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International Journal of Food Microbiology 81 (2003) 241–248

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short communication

# Occurrence of *Listeria* spp. in critical control points and the environment of Minas Frescal cheese processing

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Received 23 August 2001; received in revised form 21 January 2002; accepted 14 May 2002

**Abstract**

Critical control points (CCPs) associated with Minas Frescal cheese (a Brazilian soft white cheese, eaten fresh) processing in two dairy factories were determined using flow diagrams and microbiological tests for detection of *Listeria monocytogenes* and other species of *Listeria.* A total of 218 samples were collected along the production line and environment. The CCPs identified were reception of raw milk, pasteurization, coagulation and storage. Thirteen samples were positive for *Listeria*; 9 samples were *Listeria innocua*, 2 were *Listeria grayi* and 2 were *L. monocytogenes*. In factory A, *Listeria* was found in 50% of raw milk samples, 33.3% of curd samples, 16.7% of pasteurized milk samples, 16.7% of cheese samples and 25% of rubber pipes used to transport the whey. The microorganism was not obtained from environmental samples in this plant. In factory B, *Listeria* was found in one sample of raw milk (16.7%) and in three samples of environment (17.6%) and *L. monocytogenes* was obtained from raw milk (16.7%) and the floor of the cheese refrigeration room (14.3%). Two serotypes, 4b and 1/2a, were observed among the strains of *L. monocytogenes* isolated, both which are frequently involved in outbreaks of food-borne listeriosis and sporadic cases of the disease all over the world.


*Keywords:* *Listeria monocytogenes*; Minas Frescal cheese; Critical control points; Environmental sampling

## 1. Introduction

Minas Frescal cheese is one of the most popular cheeses produced in Brazil. Produced from cow's milk, the major characteristic is the pleasant, slightly acid taste and rich flavour. Soft, white, fresh cheeses that are subject to minimal processing before packaging are highly perishable and thus have a short shelf life, even with refrigeration. Since *Listeria monocytogenes* is psychrotrophic and can grow at low temperatures, growth of this organism on contaminated cheese can occur.

*L. monocytogenes* is a food-borne pathogen that can contaminate dairy products (Menendez et al., 1997). Outbreaks of listeriosis resulting from con-

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PII: S0168-1605(02)00223-4

sumption of dairy foods contaminated with *L. monocytogenes* have prompted concern about the behavior of this organism during processing and subsequent storage of various dairy products and about control of the hazard the bacterium poses to the dairy industry. Of particular importance is how milk, including that used to manufacture cheese and other cultured dairy products, can serve as a primary vehicle for transmission of the organism (Rosenow and Marth, 1987).

Although *Listeria* is inactivated under normal conditions of pasteurization (Schaack and Marth, 1988), problems can arise from postpasteurization contamination. Bacteria can enter cheese at many stages during its processing. The environmental diversity of dairy processing plants provides the microorganism with various sites for colonization. Any pathogen existing in raw milk can potentially make its way into the environment of plants processing cheese (Cotton and White, 1992).

In recent years, the hazard analysis critical control point (HACCP) concept has been proposed as the best approach to assure food safety. Inclusion of *L. monocytogenes* in the list of organisms subject to HACCP has recently driven the search for detection methods suitable for on-line monitoring (Almeida and Almeida, 2000). The concept of HACCP is a preventive, structured, systematic and documented approach to ensure food safety (Buchanan, 1990; Montarjemi et al., 1996). It is generally recognized that the production of cheeses with desirable organoleptic characteristics and which are safe for consumption can only be assured when certain factors are continuously controlled and tested: the microbiological quality of the raw milk; pasteurization of the raw milk prior to cheese production, prevention of recontamination after pasteurization of the milk and predominance of desirable microbial flora during storage (Zottola and Smith, 1993).

Despite the risk presented by *L. monocytogenes* in dairy products, there are few studies on the incidence of this microorganism at critical control points in the line of cheese processing in Brazil. Thus, the objectives of this work were to evaluate the hazard of *Listeria* species occurrence associated with Minas Frescal cheese processed in two factories in Bahia, Brazil and to assess the critical control points in the processing of this food.

## 2. Materials and methods

### 2.1. Plants evaluated

The factories studied in this work were located in Bahia (Brazil). Factory A uses approximately 10,000 l of milk daily in cheese production while Factory B uses approximately 2000 l daily.

### 2.2. Determination of CCPs

Flow diagrams of processing of Minas Frescal cheese were constructed in order to provide a clear, simple description of the steps involved in the process at the two factories (ICMSF, 1988).

To determine CCPs, the decision tree described by ONU/Organizacion Mundial de la Salud (OMS) (1991) was used.

### 2.3. Sampling procedures

During the study, seven visits were made to each factory between August 1999 and February 2000. Fifty-four food samples, 107 equipment samples, 22 worker handling samples and 35 environmental samples were taken.

Portions of 100 g or 100 ml of food were aseptically collected at critical control points previously identified (Messer et al., 1992). Samples obtained from sites in plant areas including floors, drainage and wooden shelves were classified as "environmental" samples and those obtained from the product contact surfaces of utensils and equipment were classified as "equipment" samples. The samples of environment, equipment and worker handling surfaces were collected using the sponge method (Quevedo et al., 1977). Environmental samples were obtained by sponging an area approximately 2 by 3 ft ($0.6 \times 1.0$ m), whereas equipment samples were obtained by sponging the same area or a complete surface. All sponge samples were placed in sterile whirlpak bags, kept at 4 °C, and processed within 24 h of collection.

### 2.4. Laboratory procedures

Sponges were tested for inhibitory properties against *Listeria* species (Daley et al., 1995).

The sponges were transferred to sterile bags containing 100 ml of University of Vermont modified *Listeria* enrichment broth (UVM, Difco) (Donnelly et al., 1992) with 0.5% sodium thiosulphate to neutralize chlorine compounds used for cleaning and incubated for 24–48 h at 30 °C.

For the food samples, 25 g or 25 ml of samples were blended with 225 ml of *Listeria* enrichment broth (LEB, Difco) (Donnelly et al., 1992) and incubated for 24–48 h at 30 °C.

After enrichment, all samples of food were plated on lithium chloride–phenylethanol–moxalactam medium containing ferric citrate ammoniac 0.05% and esculin 0.1%, supplemented with antibiotics (LPM, Difco) and Oxford medium (OXA, Difco) and incubated for 48 h at 30 and 35 °C, respectively.

For equipment, worker handling and environmental testing, secondary enrichment was performed in Fraser broth. After this, blackened Fraser broth was streaked onto LPM and modified OXA (Difco) following incubation under the same conditions previously described. At the same time as the Fraser broth medium was inoculated, a loopful of each primary enrichment broth was also streaked onto a plate of modified OXA and LPM (Donnelly et al., 1992).

Five morphologically typical colonies from each plate producing a black halo from esculin hydrolysis

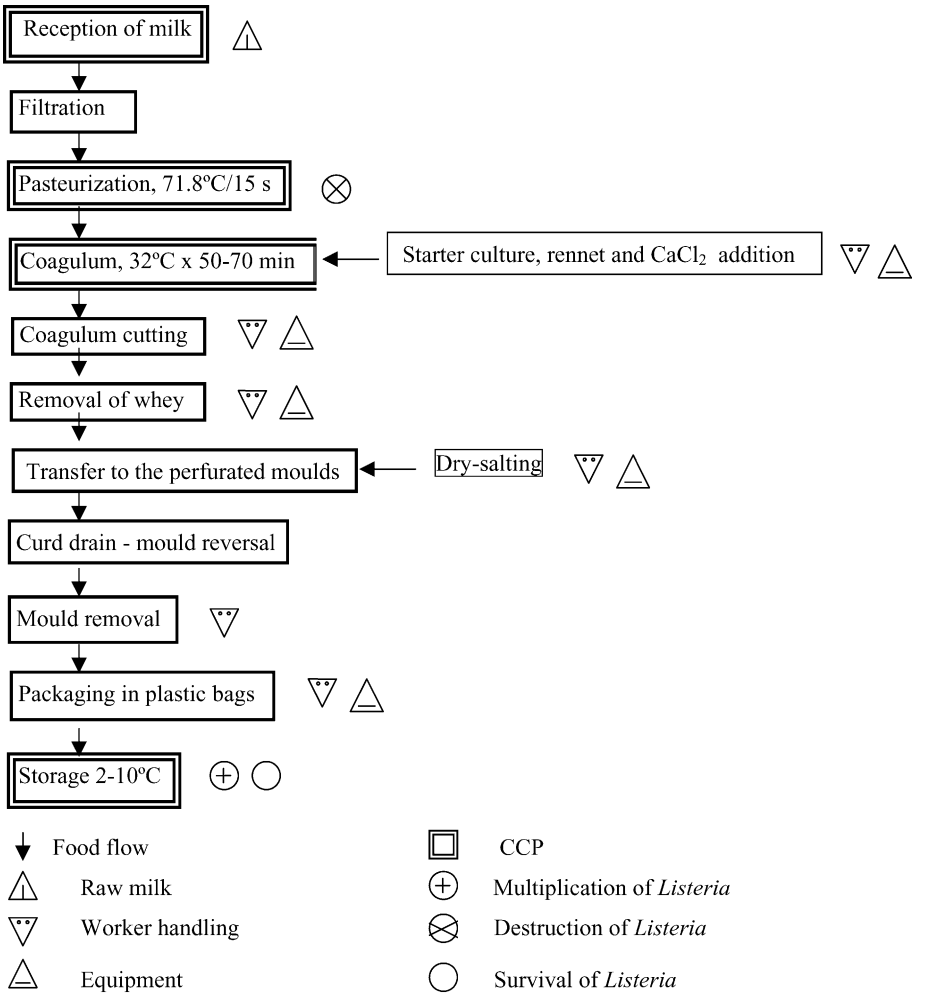


Fig. 1. Flow diagram of Minas Frescal cheese production and critical control points in factory A.

were subjected to the following tests: Gram stain, tumbling motility at 21 °C, catalase reaction, β-haemolysis on 5% horse blood agar plates, CAMP reaction with *Staphylococcus aureus* ATCC 25923 and *Rhodococcus equi* ATCC 33701, carbohydrate fermentation (rhamnose, xylose, mannitol, maltose and glucose), nitrate reduction and Voges–Proskauer test. *L. monocytogenes* Scott A serovar 4b, *Listeria innocua* (HPB 124), *Listeria grayi* (HPB 29), *Listeria welshimeri* (HPB 32) and *Listeria seeligeri* (HPB 62) provided by J.M. Farber, from Health Products and Food Branch of Health Canada, were used in control tests. Serological slide agglutination tests were done according to Seeliger and Hohne (1979) on all isolates presumed to be *Listeria*, using commercially prepared antisera (Difco).

## 3. Results and discussion

### 3.1. Minas Frescal cheese flow diagrams

Flow charts and critical control points for the processing of Minas Frescal cheese are presented in Figs. 1 and 2. Laboratory results are listed in Tables 1 and 2.

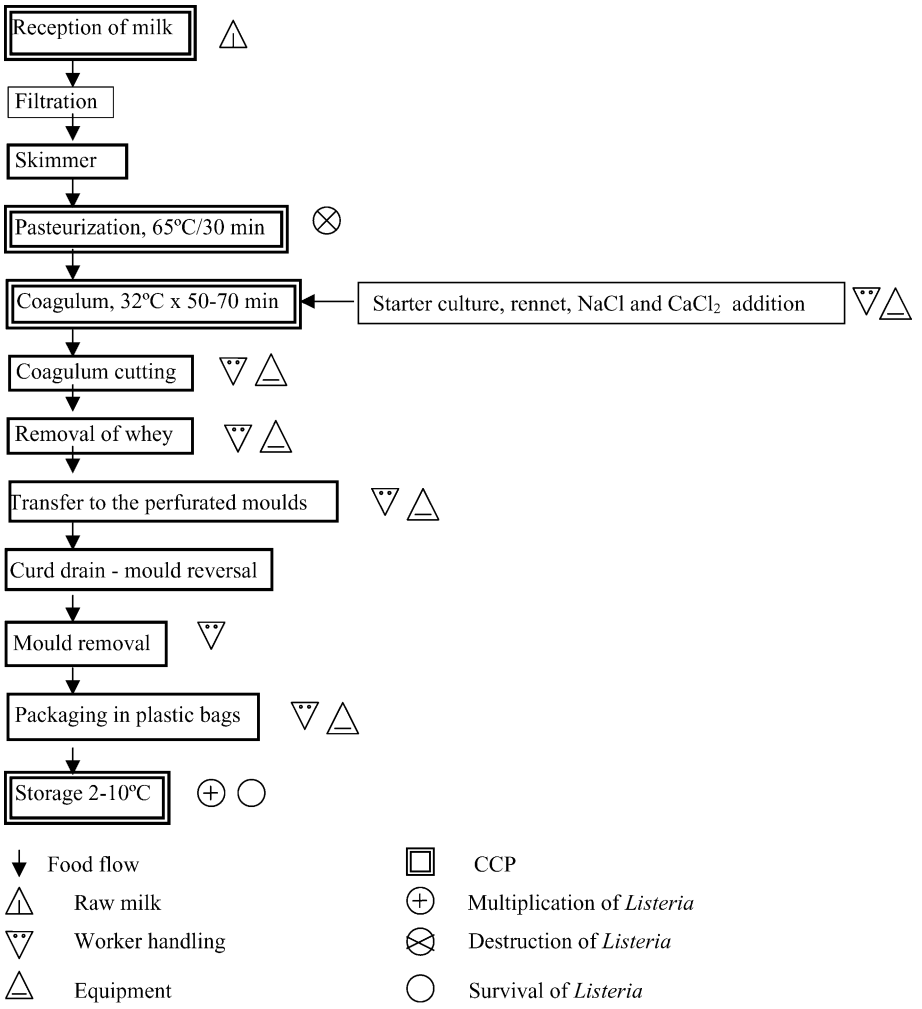


Fig. 2. Flow diagram of Minas Frescal cheese production and critical control points in factory B.

Table 1
Incidence of *Listeria* in the processing of Minas Frescal cheese in factory A

| Samples | *L. monocytogenes* | *L. innocua* | *L. grayi* |
|---|---|---|---|
| Reception and pasteurization areas | | | |
| Raw milk (6)[a] | 0 | 2 | 1 |
| Pasteurized milk (6) | 0 | 1 | 0 |
| Floor (1) | 0 | 0 | 0 |
| Drains (2) | 0 | 0 | 0 |
| Cheese manufacturing room | | | |
| Curd (6) | 0 | 1 | 1 |
| Cheese (6) | 0 | 1 | 0 |
| Steam-jacketed cheese vat (6) | 0 | 0 | 0 |
| Stirrer (6) | 0 | 0 | 0 |
| Knifes (7) | 0 | 0 | 0 |
| Milk pipes (4) | 0 | 1 | 0 |
| Cloths bags (6) | 0 | 0 | 0 |
| Curd moulds (6) | 0 | 0 | 0 |
| Lid moulds (6) | 0 | 0 | 0 |
| Wire knifes (6) | 0 | 0 | 0 |
| Hands and gloves (11) | 0 | 0 | 0 |
| Floor (1) | 0 | 0 | 0 |
| Drains (5) | 0 | 0 | 0 |
| Cheese packaging room | | | |
| Floor (1) | 0 | 0 | 0 |
| Drains (2) | 0 | 0 | 0 |
| Cheese refrigeration rooms | | | |
| Wooden shelves (3) | 0 | 0 | 0 |
| Floor (3) | 0 | 0 | 0 |
| Totals (100) | 0 | 6 | 2 |

[a] Number of samples tested.

After milking, the raw milk was transported to the dairy factories at temperature of approximately 20–25 °C (factory A) and 21–31 °C (factory B). After reception, milk was filtered and pasteurized at 71.8 °C × 15 s (factory A) or at 65 °C × 30 min (factory B) and cooled to 32–36 °C (factory A) or 35–38 °C (factory B). At this temperature, a starter culture, rennet and $CaCl_2$ were added and the milk coagulated in 50–70 min. The coagulum was cut using a 2-cm wire knife, agitated for 20–30 min to separate the whey, transferred in thin layers into perforated moulds and then salt was added. The moulds were 15.5 cm in length and 11.0 cm in diameter. The curd was drained without pressing until firm enough to e removed remove from the moulds (20–24 h). The cheese was then packaged into plastic bags and stored at 2–10 °C. The cheeses can be consumed 1 month after manufacture (Figs. 1 and 2). In factory B the raw milk was skimmed before pasteurization and NaCl was added with the rennet, $CaCl_2$ and starter.

### 3.2. Analysis of Minas Frescal cheese production CCPs

Contamination of milk by *L. monocytogenes* can occur from mastitic animals and from environmental sources such as silage and soil during milking. Animal feed must be controlled because the presence of *L.*

Table 2
Incidence of *Listeria* in the processing of Minas Frescal cheese in factory B

| Samples | *L. monocytogenes* | *L. innocua* | *L. grayi* |
|---|---|---|---|
| Reception and pasteurization areas | | | |
| Raw milk (6)[a] | 1 | 0 | 0 |
| Skim milk (6) | 0 | 0 | 0 |
| Pasteurized milk (6) | 0 | 0 | 0 |
| Floor (1) | 0 | 0 | 0 |
| Drains (1) | 0 | 0 | 0 |
| Cheese manufacturing room | | | |
| Curd (6) | 0 | 0 | 0 |
| Cheese (6) | 0 | 0 | 0 |
| Steam-jacketed cheese vat (6) | 0 | 0 | 0 |
| Stirrer (6) | 0 | 0 | 0 |
| Milk pipes (6) | 0 | 0 | 0 |
| Sieves, pots and jars (18) | 0 | 0 | 0 |
| Curd mould (6) | 0 | 0 | 0 |
| Wire knifes and knifes (12) | 0 | 0 | 0 |
| Hands and gloves (11) | 0 | 0 | 0 |
| Floor (3) | 0 | 0 | 0 |
| Drains (4) | 0 | 0 | 0 |
| Cheese packaging room | | | |
| Boxes and plastic bags (12) | 0 | 0 | 0 |
| Floor (1) | 0 | 0 | 0 |
| Drains (1) | 0 | 0 | 0 |
| Cheese refrigeration room | | | |
| Wooden shelves (2) | 0 | 0 | 0 |
| Floors (2) | 1 | 2 | 0 |
| Drains (2) | 0 | 1 | 0 |
| Totals (118) | 2 | 3 | 0 |

[a] Number of samples tested.

*monocytogenes* from improperly fermented silage has been documented (Fenlon, 1986). An increase in somatic cells indicates an unhealthy animal. Additional sources of contamination can occur from handling milk en route and at the dairy factory. The milk should be obtained from healthy animals under hygienic conditions. Cleaning and antisepsis of the udder and hands of the milkman before and after milking with appropriate antiseptics constitute preventive measures. Long exposure of milk to high temperatures during transportation may favour the growth of pathogens, including *L. monocytogenes*. Control of raw milk includes the determination of milk acidity, freezing point, antibiotic and metabolic residues (Mauropoulos and Arvanitoyannis, 1999). The reception of raw milk was a stage considered a CCP because the reception was carried out at ambient temperature and the product stood there for more than 2 h. Controls of time and temperature in this stage should be established and systematically monitored to prevent creation of a hazard.

In a high-temperature short-time (HTST) system, the typical temperature–time conditions for pasteurization of milk are 72 °C for 15 s (Mauropoulos and Arvanitoyannis, 1999). The flow of milk into the pasteurizer cannot exceed the rate at which the 15-s hold is measured. In 1985, the consumption of contaminated Jalisco brand Mexican-style cheese manufactured in California was directly linked to more than 142 cases of listeriosis, including 48 deaths (Linnan et al., 1988). According to the Center for Disease Control in the United States, a leak in the pasteurization of the milk used to produce the cheese was attributed to excessive volume of milk in the pasteurizer. Pasteurization must ensure that pathogenic microorganisms do not survive the process in order to reduce public health risk.

For Minas Frescal cheese, the coagulation step was considered a CCP because the temperature was controlled in order to promote the starter growth and the addition of the rennet. The preventive measures at this stage consist of monitoring the temperature of milk and controlling the development of acidity (pH reaches 5.0–6.1 in factory A and 4.9–6.2 in factory B).

The other stages, such as coagulum cutting, removal of whey, transfer to the moulds and packaging were considered critical points because of the possibility of introduction of *Listeria* from utensils and worker handling. The storage of cheese (2–8 °C) was considered a CCP because the reduced temperature delays the growth of *L. monocytogenes*.

### 3.3. Occurrence of Listeria in CCPs and the environment

A total of 218 samples were collected and 13 different isolates of *Listeria* species were identified in both factories. The species most often isolated was *L. innocua*, which accounted for 9 of the 13 (70%) isolates in both factories. Fifteen percent of the isolates were *L. monocytogenes* and the same percentage were *L. grayi*. *L. monocytogenes* was obtained from raw milk (serotype 4b) and the floor of the milk storage room (serotype 1/2a) in factory B (Table 2).

In factory A, 50% of the raw milk samples were *Listeria* positive and in factory B the bacteria was isolated in 16,7% of the raw milk samples.

*L. innocua* was detected in raw milk, pasteurized milk, curd and cheese and in the milk pipes used to transport the whey in the plant A (Table 1). In factory B, *L. innocua* was found in three samples taken from floors and drains of the cheese storage room and *L. monocytogenes* was found in one sample (Table 2).

Two serotypes, 4b (raw milk) and 1/2a (floor) were observed among the strains of *L. monocytogenes* isolated in factory B. Both of these serotypes are frequently involved in outbreaks of food-borne listeriosis and sporadic cases of disease all over the world. The presence of *L. monocytogenes* in 16.7% of raw milk and in 14.3% of floor samples in plant B indicated the need for frequent monitoring at the milk pasteurization stage and during cleaning and sanitizing operations of equipment and environments in order to avoid the occurrence of cross contamination of the product.

Minas Frescal cheese has a low pH (4.9–5.3), high water activity and moisture levels (55–58%) that could favor the survival of *L. monocytogenes*. Previous research conducted in Brazil by Destro et al. (1991) detected *L. monocytogenes* in 10% of manufactured Minas Frescal cheese, while Cassarotti et al. (1994) failed to detect *Listeria* in 20 samples of the same type of cheese.

According to results obtained by Silva et al. (1998), among the different types of cheese investigated in Brazil, Minas Frescal showed the highest

frequency of *L. monocytogenes*, which was in 7 of the 17 (41.17%) samples tested.

Of 107 samples taken from equipment from the two plants, only one (0.9%) was positive for *Listeria*. Surface samples from workers' hands and gloves were negative for *Listeria*. In a study conducted by Menendez et al. (1997), only 3% of samples taken from equipment in 18 cheese factories, were found to be positive.

An evaluation of plants in California producing milk products (Walker et al., 1991) revealed that 12% of the samples obtained from 39 different plants were contaminated with *Listeria*. Klausner and Donelly (1991) and Charlton et al. (1990) surveyed the environment of a variety of dairy plants including processing, fluid milk, frozen milk products, cheese and other cultured products. Both groups reported an overall incidence of *Listeria* ranging from 12.6% to 17.5%.

In this study, we found *Listeria* in 11.4% of the samples from the environment; of these 2.9% were *L. monocytogenes*. In the Menendez study, the total percentage of environmental samples testing positive for *Listeria* spp. was 14.4%, higher than that reported by Nelson (1990). However, percentages of positive samples of *L. monocytogenes* were similar, 2.4% by Menendez et al. (1997) and 2.2% for the data obtained by Nelson (1990). Pritchard et al. (1995) evaluated the environment of plants processing fluid milk, cheese and noncheese cultured products and they identified *Listeria* in 35.3% of environmental samples tested.

The presence of *Listeria* species in the production line indicates that postprocessing contamination can occur. Therefore, every effort must be made to control this organism in cheese manufacturing by safer handling of raw milk, effective destruction of microorganisms through heat processing and proper cleaning and sanitation of CCPs to prevent recontamination of the heated product with *L. monocytogenes*. It is evident that development and use of the HACCP concept is urgently needed for all processing plants showing the degree and level of contamination observed in this study.

## Acknowledgements

We are grateful to Dr. Ernesto Hofer (Brazil) for performing serological characterization of *Listeria* species and to Dr. J.M. Farber (Canada) for providing the strains of *Listeria*. CAPES, Brazil supported this study.

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