

Determination and characterization of pathogens found in dairy products

Determinação e caracterização de agentes patogênicos encontrados em produtos lácteos

RIALA6/1231

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Recebido: 14.08.2009 – Aceito para publicação: 23.11.2009

ABSTRACT

The quality of dairy products was investigated on 63 samples, being 24 nonindustrial-type ice creams, 19 pasteurized milk creams and 20 of cheese pâtés. The samples were analyzed for searching thermo-tolerant coliforms, coagulase positive *Staphylococcus*, *Salmonella* spp., molds and yeasts, *Bacillus cereus* and *Listeria monocytogenes*. Additionally, characterization of the enterotoxigenic potential of *B. cereus* for the codified genes of enterotoxins NHE and HBL was also carried out by PCR technique; also the molecular subtyping of *L. monocytogenes* allelic polymorphism of virulence genes *actA* and *hly* was performed. Thirty-three percent (8/24) of ice cream samples and 15.8% (3/19) of milk cream samples did not comply with the microbiological standards established by RDC N°12/2001 of ANVISA, because these products showed thermo-tolerant coliform counts above the established limits. *L. monocytogenes* was detected in one milk cream sample and those bacteria isolates were identified as belonging to the lineage I, which is potentially pathogenic to human. Potentially enterotoxigenic *B. cereus* was isolated from the three kinds of products analyzed in the present study. The degree of microbiological contamination found in this study reflects the inadequate hygienic-sanitary conditions in processing the dairy products, indicating the necessity of a best action, surveillance and control by sanitary authorities.

Keywords. *Listeria monocytogenes*, *Bacillus cereus*, Dairy Products, Pathogenicity, Polymerase Chain Reaction.

RESUMO

A qualidade de produtos derivados do leite foi verificada em 63 amostras, sendo 24 de sorvetes artesanais, 19 de cremes de leite pasteurizados e 20 de pâtés de queijo. As análises microbiológicas foram realizadas para averiguar a presença de coliformes termotolerantes, estafilococos coagulase positiva, *Salmonella* spp., bolores e leveduras, *Bacillus cereus* e *Listeria monocytogenes*. Além disso, foram realizados ensaios para efetuar a caracterização do potencial enterotoxigênico de *B. cereus* quanto aos genes codificadores das enterotoxinas NHE e HBL por meio de técnica PCR e a subtipagem molecular dos genes *actA* e *hly* de *L. monocytogenes*. As 33% (8/24) de amostras de sorvetes e 15,8% (3/19) de amostras de cremes de leite estavam em desacordo com os padrões microbiológicos estabelecidos para coliformes termotolerantes, de acordo com a RDC N°12/2001 da ANVISA. A presença de *L. monocytogenes* foi observada em apenas uma amostra de creme de leite e os isolados da bactéria foram identificados como pertencentes à linhagem I, que é potencialmente patogênica ao homem. *B. cereus* com potencial enterotoxigênico foi isolado nos três grupos de alimentos estudados. O perfil de contaminação microbiológica observado nos produtos avaliados neste estudo serve de alerta para que os produtores melhorem as condições higiênico-sanitárias no processamento e da necessidade das autoridades sanitárias em estabelecer maior controle desses produtos.

Palavras-chave. *Listeria monocytogenes*, *Bacillus cereus*, Produtos Lácteos, Patogenicidade, Reação em Cadeia da Polimerase.

INTRODUCTION

Milk and dairy products constitute an excellent medium for the development of deteriorative and pathogenic microorganisms. The presence of pathogens in milk products has been a concerning question of public health, since they have contributed to innumerable outbreaks of foodborne diseases¹.

The genera *Salmonella*, *Staphylococcus*, *Bacillus* and *Listeria* are amongst the main microorganisms involved in the contamination of dairy products. Infections caused by the genera *Salmonella* are currently considered as the most important causes of foodborne diseases², and outbreaks involving *Salmonella enteritidis* in ice creams have been reported in the United States³ and England^{4,5}.

Another important microorganism prevalent in ice creams is *Staphylococcus aureus*^{6,7,8,9}, which is the agent of intoxication caused by the ingestion of a thermostable toxin preformed in foods. In Brazil, the presence of toxigenic *Staphylococci*, both coagulase positive and negative, has led to two outbreaks associated with the consumption of fresh Minas-type cheese and raw milk, which was evidenced as a microorganism frequently isolated in these foods^{10,11}.

Contamination by *Bacillus cereus* is highly significant, not only because it is widely spread in the environment, presenting spores resistant to heat treatment¹², but also due to its potential to cause emetic diseases and diarrhea. Gastroenteritis is attributed to the production of enterotoxins, including the hemolytic (HBL) and non-hemolytic (NHE) complexes¹³. Amongst other foods, this disease has been associated with the consumption of milk and dairy products² and some studies have isolated the microorganism in pâtés and ice creams^{14,15,16}, dairy creams¹⁷, and raw and pasteurized milk^{18,19,20}.

Listeria monocytogenes is considered to be a pathogen of considerable importance in public health, causing infection in humans which, due to the gravity of the disease, can lead to death¹². This pathogen has been detected in various foods, but is more common in raw milk and cheeses^{21,22,23,24}.

Genetic studies of virulent genes in *L. monocytogenes* isolates have shown the existence of three genetically distinct lineages, and by the assessment of the allelic polymorphism of the virulence genes *actA* and *hly*, may be divided into lineages I, II and III, with

lineage I being considered to be the one with the greatest pathogenic potential for humans²⁵.

Considering the important participation of dairy products in the population nutrition and the lack of surveys with respect to the innocuousness of ice creams, dairy creams and pâtés in Brazil, the aim of this study was to identify the hazards associated with the consume of these products, sold in the city of Campinas-SP, and the consequent compliance with the microbiological pattern established by the Brazilian National Agency for Sanitary Surveillance (ANVISA), the RDC 12/01²⁶; in addition, to verify the presence of *Bacillus cereus* potentially enterotoxigenic and *Listeria monocytogenes* in these products.

MATERIAL AND METHODS

Sampling

Sixty-three samples of dairy products commercialized at Campinas city (SP, Brazil) were analyzed, consisting of 24 samples of artisan-type ice creams with three different flavors produced in four local ice cream manufacturers, 19 samples of pasteurized dairy creams from six different brands and 20 samples of cheese pâtés from different brands and with varied flavors, sold in supermarkets. The samples were transported to the laboratory in isothermal boxes with ice. Ice creams were stored at freezing temperature (-17°C), and the other products at 4°C, until analyzed.

Microbiological analyses

The microbiological analyses for the thermo-tolerant coliforms, coagulase positive staphylococcal count, detection of *Salmonella* spp., *Bacillus cereus* count and the yeast and mold count were all carried out according to the methodologies commended by the *American Public Health Association*²⁷. The presence of *Listeria monocytogenes* was evaluated using the methodology proposed by the *Canadian Health and Food Branch*²⁸.

The thermo-tolerant coliforms count was carried out using the Most Probable Number (MPN) technique in a series of three tubes containing lauryl sulphate tryptose broth (LST, Oxoid), incubated at 35°C/24-48h, followed by transference to EC broth (Merck) and incubation at 45°C/24-48h. For the *B. cereus*, coagulase positive *Staphylococcus* and yeast and mold counts, the surface streaking technique was used on mannitol egg yolk polymixin agar (MYP, Difco) with incubation at

30°C/24h, Baird Parker agar (BP, Difco) with incubation at 35°C/48h, and dichloran Bengal rose chloramphenicol agar (DBRC, Oxoid) with incubation at 25°C/4 days, respectively. *B. cereus* and *Staphylococcus* spp. typical colonies were identified morphologically by the Gram stain and other specific biochemical tests²⁷.

To detect *Salmonella* spp., 25 grams of sample, pre-enriched in buffered peptone water (Oxoid) were incubated at 35°C/24h and then transferred to tetrathionate selective enrichment broth (Difco) and Rapaport Vassiliadis (Oxoid), with incubation at 35°C/24h and 42°C/24h, respectively. They were then transferred to the selective media xylose lysine desoxycholate agar (XLD, Difco), bismuth sulphite agar (BS, Difco) and Hektoen enteric agar (HE, Acumedia, Michigan) and incubated at 35°C/24h. Typical colonies were transferred to triple sugar iron agar (TSI, Difco) and lysine iron agar (LIA, Difco) and incubated at 35°C/24h. The isolates showing typical reactions in TSI and LIA were identified by complementary biochemical assays and serology²⁷.

The research of *L. monocytogenes* was carried out using 25 grams of samples, which were enriched in modified Listeria enrichment broth (LEB, Difco), incubated at 30°C/24-48h, followed by enrichment in modified Fraser broth (MFB, Difco), incubated at 35°C/24-48h. For isolation purposes, lithium chloride phenyl ethanol moxalactam agar (LPM, Difco) and Oxford agar (OXA, Difco) were used, and incubated at 30°C/48h and 35°C/48h, respectively. Typical colonies were identified by the morphological characteristics and biochemical tests²⁸.

Characterization of the enterotoxigenic potential of *B. cereus*

The enterotoxigenic potential of 25 isolates of *B. cereus* was evaluated by determining the presence of the genes *nheA*, *nheB* and *nheC*, codifiers for NHE complex, and *hblA*, *hblC* and *hblD* codifiers for HBL complex by the PCR (*Polymerase Chain Reaction*) technique. The isolates were inoculated in brain heart infusion broth (BHI, Difco) supplemented with 1% of the glucose (Difco) and incubated in shaker at 200rpm, at 32°C for 18 hours. To DNA extraction, 250 µl of the broth were centrifuged at 13.000 x g for 10 min. The supernatant was discharged, the bacteria cells were suspended in TE buffer [(Tris (Merck) 10mM, EDTA (Sigma) 1mM, pH=7,5] and boiled in water bath at 100°C for 10 minutes

as previously utilized²⁹. The primers and PCR reaction were carried out as described by Hansen; Hendriksen¹³.

Subtyping of *L. monocytogenes*

The molecular subtyping of 10 isolates of *L. monocytogenes* obtained from a sample of pasteurized dairy cream was carried out by evaluating the allelic polymorphism of the genes *actA* and *hly*. The gene *actA* was characterized by the PCR technique and the *hly* gene by the PCR-RFLP (Restriction Fragment Length Polymorphism) technique using the restriction enzymes *HhaI* and *HpaII*²⁵.

RESULTS AND DISCUSSION

The microbiological standards established for commercialized products in Resolution RDC nº 12/01 of the Brazilian National Agency for Sanitary Surveillance (ANVISA)²⁶, were adopted as the reference for the thermo-tolerant coliforms, coagulase positive *Staphylococcus*, *Salmonella* spp. and *B. cereus* counts in the samples of ice creams, dairy creams and pâtés. The frequency of samples not compliant with the patterns established for the foods analyzed, is shown in Table 1.

The percentage of samples not compliant with regulation was higher for ice creams, followed by dairy creams and pâtés. Thirty-three percent (8/24) of ice cream samples and 15.8% (3/19) of dairy cream exceeded the standard pattern by containing 1.7 log MPN/g and 1 log MPN/g of thermo-tolerant coliforms, respectively. The count of thermo-tolerant coliforms ranged from <0.5 to 3.4 log MPN/g in ice creams and <0.5 to 3.1 log MPN/g in dairy creams.

Another work in Brazil showed 58% of artisan-type ice creams over the maximum acceptable limit established by ANVISA for thermo-tolerant coliforms⁷. In Korea, Jo et al.³⁰ found coliforms counts in ice creams ranging from 1.7 to 2.4 log CFU/g. The high quantity of samples not compliant with the legal patterns found for thermo-tolerant coliforms in ice cream and dairy creams indicates contamination of fecal origin during processing, suggesting the presence of enteropathogens.

However, 5% (1/20) of the pâté samples were out of pattern (3 log CFU/g) by containing 4.5 log CFU/g of *B. cereus*. In contrast to the samples of dairy creams and ice creams, they did not present thermo-tolerant coliforms above the limit established for this product (2 log MPN/g).

Table 1. Percentage of samples not compliant with the microbiological criteria established in RDC 12/2001(Brazil)

Food	Number of samples	Number of non-compliant samples (%)			
		Thermo-tolerant coliforms	Coagulase positive <i>Staphylococcus</i>	<i>Salmonella</i> spp.	<i>Bacillus cereus</i>
Ice cream	24	8 (33.3)	0 (0.0)	0 (0.0)	Nd*
Dairy cream	19	3 (15.8)	0 (0.0)	0 (0.0)	Nd*
Pâté	20	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)

*Not determined as reference microbiological pattern (RDC N°12/01).

For the other established patterns, all the samples exhibited counts of coagulase positive *Staphylococcus* less than the legal limit of 2.0 log CFU/g, and absence of *Salmonella* spp. (**Table 1**), thus complying with the legislation. André et al.¹⁰, to the contrary, found 66.7% and 70.8% of samples positive for *S. aureus* in raw milk and Minas-type fresh cheese manufactured in Goiás, Brazil.

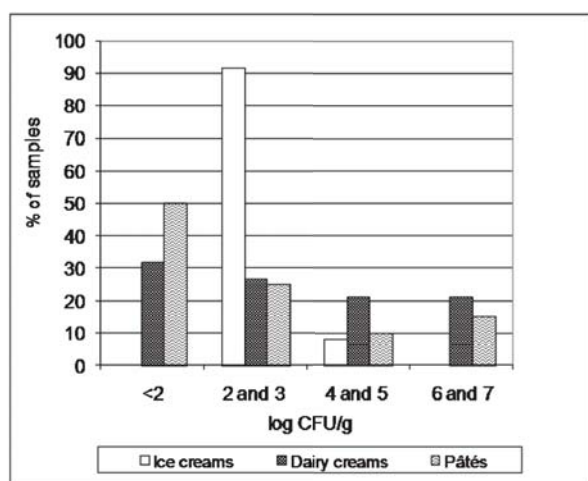


Figure 1. Frequency of Molds and Yeasts detected in milk products.

Determinations of molds and yeasts, *B. cereus* and *L. monocytogenes* in ice creams and pasteurized dairy creams, complementary to those required by Brazil regulation, were performed to establish largeness microbial profile for these products. The quantification of molds and yeasts, considered as indicators of the environmental conditions during processing, indicated the high counts (**Figure 1**). Variations ranged from 2.0

to 4.4 log CFU/g for ice creams, <2.0 to 6.5 log CFU/g for dairy creams and <2 to 7.7 log CFU/g for pâtés. In ice creams, the results were similar to those reported by Warke et al.³¹ and Kanbakan et al.⁹, and than the ones found by Jo et al.³⁰.

The elevated numbers of fecal coliforms, yeasts and molds found in the products evaluated, showed the need for control practices with respect to environmental contamination and the hygienic-sanitary condition of the production line, and also during the commercialization and handling of these products.

The complementary analysis for *B. cereus* were carried out since this microorganism is widely distributed in the environment and also because its spores are heat resistant and can survive pasteurization, which contributes to their presence in foods^{18,19}. **Figure 2** shows the results of the *B. cereus* counts found in the samples, varying from <2 to 3.1 log CFU/g in the ice creams, a result similar to that found by Warke et al.³¹. The count of 4.5 log CFU/g found in a single sample of pâté was similar to others values previously described^{14,16}. Dairy cream samples revealed higher counts (up to 7.4 log CFU/g), and for 10,5% (2/19) of the samples, the count level may represent risk for the development of diarrheic syndrome to consumer once the infective dose of *B. cereus* have been associated to 5 log of feasible cells³². This pathogen was also found in significant amounts in raw and pasteurized milk¹⁸ and other dairy products such as fresh Minas-type cheese and ricotta^{33,34}.

The present study and others already published^{18,33,34} evidence the need for authorities and producers to adopt *B. cereus* as a reference in microbiological hazard control, particularly for dairy products.

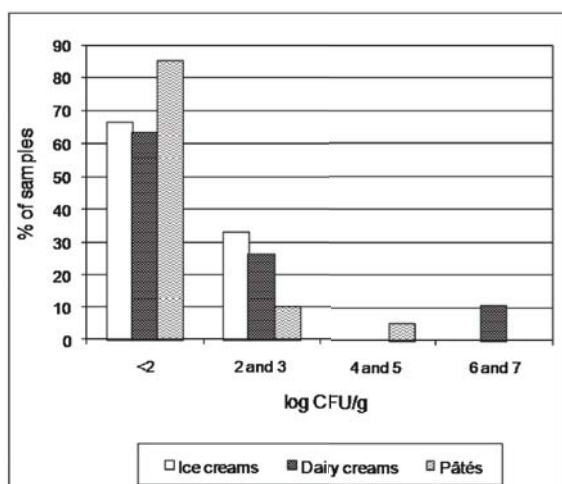


Figure 2. Frequency of *B. cereus* detected in milk products.

The characterization of the enterotoxigenic potential of *B. cereus* strains, performed via PCR of the coding genes of the main enterotoxins, NHE and HBL is shown in **Table 2**. From the 25 isolates of *B. cereus* proceeding from the milk products, 100% (25/25) were positive for at least one of the six genes tested, 32% (8/25) were positive for the three genes coding for the HBL hemolysin (*hblA*, *hblD* e *hblC*), and the three genes coding for the NHE enterotoxin (*nheA*, *nheB* e *nheC*) were detected in 76% (19/25) of the isolates. Other works with dairy products also observed the prevalence

of NHE complex genes in *B. cereus* isolates^{15,18,19,20,29,33}, confirming that these genes appear to be present in the majority of the isolates of this pathogen³⁵.

Among the 25 isolates of *B. cereus*, 6 distinct toxigenic profiles were characterized (**Table 2**). For ice creams, there was predominance of only one of the genes that code the NHE complex (61.5%). The isolates of dairy creams showed higher profile scope, and 33.4% had all the analyzed genes. Among the pâtés, only the three genes that express the NHE complex were detected in two isolates, and all the genes (HBL and NHE complexes) were detected in only one of the isolates.

The maximum biological activity of enterotoxins (HBL and NHE) depends on the presence of three proteins^{36,37}. Therefore, most of the isolates exhibit at least three HBL and/or NHE genes, and these are potentially capable of producing enterotoxins in favorable conditions.

The diversity among strains may increase the chances of the persistent environmental contamination to reach the finished products³⁸. The food exposure to air environmental during extended time, to improper hygiene conditions on counters and equipment, and to abusive temperatures are frequently associated to eventual contaminations by foodborne pathogens and enterotoxin production by *B. cereus*^{18,19,20}.

L. monocytogenes may be found in several foods, including pâtés^{39,40}, ice creams^{8,31,41} and dairy creams⁴¹. In our work, it was isolated only in one sample of pasteurized dairy cream and *Listeria welshimeri* was

Table 2. Enterotoxigenic profile of *B. cereus* isolated from ice creams, pâtés and pasteurized dairy creams

HBL			NHE			Foods		
<i>hblA</i>	<i>hblC</i>	<i>hblD</i>	<i>nheA</i>	<i>nheB</i>	<i>nheC</i>	Ice cream	Dairy cream	Pâté
+	+	+	+	+	+	23.1% (3/13)	33.4% (3/9)	33.3% (1/3)
-	-	-	+	+	+	61.5% (8/13)	22.2% (2/9)	66.7% (2/3)
-	-	-	+	-	+	7.7% (1/13)	22.2% (2/9)	0
-	+	+	-	-	-	7.7% (1/13)	0	0
+	+	+	+	+	-	0	11.1% (1/9)	0
-	-	+	+	+	-	0	11.1% (1/9)	0

Table 3. Frequency of *Listeria* species detected in milk product samples

Food	Number of samples analyzed	Number of positive samples (%)	Species
Dairy cream	19	1(5.25) 1(5.25)	<i>L. monocytogenes</i> <i>L. welshimeri</i>
Ice cream	24	1 (4.1)	<i>L. welshimeri</i>
Pâté	20	0	-

detected in two samples, one of dairy cream and one of ice cream (**Table 3**). Similarly, *L. monocytogenes* has been detected recently, with low frequencies (2.35% - 5.1%), in other dairy products such as cheeses, raw milk, yogurt and butter^{21,22,24,33}.

L. monocytogenes isolates from the dairy cream sample were genetically characterized by studying the allelic polymorphism of virulence genes *actA* and *hly*. The gene *actA* has 2 alleles named as types 3 and 4, according to the presence of 3 or 4 proline-rich repeats regions²⁵, and the type 4 was found among the isolates investigated. PCR-RFLP distinguishes 8 alleles for gene *hly*; allele 1 was identified in all the isolates evaluated, thus belonging to lineage I, and considered particularly pathogenic to human.

The analyses of *actA* and *hly* types enable us to separate *L. monocytogenes* in three lineages²⁵. Similarity in works with ricotta cheese and Latin-type fresh cheese, all the isolates were also lineage I^{23,33}. Based on the genetic characterization of the isolates, the pasteurized dairy cream may represent a product that exposes the consumer to risks, due to the presence of lineage I, potentially pathogenic and previously associated to human listeriosis outbreaks⁴².

This investigation has a great public health importance and must have the attention of dairy industries to apply the Good Manufacture Practices throughout the production chain, and of the competent authorities of public health, that must establish more strict microbiological parameters and controls to guarantee safety products to consumer and decrease the incidence of food poisonings and costs in the health sector.

CONCLUSIONS

Our findings highlights the high rate of thermo-tolerant coliforms contamination in artisan-type ice cream samples, far exceeding in some cases the regulation limit. Regarding the pâtés samples, the different rates of molds and yeasts and *B. cereus* may be associated to high environmental contamination. Further the pasteurized dairy creams were the products that showed a great potential risk due to the high contamination level of potentially enterotoxigenic *B. cereus* and the presence of *L. monocytogenes* virulent strain.

REFERENCES

- De Buyser ML, Dufour B, Marie M, Lafarge V. Implication of milk and milk products in food-borne disease in France and different industrialized countries. *Int J Food Microbiol* 2001; 67 (1-2): 1-17.
- Germano PML, Germano MIS. Higiene e Vigilância Sanitária de Alimentos. São Paulo: Varela, 2001.
- Henessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, Feldman J, Coleman WW, Edmonson LM, MacDonald KL, Osterholm MT. A national outbreak of *Salmonella enteritidis* infections from ice cream. *N Engl J Med* 1996; 334 (20): 1281-6.
- Djuretic T, Wall PG, Nichols G. General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. *Commun Dis Rep CDR Wkly* 1997; 7: 41-5.
- Dodhia H, Kearney J, Warburton F. A birthday party, home-made ice cream, and an outbreak of *Salmonella enteritidis* pahge type 6 infection. *Commun Dis Public Health* 1998; 1: 31-4.
- Armondes MP, Issy PN, André MCDPB, Serafini AB. Aspectos higiênico-sanitários de sorvetes produzidos artesanalmente na cidade de Goiânia, GO. *Hig Alim* 2003; 17 (107): 86-94.
- De Farias FE, Da Silva WR, Botelho ACN, Da Hora IMC, Kronenberger G, Da Cruz AG. Microbiological quality of ice creams

- commercialized in some cities in the state of Rio de Janeiro, Brazil. *Int J Dairy Technol* 2006; 59 (4): 261-4.
8. El-Sharef N, Ghenghesh KS, Abognah YS, Gnan SO, Rahouma A. Bacteriological quality of ice cream in Tripoli-Libya. *Food Control* 2006; 17: 637-41.
 9. Kanbakan U, Çon AH, Ayar A. Determination of microbiological contamination sources during ice cream production in Denizli, Turkey. *Food Control* 2004; 15: 463-70.
 10. André MCDPB, Campos MRH, Borges LJ, Kipnis A, Pimenta FC, Serafini AB. Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and Minas Frescal cheese by antibiogram and pulsed-field gel electrophoresis following SmaI digestion. *Food Control* 2008; 19: 200-07.
 11. Carmo LS, Dias RS, Linardi VR, Sena MJ, Santos DA, Faria ME, Pena EC, Jett M, Heneine LG. Food poisoning due to enterotoxigenic strains of *Staphylococcus* present in Minas cheese and raw milk in Brazil. *Food Microbiol* 2002; 19: 9-14.
 12. Franco BDGM, Landgraf M. *Microbiologia dos Alimentos*. São Paulo: Atheneu, 2005.
 13. Hansen BM, Hendriksen NB. Detection of Enterotoxic *Bacillus cereus* and *Bacillus thuringiensis* Strains by PCR Analysis. *Appl Environ Microbiol* 2001; 67: 185-9.
 14. Rosenquist H, Smidt L, Andersen SR, Jensen GB, Wilcks A. Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food. *FEMS Microbiol Lett* 2005; 250: 129-36.
 15. Wijnands LM, Dufrenne JB, Rombouts FM, Veld PH, Leusden FM. Prevalence of potentially pathogenic *Bacillus cereus* in food commodities in the Netherlands. *J Food Prot* 2006; 69 (11):2587-94.
 16. Wong TL, Carey-Smith GV, Hollis L, Hudson JA. Microbiological survey of prepackaged pâté and ham in New Zealand. *Lett Appl Microbiol* 2005; 41: 106-11.
 17. Arnensen LPS, O'Sullivan K, Granum PE. Food poisoning potential of *Bacillus cereus* strains from Norwegian dairies. *Int J Food Microbiol* 2007; 116: 292-6.
 18. Bartoszewicz M, Hansen BM, Swiecicka I. The members of *Bacillus cereus* group are commonly present contaminants of fresh and heat-treated milk. *Food Microbiol* 2008; 25: 588-96.
 19. Svensson B, Monthán A, Guinebretière MH, Nguyen-Thé C, Christiansson A. Toxin production potential and the detection of toxin genes among strains of the *Bacillus cereus* group isolated along the dairy production chain. *Int Dairy J* 2007; 17: 1201-08.
 20. Zhou G, Liu H, He J, Yuan Y, Yuan Z. The occurrence of *Bacillus cereus*, *B. thuringiensis* and *B. mycoides* in Chinese pasteurized full fat milk. *Int J Food Microbiol* 2008; 121: 195-200.
 21. Aygun O, Pehlivanlar S. *Listeria* spp. in the raw milk and dairy products in Antakya, Turkey. *Food Control* 2006; 17: 676-9.
 22. Colak H, Hampikyan H, Bingol EB, Ulusoy B. Prevalence of *L. monocytogenes* and *Salmonella* spp. in Tulum cheese. *Food Control* 2007; 18: 576-9.
 23. Kabuki DY, Kuaye AY, Wiedmann M, Boor KJ. Molecular subtyping and tracking of *Listeria monocytogenes* in latin-style fresh-cheese processing plants. *J Dairy Sci* 2004; 87: 2803-12.
 24. Kalorey DR, Warke SR, Kurkure NV, Rawool DB, Barbudde SB. *Listeria* species in bovine raw milk: A large survey of Central India. *Food Control* 2008; 19: 109-12.
 25. Wiedmann M, Bruce JL, Keating C, Johnson AE, McDounough PL, Batt CA. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. *Infect Immun* 1997; 65 (7): 2707-16.
 26. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC n° 12 de 02 de janeiro de 2001. Aprova regulamento técnico sobre padrões microbiológicos em alimentos. Diário Oficial [da] republica federativa do Brasil. Brasília, DF, 10 de jan. 2001, Seção 1.
 27. Downes FP, Ito K. *Compendium of methods for the microbiological examination of foods*. 4th edition, Washington: American Public Health Association, 2001, 676p.
 28. Pagotto F, Daley E, Farber J, Warburton, D. Isolation of *Listeria monocytogenes* from all food and environmental samples. In: CANADA. Health Products Food Branch. *Compendium of analytical methods: laboratory procedures of microbiological analytical of foods*, [MFHPB 30] Ottawa, 2001. Available from: <http://www.hc-sc.gc.ca>
 29. Kabuki DY, Soares CM, Souza RM, Kuaye AY. Avaliação do perfil enterotoxigênico de *Bacillus cereus* isolados de queijos Minas frescal. XX Congresso Brasileiro de Ciência e Tecnologia de Alimentos, Curitiba, PR, 2006.
 30. Jo C, Kim H-J, Kim D-H, Lee W-K, Ham, J-S, Byun, M-W. Radiation sensitivity of selected pathogens in ice cream. *Food Control* 2007; 18: 859-65.
 31. Warke R, Kamat A, Kamat M, Thomas P. Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. *Food Control* 2000; 11: 77-83.
 32. Kramer JM, Gilbert RJ. *Bacillus cereus* and other *Bacillus* species. In: Doyle MP, ed. *Foodborne bacterial pathogens*. New York: Marcel Dekker, 1989, p. 21-69.
 33. Esper LMR. Diagnóstico da qualidade de ricotas comercializadas no município de Campinas - SP. [dissertação] Campinas, São Paulo: Universidade Estadual de Campinas (UNICAMP); 2006.
 34. Kabuki DY, Rocha JAK, Kuaye AY. Ocorrência de *Bacillus cereus* em queijos Minas frescal elaborados por diferentes processos tecnológicos. XXIII Congresso Brasileiro de Microbiologia, Santos, SP, 2005.
 35. Granum PE. *Bacillus cereus*. In M. P. Doyle (Ed.), *Food microbiology: Fundamentals and frontiers*. Washington, DC, USA: ASM Press, p.373-81, 2001.
 36. Beecher DJ, Schoeni JL, Wong ACL. Enterotoxic activity of hemolysin BL from *Bacillus cereus*. *Infect Immun* 1995; 63(11): 4423-8.
 37. Schoeni JL, Wong ACL. *Bacillus cereus* food poisoning and its toxins. *J Food Prot* 2005; 68(3): 636-48.
 38. Guinebretière MH, Nguyen-The C. Sources of *Bacillus cereus* contamination in a pasteurized zucchini purée processing line, differentiated by two PCR-based methods. *FEMS Microbiol Ecol* 2003; 43(2): 207-15.
 39. Nichols G, McLauchlin J, Louvois J. The contamination of pâté with *Listeria monocytogenes*. Results from the 1994 European Community – Coordinated Food Control Program for England and Wales. *J Food Prot* 1998; 61(10): 1299-1304.
 40. Dominguez C, Gomez I, ZumalacarreGUI J. Prevalence and contamination levels of *Listeria monocytogenes* in smoked fish and pâté sold in Spain. *J Food Prot* 2001; 64 (12): 2075-7.
 41. Kiss R, Tircza T, Szita G, Bernath S, Csikó G. *Listeria monocytogenes* food monitoring data and incidence of human listeriosis in Hungary. *Int J Food Microbiol* 2004; 112: 71-4.
 42. Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. *Microbes Infect* 2007; 9: 1236-43.