BRAZILIAN JOURNAL OF MICROBIOLOGY

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Food Microbiology

Performance of two alternative methods for *Listeria* detection throughout Serro Minas cheese ripening

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ARTICLE INFO

Article history:
Received 12 March 2015
Accepted 4 January 2016
Available online 22 April 2016

Associate Editor: Mariza Landgraf

Keywords:
Listeria
Raw milk cheese
Alternative methods

ABSTRACT

The ability of pathogens to survive cheese ripening is a food-security concern. Therefore, this study aimed to evaluate the performance of two alternative methods of analysis of *Listeria* during the ripening of artisanal Minas cheese. These methods were tested and compared with the conventional method: *Lateral Flow System*™, in cheeses produced on laboratory scale using raw milk collected from different farms and inoculated with *Listeria innocua*; and VIDAS®-LMO, in cheese samples collected from different manufacturers in Serro, Minas Gerais, Brazil. These samples were also characterized in terms of lactic acid bacteria, coliforms and physical–chemical analysis. In the inoculated samples, *L. innocua* was detected by *Lateral Flow System*™ method with 33% false-negative and 68% accuracy results. *L. innocua* was only detected in the inoculated samples by the conventional method at 60-days of cheese ripening. *L. monocytogenes* was not detected by the conventional and the VIDAS®-LMO methods in cheese samples collected from different manufacturers, which impairs evaluating the performance of this alternative method. We concluded that the conventional method provided a better recovery of *L. innocua* throughout cheese ripening, being able to detect *L. innocua* at 60-day, aging period which is required by the current legislation.

Introduction

Artisanal cheeses are widely appreciated and constitute a specific group of cheeses produced on farms on a small scale using traditional techniques.[1] In addition to their cultural, social and economic relevance, these cheeses also have a complex microbial ecosystem associated with raw milk, cattle management and changes that occur in this food matrix during ripening, which contribute to the unique sensory characteristics of this product.[2–5] Traditional Brazilian cheese includes varieties classified according to their region in Minas Gerais, and the most important varieties are produced in Serro, Canasta, Cerrado and Araxá.[5–7] Serro Minas cheese is usually

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http://dx.doi.org/10.1016/j.bjm.2016.04.006

made from raw bovine milk with addition of the "pingo", a natural fermentation starter originated from whey collected from successful cheese production from the previous batch.[8]

There is a clear risk of pathogen transmission in the production of artisanal cheese.[9] Loncarevic et al.,[10] for example, found *Listeria monocytogenes* in 42% of cheeses made from raw milk and in 2% of cheeses made from pasteurized milk. *L. monocytogenes* is a Gram-positive bacteria and causal agent of listeriosis whose clinical symptoms may include gastrointestinal diseases, meningitis, septicemia or even death.[11]

In industrialized countries, milk and dairy products are involved in 2–6% of outbreaks of foodborne illnesses[12] and *L. monocytogenes* is one of the major pathogens involved in these outbreaks.[13] Throughout the world, 261 clinical cases and 18 deaths were caused by listeriosis outbreaks associated with raw milk or raw milk cheese from January 2000 to 2010.[14] Annually, *L. monocytogenes* is responsible for approximately 2500 cases of listeriosis, 2289 hospitalizations and 449 deaths in the United States.[15]

To avoid illnesses in the consumption of artisanal cheeses, it is recommended in addition to the adoption of Good Manufacturing Practices and Hazard Analysis and Critical Control Point tools[16] that the cheeses be aged for 60 days prior to commercialization.[17,18] Brazilian law was recently changed, thus allowing raw milk cheeses be matured for a period less than 60 days, if the provided technical and scientific studies demonstrate that reducing the maturation period does not compromise the quality and safety of the product.[19] This rule is based on the assumption that even if pathogenic microorganisms were initially present in raw milk, they would be inactivated by changes throughout ripening,[20] which include low pH, water activity, high salt content and a competitive environment.[21] However, studies suggest that if pathogenic bacteria are present in the milk prior to cheese production, they could still survive.[22–24] Safe *L. monocytogenes* levels can vary until 100 CFU/g, only for products where the growth of *L. monocytogenes* is maintained in this limit until the end of its shelf life,[25] to absent in 25 g.[26,27]

The current legislation on food and health suggests an increased need for sample collection and analytical methods that are faster, cost-effective and easy to apply in the industry.[26,28] Therefore, alternative pathogen detection methods in food have proven to be positive for the industry because of their practicality, agility and potential for automation.[29] These methods eliminate some steps relative to conventional methodologies, such as selection of typical colonies on selective culture media and morphological, biochemical and serological tests.[28] Current molecular methods based on the amplification of target DNA by PCR and immunodetection based on the antigen–antibody reaction are the main alternative methods for pathogen detection.[30–34] The analytical methods must also be suited to the food matrix and have good performance attributes such as a low detection limit and high sensitivity, specificity and accuracy. Emphasis is given to the adequacy of the pathogen detection methods to the intrinsic feature of the food matrix, since the competing microbiota[35] and physical–chemical can interfere with performance of these methods. So here, we showed a study comparing the performance of two alternative methods of analysis of *Listeria* against the conventional method throughout artisanal Minas cheese ripening, also taking into account the influence of the intrinsic characteristics of these samples in the analyses.

Materials and methods

Detection of L. innocua by the conventional and immunoanalytical methods in artificially contaminated artisanal Minas cheese samples

Fifteen artisanal Minas cheese samples were produced on laboratory scale from raw milk obtained from three suppliers in the Serro region and was artificially contaminated with 10 CFU/mL of *L. innocua* ATCC 33090 as a surrogate for *L. monocytogenes*. The cheese samples were manufactured as described by Pinto et al.[23] Negative controls were also produced with raw milk not inoculated with *L. innocua*.

The survival of *L. innocua* was evaluated using conventional and immunoanalytical methods at five different times of ripening (5, 15, 30, 45 and 60 days). In each period, three independent samples were evaluated.

To detect *L. innocua* using conventional method,[36] 25 g of the cheese were homogenized in 225 mL of *Listeria* Enrichment Broth – LEB (Acumedia, Lansing, USA), and after incubation for 20–24 h at 30 ± 1 °C, 0.1 mL aliquots were transferred to 10 mL of supplemented Fraser broth (Oxoid, Basingstoke, UK). After incubation for 25 ± 1 h at 30 ± 1 °C, selective plating was performed in Oxford agar (Difco, Sparks, USA) and Palcam agar (Merck, Darmstadt, Germany). Typical *Listeria* sp. colonies were selected on TSA agar (Oxoid) containing 6% (w/v) yeast extract (MicroMed, Rio de Janeiro, Brazil) and submitted to biochemical characterization. Biochemical tests included catalase, Gram stain, motility, nitrate reduction, methyl red, Voges Proskauer, carbohydrate fermentation in phenol-red broth with xylose (Vetec, Rio de Janeiro, Brazil), rhamnose (Merck), mannitol (Merck) and alpha-hemolysis in Columbia agar (Oxoid) supplemented with 5% (v/v) defibrinated sheep blood.

The immunoassay method *Listeria* Test Kit PN 18220002 DuPont™ Lateral Flow System™ (DuPont Qualicon, Wilmington, USA) was also used to detect *Listeria* sp. in the same samples previously described, according to the manufacturer's recommendations. Aliquots of the enrichment broth were boiled in a water bath for 15 min, transferred to microtubes containing immobilized anti-*Listeria* sp. antibodies and then the results were read after 10 min at room temperature.

Detection of L. monocytogenes by the conventional and immunoanalytical methods in artisanal Minas cheese samples

A total of 48 samples of Serro Minas cheese with different ripening times were collected from different manufacturers in Serro, Minas Gerais, Brazil. Half of these samples had ripening times less than 60 days and the other samples were greater than 60 days. The analysis of *L. monocytogenes* was performed according to the conventional method described above. To detect *L. monocytogenes* by the VIDAS®-LMO method, from bioMérieux, Marcyl'Etoile, France,[37] 25 g of cheese