ENTEROXIGENIC STAPHYLOCOCCUS SPP. AND OTHER MICROBIAL CONTAMINANTS DURING PRODUCTION OF CANASTRA CHEESE, BRAZIL

Beatriz M. Borelli; Elaine G. Ferreira; Inayara C. A. Lacerda; Deise A. Santos; Luiz S. Carmo; Ricardo S. Dias; Maria Crisolita C. Silva; Carlos A. Rosa

1 Departamento de Microbiologia, ICB, CP. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; 2 Laboratório de Enterotoxinas, Fundação Ezequiel Dias, Belo Horizonte MG, Brazil

Submitted: January 03, 2006; Returned to authors for corrections: March 23, 2006; Approved: October 13, 2006

ABSTRACT

Canastra cheese is produced from raw cow’s milk, and it is made at the farmhouse level using artisanal procedures and natural starters. The aim of this work was to determine the main hygienic-sanitary indicators and enterotoxigenic staphylococcal strains present during the manufacturing of traditional cheese of Serra da Canastra region, Minas Gerais state, Brazil. Samples from 10 farms were studied, and they included: water employed in the process, raw milk, natural starters, cheese curd before salting and cheese after five days of ripening. All water samples exhibited faecal coliform contamination above the maximum acceptable value recommended by Brazilian standards. Pseudomonas aeruginosa and sulfite-reducing clostridia were also isolated from the water samples. In five samples of raw milk faecal coliform were above the limits allowed by the Brazilian legislation. The counts of Staphylococcus spp. in milk were between <2.0 to 4.9 log.cfu.ml⁻¹. The counts of microbiological indicators were higher in natural starters and curd. High levels of faecal and total coliform, as well as molds, were found in the cheese samples. In all cheeses analyzed Staphylococcus spp. were found in levels above 5.0 log.cfu.g⁻¹. The enterotoxins (SE) most frequently produced by Staphylococcus spp. strains were SEB and SEC. A high number of coagulase negative Staphylococcus strains were also enterotoxin producers. None of the samples contained Salmonella spp. or Listeria spp. These results point out a need for improvements in the production process of the artisanal cheese produced at Serra da Canastra in Brazil.

Key words: Artisanal cheese, coliforms, Staphylococcus, enterotoxin

INTRODUCTION

Serra da Canastra cheese is traditionally made with raw cow’s milk employing natural starters (indigenous lactic acid bacteria) and commercial rennet. The cheeses produced in the region of Serra da Canastra have been manufactured in a traditional empirical manner for more than 200 years in Minas Gerais state, Brazil (25). The natural starter used in its production is composed by species of Lactobacillus, Lactococcus and Streptococcus, with counts of approximately 8 log cfu.ml⁻¹, and it is produced by dropping whey from previous cheese covered with salt (B. M. Borelli & C. A. Rosa, unpublished data). The production of Canastra cheese is about 375.5 tons per month, being the main economic activity of many families of the region (14). Nevertheless, there is no standardization of the manufacturing process, especially regarding time of coagulation, natural starter used, pressing, salt and humidity in the final product. Thus, it is possible to find Canastra cheeses in the market having different and characteristic flavors and aromas.

As Canastra cheese is an artisanal product, the production process does not follow safety standards regularly. Use of raw milk, for example, is a cause for worry because it constitutes an important source of pathogens such as Staphylococcus aureus, Escherichia coli, Salmonella spp., Shigella spp., Listeria monocytogenes, among others (6,16,20). The presence of S. aureus and the possibility of staphylococcal toxin production...
represent a potential risk for public health (3,4,17,22). Staphylococcal enterotoxins (SEs) are a family of nine thermostable, pepsin-resistant exoproteins forming a single chain with a molecular weight ranging from 26,000 to 29,600 Da (10,17). They are identifiable serologically and known as SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI and SEJ. Most staphylococcal strains are able of producing one or more enterotoxins, which are the cause of the gastrointestinal symptoms, that include vomiting with or without diarrhea, observed during intoxications (17). Staphylococcus spp. coagulase negative strains that produce enterotoxins have been isolated, but there is very little information about their role in food poisoning (10). In Brazil, the commercialization of Canastra cheese produced from raw milk is common, although Brazilian food safety regulations for cheeses made from raw milk recommend that those products be made available only after ripening periods of at least 60 days (7). There is little information available on the microbiological characteristics of artisanal Brazilian cheeses (13,23). The aims of this study were: (i) to determine counts of total and faecal coliforms, aerobic mesophilic bacteria, Staphylococcus spp. and S. aureus, molds; (ii) to establish the presence of Salmonella spp. and Listeria spp. and (iii) to investigate the evolution of enterotoxigenic Staphylococcal strains during the manufacturing of the artisanal cheese produced in region of Serra da Canastra, Brazil.

MATERIAL AND METHODS

Sample collection

Samples were obtained from ten different farms (A to J) in the city of São Roque de Minas, State of Minas Gerais, Brazil, in December 1999 and February 2000. Water, raw milk, natural starter, cheese curd before salting and cheese after five days of ripening at room temperature from each farm were sampled aseptically, and transported to the laboratory under refrigeration for microbiological analyses.

Microbiological analysis

Water samples

Water samples were surveyed for the presence of total and faecal coliforms, sulfate-reducing clostridia, enterococci and Pseudomonas aeruginosa, according to standard methods (11) and compared to safety standards of the Brazilian Health Legislation (8). Total and faecal coliform, enterococci and P. aeruginosa counts were determined by the most probable number (MPN) method and enumeration of sulfate-reducing clostridia was made in reinforced clostridial medium (11).

Milk, natural starter, curdle and cheese samples

Aliquots of 10 ml of milk and natural starter were diluted in 90 ml sterile 0.1% buffered peptone water before inoculation. For curdle and cheese 25g portions were homogenized with 225 ml of 0.1% buffered peptone water in a Stomacher 400 Lab Blender (London, UK) for 1 minute and decimal dilutions were prepared therefore using the same diluent. Aerobic mesophilic bacteria were determined on plate count agar (Difco Laboratories, Detroit, USA); occurrence of molds was determined on potato dextrose agar (Difco) acidified with 10 ml/l of 10% (w/v) sterile tartaric acid by pour plating. The plates were incubated at 30°C for 48 h for mesophilic microorganisms, and 25°C for five days for molds (11). All microbiological analyses were performed in triplicate.

Total and faecal coliform counts were determined by the Most Probable Number (MPN) method using a three-tube series. Total coliforms were enumerated in 2% bile brilliant green broth (Difco), incubated at 37°C for 24-48 h; faecal coliforms were determined in EC broth (Difco) incubated at 44°C for 24 h (11).

Salmonella detection was carried out after pre-enrichment in buffered peptone-water and enrichment in selenite cystine broth (Biobrás, MG, Brazil) and Rapaport-Vassiliads broth (Biobrás), incubated at 35°C and 42°C for 24 h, respectively. Enrichment cultures were streaked onto Salmonella-Shigella agar (Biobrás) and Hektoen enteric agar (Biobrás). The plates were incubated at 35°C for 48 h (11). The typical colonies were identified by Triple Sugar Iron (TSI) agar (Oxoid, Ltd. Basingstoke, Hampshire, England), Lyssine Iron Agar (LIA) (Oxoid) fermentation tests, urease test (Urea Broth, Oxoid) and serological tests such as polyvalent flagellar (H) and polyvalent somatic (O) tests (murex Salmonella polyvalent agglutinating sera) (11).

The presence of Listeria was established by homogenization of either 25g or 25ml of the sample with 225ml primary Listeria enrichment broth (LEB I, Merck, Darmstadt, Germany) in a Stomacher 400 Lab Blender. The enrichment broth was incubated at 35°C for 48 h. LEB I cultures were transferred to secondary Listeria enrichment broth (LEB II, Merck) and incubated at 35°C for 48 h. After incubation, LEB II cultures were streaked onto Palcam agar (Sigma, St. Louis, MO, USA) and Oxford agar (Sigma) and the plates were incubated at 35°C for 48 h and analyzed for the presence of Listeria colonies (11). The purified isolates were identified by examination of TSAYE (triplicate soy agar plus 0.6% yeast extract) plates with oblique Henry illumination, Gram staining, examination of catalase activity, rotating or tumbling motility, hemolysis zone on blood agar, motility in SIM medium (hydrogen sulfide production, indole formation and motility) (Oxoid) for typical umbrella shape, and carbohydrate fermentation tests in Purple Carbohydrate Broth, and the CAMP test (11).

Staphylococcus spp. were counted on Baird-Parker agar (Biobrás) with added egg yolk tellurite, incubated at 37°C for 48 h. After growth, Staphylococcus colonies were counted and classified as typical for S. aureus (jet black to dark gray, smooth, convex, entire margins with an opaque zone, clear halo beyond the opaque zone) and atypical (jet black to dark gray colonies,
Contaminants in canastra cheese

entire margin without a halo). Ten colonies from each sample (5 typical and 5 atypical) were selected and transferred to individual tubes with nutrient agar (stock culture), and tested for coagulase, thermonuclease (TNase), anaerobic fermentation of glucose and mannitol, and production of hemolysin on sheep’s blood agar (11).

Toxin production by Staphylococcus species

Strains of Staphylococcus spp. from the same sample and exhibiting similar physiological and biochemical profiles were pooled for testing. Individual pools comprised one to ten strains. Enterotoxin quantification was performed using the membrane-over-agar method with subsequent optimum-sensitivity-plating as described by Bergdoll (5).

RESULTS AND DISCUSSION

Water used in the manufacture of Canastra cheese exhibited high levels of contamination by coliform bacteria (Table 1). None of the water samples tested was in conformity with the standards set by the Brazilian’s Ministry of Health (8), which defines the absence of faecal coliforms or E. coli in 100 ml of water as standard. Also, the presence of P. aeruginosa and sulfite reducing clostridia would offer risk to public health, probably leading to contamination of cheese, with consequent reduction of shelf life. Sulfite reducing clostridia are able to produce enzymes that cause late swelling in cheeses (12,19,21). Pseudomonas aeruginosa also produces thermoresistant proteases and lipases that could alter organoleptic properties of the final product. In addition, these bacteria represent a serious risk to public health since P. aeruginosa is frequently associated to infections in immunocompromised patients (15). Our results suggest that the water employed in cheese manufacturing could represent a source of contamination of the final product.

Microbiological analysis of raw milk revealed that 40% of samples did not exhibit detectable counts of faecal coliforms (less than 0.3 MPN.ml⁻¹) (Table 2). The other 60% of samples exhibited faecal coliform counts varying from 2.3 to 24.0 MPN.ml⁻¹. These counts were lower than those observed by Tornadijo et al. (24) in raw cow’s milk. The counts of aerobic mesophilic bacteria and Staphylococcus spp. present in the raw milk ranged from 2.6 to 7.0 and <2.0 to 4.9 log cfu.ml⁻¹, respectively (Table 2). The counts of faecal coliforms, aerobic mesophilic bacteria and Staphylococcus spp. in milk are not related only to the poor hygienic conditions during milking; they are also associated to storage temperature of milk, without refrigeration before cheese making. Our results show that milk is a source of contamination of artisanal cheeses; thus, control measures in milking are needed for a better quality of the product.

Table 1. Microbial counts obtained of water from ten cheese producing farms in the region of the Serra da Canastra, MG, Brazil.

| Farms | Total coliforms (MPN/100 ml)¹ | Faecal coliforms (MPN/100 ml) | Pseudomonas aeruginosa (MPN/100 ml) | Enterococci (MPN/100 ml) | Sulfite reducing Clostridium (MPN/100 ml) |
|-------|-------------------------------|-------------------------------|------------------------------------|--------------------------|-----------------------------------------|
| A     | >16.0                         | >16.0                         | 5.1                                | <2.2                     | <2.2                                    |
| B     | >16.0                         | >16.0                         | >16.0                              | <2.2                     | <2.2                                    |
| C     | >16.0                         | >16.0                         | 9.2                                | <2.2                     | <2.2                                    |
| D     | >16.0                         | >16.0                         | 16.0                               | <2.2                     | <2.2                                    |
| E     | >16.0                         | >16.0                         | >16.0                              | <2.2                     | <2.2                                    |
| F     | 16.0                          | 16.0                          | 16.0                               | <2.2                     | 2.2                                     |
| G     | >16.0                         | 2.2                           | >16.0                              | <2.2                     | 5.1                                     |
| H     | >16.0                         | 16.0                          | <2.2                               | <2.2                     | 16.0                                    |
| I     | 16.0                          | 16.0                          | 5.1                                | <2.2                     | 5.1                                     |
| J     | >16.0                         | >16.0                         | >16.0                              | <2.2                     | 2.2                                     |

¹ MPN: Most Probable Number.
log cfu g⁻¹, in most samples (Table 2). These results were similar to those found in other cheeses, such as Serrano (23) and Tetila (16). High levels of contamination with Staphylococcus spp. were also found in curd and cheese samples (Table 2). About 70% of the Canastra cheeses were contaminated with S. aureus with population counts varying from less than 4.8 log cfu g⁻¹ to 6.3 log cfu g⁻¹ (Table 2). Albenzio et al. (2) observed that the Italian Canestrato Pugliese cheeses produced with raw milk exhibited Staphylococcus counts around 4.1 log cfu g⁻¹. Staphylococcus aureus is often found in raw milk and in cheese-making environment. This microorganism is salt-tolerant and has the ability to grow under very different conditions; low acid production may allow staphylococci to grow and produce enterotoxins (4, 18). Salmonella spp. and Listeria spp. were not found in samples of raw milk, natural starter, curd or in cheese collected in this study.

Out of the 75 Staphylococcus spp. pools tested for the production of enterotoxin and TSST-1 toxin, 70 (93.3%) produced at least one of those toxins (Table 3). Cardoso et al. (9) found that 65% of 127 strains of S. aureus isolated from milk of cows suffering from mastitis produced either enterotoxin or TSST-1 toxin; the latter was most frequently produced (47% of positive samples). Carmo et al. (10), studying an outbreak of food poisoning caused by ingestion of raw milk and Minas cheese, isolated SEA, SEB and SEC from enterotoxigenic strains of S. aureus.

Although S. aureus is the most common staphylococcal species involved in outbreaks of food poisoning, two other coagulase positive species, S. intermedius and S. hyicus, have been cited as enterotoxigenic (1, 4). In our study, S. intermedius and S. hyicus isolated from milk and curd have produced enterotoxin B and C. Most pools of S. hyicus producing enterotoxin were from milk, curd and cheese samples. Pools of

---

Table 2. Microbial counts obtained for samples of raw milk, natural starter, curdle and Canastra cheese from ten farms in the region of the Serra da Canastra, MG, Brazil.

| Microorganisms     | Farms       |
|--------------------|-------------|
|                    | A | B | C | D | E | F | G | H | I | J |
| Raw Milk           |   |   |   |   |   |   |   |   |   |   |
| Total coliforma    |   |   |   |   |   |   |   |   |   |   |
| Faecal coliforma   |   |   |   |   |   |   |   |   |   |   |
| Staphylococcus spp.|   |   |   |   |   |   |   |   |   |   |
| Staphylococcus aureus |   |   |   |   |   |   |   |   |   |   |
| Mesophilic bacteria|   |   |   |   |   |   |   |   |   |   |
| Natural starter    |   |   |   |   |   |   |   |   |   |   |
| Total coliforma    |   |   |   |   |   |   |   |   |   |   |
| Faecal coliforma   |   |   |   |   |   |   |   |   |   |   |
| Staphylococcus spp.|   |   |   |   |   |   |   |   |   |   |
| Staphylococcus aureus |   |   |   |   |   |   |   |   |   |   |
| Moulds             |   |   |   |   |   |   |   |   |   |   |
| Curd               |   |   |   |   |   |   |   |   |   |   |
| Total coliforma    |   |   |   |   |   |   |   |   |   |   |
| Faecal coliforma   |   |   |   |   |   |   |   |   |   |   |
| Staphylococcus spp.|   |   |   |   |   |   |   |   |   |   |
| Staphylococcus aureus |   |   |   |   |   |   |   |   |   |   |
| Moulds             |   |   |   |   |   |   |   |   |   |   |
| Cheese             |   |   |   |   |   |   |   |   |   |   |
| Total coliforma    |   |   |   |   |   |   |   |   |   |   |
| Faecal coliforma   |   |   |   |   |   |   |   |   |   |   |
| Staphylococcus spp.|   |   |   |   |   |   |   |   |   |   |
| Staphylococcus aureus |   |   |   |   |   |   |   |   |   |   |
| Moulds             |   |   |   |   |   |   |   |   |   |   |

a MPN g⁻¹ or ml⁻¹ (Most probable number); b log cfu g⁻¹ or ml⁻¹ (Colony forming units).
Contaminants in canastra cheese

| Species                              | Number of tested “pools” | SEA | SEB | SEC | SED | TSST-1 | Number of “pools” negative for toxin |
|--------------------------------------|--------------------------|-----|-----|-----|-----|--------|--------------------------------------|
| Milk                                 | 15                       | 3   | -   | 2   | 3   | -      |                                      |
| Staphylococcus aureus                | 3                        | -   | 2   | 3   | -   | -      |                                      |
| Staphylococcus coagulase negative    | 7                        | -   | 5   | 3   | 1   | 3      | 1                                    |
| S. hyicus                           | 5                        | -   | 4   | 2   | 1   | 1      | -                                    |
| Natural starter                      | 15                       | 5   | 2   | 2   | 2   | -      | -                                    |
| S. aureus                           | 9                        | -   | 7   | 7   | 1   | 3      | -                                    |
| S. coagulase negative               | 1                        | -   | 1   | 1   | -   | -      | -                                    |
| S. intermedius                      | 13                       | 1   | 9   | 7   | -   | 1      | 1                                    |
| Staphylococcus coagulase negative    | 5                        | -   | 4   | 2   | -   | 1      | 1                                    |
| S. hyicus                           | 7                        | 1   | 5   | 6   | 2   | 3      | -                                    |
| S. intermedius                      | 1                        | -   | 1   | 1   | 1   | -      | -                                    |
| Curd                                | 26                       | 1   | 9   | 7   | -   | 1      | 1                                    |
| S. aureus                           | 13                       | 1   | 9   | 7   | -   | 1      | 1                                    |
| S. coagulase negative               | 5                        | -   | 4   | 2   | -   | 1      | 1                                    |
| S. hyicus                           | 7                        | 1   | 5   | 6   | 2   | 3      | -                                    |
| S. intermedius                      | 1                        | -   | 1   | 1   | 1   | -      | -                                    |
| Cheese                              | 19                       | 2   | 7   | 3   | 1   | 1      | 1                                    |
| S. aureus                           | 10                       | 2   | 7   | 3   | 1   | 1      | 1                                    |
| S. coagulase negative               | 7                        | -   | 7   | 6   | 2   | 3      | -                                    |
| S. hyicus                           | 2                        | -   | 1   | 1   | 2   | 2      | -                                    |
| Total                               | 75                       | 6   | 55  | 44  | 9   | 18     | 5                                   |

* Individual pools comprised one to ten strains of Staphylococcus spp. from the same sample and exhibiting similar physiological and biochemical profiles.

coagulase negative Staphylococcus, mainly from natural starter and cheese samples, were positive for production of enterotoxin B, C and D, and TSST-1 toxin. Carmo et al. (10) found that coagulase negative strains, from raw milk samples that were associated to food poisoning, produced enterotoxins C and D. These findings point to the need for research on toxin production by coagulase negative Staphylococci even if there are no legal requirements for that. Detection of enterotoxigenic strains of coagulase negative Staphylococcus is of great importance for public health, although a Brazilian standard for those microorganisms has not yet been defined. The presence of enterotoxigenic Staphylococcal strain in milk and cheeses represents a risk to for consumers, even in low numbers (10,17). It is therefore imperative to ensure that milk used in the manufacturing is of the highest bacteriological quality. The use of pasteurized milk could be an alternative to improve the microbial quality of the Canastra cheese, associated to specific starter strain.

We conclude that sanitary measures are needed to improve the hygienic conditions during milking and manufacturing cheese in order to guarantee the quality of this highly popular cheese in Brazil. These measures must include a program of sanitary education for the milking personnel and cheese producers with emphasis on hygiene standards as well as technical and practical aspects of milking.

ACKNOWLEDGMENTS

This work was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brasil (CNPq - Process no. 62.0477/98-2), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

RESUMO

Staphylococcus spp. enterotoxigênicos e outros contaminantes microbiológicos durante a produção de queijo Canastra, Brasil

O queijo Canastra é produzido a partir de leite cru, e é fabricado em fazendas utilizando procedimentos artesanais e culturas naturais de soro como iniciadoras. O objetivo deste trabalho foi determinar os principais indicadores higiênico-sanitário e as linhagens enterotoxigenicas de Staphylococcus
presentes durante a fabricação do queijo tradicional da região da Serra da Canastra, Minas Gerais, Brasil. Amostras provenientes de 10 fazendas foram estudadas, e estas incluíram: a água utilizada no processo, o leite cru, o soro iniciador, a coalhada antes da salga, e o queijo após cinco dias de cura. Todas as amostras de água apresentaram contaminação por coliformes fecais acima do valor máximo recomendado pelos padrões brasileiros. Pseudomonas aeruginosa e clostrídios sulfito-redutores também foram isolados das amostras de água. Em cinco amostras de leite cru os coliformes fecais apresentaram-se acima dos limites permitidos pela legislação brasileira. As contagens de Staphylococcus spp. foram variadas entre <2,0 a 4,9 log ufc ml⁻¹. As contagens dos indicadores microbiológicos foram maiores no soro iniciador do que na massa coagulada. Níveis altos de contaminação por coliformes totais e fecais, como também bolores, foram observados nas amostras de queijo. Em todos os queijos estudados Staphylococcus spp. foram encontrados em níveis acima de 5,0 log ufc g⁻¹. As enterotoxinas mais frequentes produzidas pelas linhagens de Staphylococcus spp. foram B e C. Um número elevado de linhagens de Staphylococcus coagulase-negativa foram também produtoras de enterotoxinas. Em nenhuma das amostras foi isolada Salmonella spp. ou Listeria spp. Estes resultados mostram a necessidade de melhorias no processo de produção do queijo artesanal produzido na Serra da Canastra, Brasil.

Palavras-chave: Queijo artesanal, coliformes, Staphylococcus, enterotoxinas

REFERENCES

1. Adesiyun, A.A.; Tatini, S.R.; Hoover, D.G. (1984). Production of enterotoxin by Staphylococcus hyicus. Vet. Microbiol., 9, 487-495.
2. Albénzio, M.; Corbo, M.R.; Rehman, S.U.; Fox, P.F.; De Angelis, M.; Corsetti, A.; Sevi, A.; Gobbetti, M. (2002). Microbiological and biochemical characteristics of Canestrato Pugliese cheese made from raw milk, pasteurized milk or by heating the curd in hot whey. Int. J. Food Microbiol., 20, 35-48.
3. Balaban, N.; Rassoly, A. (2000). Staphylococcal enterotoxin. Int. J. Food Microbiol., 61, 1-10.
4. Bergdoll, M.S. (1989). Staphylococcus aureus. In: Doyle, M.P. (ed.) Foodborne bacterial pathogens. Marcel Dekker, New York, USA, p.463-523.
5. Bergdoll, M.S. (1995). Importance of staphylococci that produce nanogram quantities of enterotoxin. Zent. Bakteriol., 282, 1-6.
6. Bintsis, T.; Papademas, P. (2002). Microbiological quality of white-brined cheeses: a review. Int. J. Dairy Technol., 55, 113-120.
7. Brasil. Portaria nº 146, de 7 de março de 1996. Aprova Regulamento Técnico de Identidade e qualidade de Queijos. Diário Oficial da União. Brasília, 11 de março de 1996, seção I, 3977-3979, 1996.
8. Brasil. Portaria nº 518, de 25 de março de 2004. Estabelece os procedimentos e responsabilidades relativos ao controle e vigilância da qualidade da água para consumo humano e seu padrão de potabilidade, e dá outras providências. Ministério da Saúde, 2004. Diário Oficial da União, Brasília, 26 de março de 2004.
9. Cardoso, H.F.T.; Carmo, L.S.; Silva, N. (2000). Detecção da toxina-1 da síndrome do choque tóxico em amostras de Staphylococcus aureus isoladas de mastite bovina. Arq. Bras. Med. Vet. Zoot., 52, 15-28.
10. Carmo, L.S.; Dias, R.S.; Linardi, V.R.; Sena, M.J.; Santos, D.A.; Pena, E.C. (2002). Food poisoning due to enterotoxigenic strain of Staphylococcus present in Minas cheese and raw milk in Brazil. Food Microbiol., 19, 9-14.
11. Downes, F.P.; Ito, K. (2001). Compendium of Methods for The Microbiological Examination of Foods. American Public Health Association, Washington, D.C.
12. Furtado, M.M. (1999). Principais problemas dos queijos causas e prevenção. 1ª Edição. São Paulo: Fonte Comunicações e Editora.
13. Gomes, M.I.F.V.; Bonassi, L.A. (1995). Aspecto microbiológico do queijo Minas tipo prensado. Rev. Inst. Lat. Cândido Tostes, 50, 23-26.
14. Instituto Mineiro de Agropecuária. (1999). Programa Selo Azul de Qualidade - Projeto Pró-Queijo Canastra. Belo Horizonte, IMAsco.
15. Legnani, P.; Leoni, E.; Rapuano, S.; Turin, D.; Valenti, C. (1999). Survival and growth of Pseudomonas aeruginosa in natural mineral water: a 5-year study. Int. J. Food Microbiol., 53, 155-158.
16. Menéndez, S.; Godínez, R.; Centeno, J.A.; Rodríguez-Otero, J.L. (2001). Microbiological, chemical and biochemical characterization of ‘Tetila’ raw cows-milk cheese. Food Microbiol., 18, 151-158.
17. Normanno, G.; Finiru, A.; Virgilio, S.; Mula, G.; Dambrosio, A.; Poggio, A.; Decastelli, L.; Mioni, R.; Scuota, S.; Bolzoni, G.; Di Giannatale, E.; Salinetti, A.P.; La Salandra, G.; Bartoli, M.; Zuccon, F.; Pirino, T.; Sias, S.; Parisi, A.; Quaglia, N.C.; Celano, G.N. (2005). Coagulase-positive Staphylococci and Staphylococcus aureus in food products marketed in Italy. Int. J. Food Microbiol., 98, 73-79.
18. Olarte, C.; Sanz, S.; Gonzalez-Fandos, E.; Torre, P. (1999). Microbiological and physicochemical characteristics of Camerino cheese. Food Microbiol., 16, 615-621.
19. Papageorgiou, D.K.; Abraham, A.; Bori, M.; Doundounakis, S. (1998). Chemical and bacteriological characteristics of Pichtogalo Chania cheese and mesophilic starter cultures for its production. J. Food Prot., 61, 688-692.
20. Pinto, P.S.A.; Germano, M.I.S.; Germano, P.M.L. (1996). Queijo Minas: problema emergente da Vigilância Sanitária. Hig. Alim., 10, 22-27.
21. Soriano, J.M.; Font, G.; Rico, H.; Molto; J.C. Manes; J. (2002). Incidence of enterotoxigenic staphylococci and their toxins in foods. J. Food Prot., 65, 857-860.
22. Souza, C.F.V.; Rosa, T.D.; Avub, M.A.Z. (2003). Changes in the microbiological and physicochemical characteristics of Serrano cheese during manufacture and ripening. Braz. J. Microbiol., 34, 260-266.
23. Tornadijo, M.E.; García, M.C.; Fresno, J.M.; Carballo, J. (2001). Study of Enterobacteriaceae during the manufacture and ripening of San Simón cheese. Food Microbiol., 18, 499-509.
24. Vargas, O.L.; Porto, M.A.; Brito, A.L. (1998). Características de orígenes para queijos naturais de Minas Gerais: municípios do Serro e de São Roque de Minas. Rev. Inst. Lat. Cândido Tostes, 53, 19-49.