the absence of quality control programs in the cheese plants evaluated. Compared to *E. coli*, the higher counts of total coliforms in all samples may be related to the presence of other species classified in the coliform group, such as *Citrobacter*, *Enterobacter* and *Klebsiella* [7]. The presence of *E. coli* in cheese, and fecal contamination related to the potential presence of enteric pathogens, may indicate significant health risks. In a study evaluating Minas cheese produced in dairy plants in Minas Gerais state and also subjected to Federal Inspection Services (SIF), isolates of *E. coli* from cheese samples were classified as entero-pathogenic (EPEC), which is an important hazard to the consumer health [9].

Conclusion

Results of this trial showed a gradual increase in total coliforms and *E. coli* during the manufacture of Minas cheese, which can be attributed to failures in the good manufacture practices in the cheese plants evaluated, especially during manipulation of curds and packaging of cheeses. Given the potential involvement of *E. coli* in human diseases and its frequent occurrence in Minas cheese, there is a need for improvement of hygiene practices for its prevention in medium-scale cheese plants in Brazil.

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