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ScienceDirect
Food Control 18 (2007) 1258–1262
FOOD CONTROL
www.elsevier.com/locate/foodcont

Evolution of *Listeria monocytogenes* populations during the ripening of naturally contaminated raw ewe's milk cheese

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Received 25 April 2006; received in revised form 1 August 2006; accepted 7 August 2006

Abstract

The aim of this work was to study, *in loco*, the evolution of *Listeria monocytogenes* populations, during ripening (7, 42, 60 and 120 days) of naturally contaminated raw ewe's milk cheese. Two batches of cheese consisting of 20 or 16 cheeses were obtained from two farmstead cheesemakers, respectively. A significant increase in numbers of *L. monocytogenes* was observed for both batches, from 7 to 42 days of ripening. These results suggest that this type of cheese has potential to support the survival of *L. monocytogenes*, while stressing the importance of cheese contamination in the dairies by resident strains.

Keywords: *Listeria monocytogenes*; Cheese ripening; Environmental contamination

1. Introduction

Listeria monocytogenes is a foodborne bacterium recognised as pathogenic for both humans and animals. Because of its versatility, *Listeria* is able to persist in the food industry environment, for several years (Unnerstad et al., 1996), probably in a biofilm state. Post-processing contamination of food with *L. monocytogenes* is a critical problem of public health. Several outbreaks of listeriosis were linked with the consumption of minimally processed and ready to eat (RTE) foods (Aureli et al., 2000; Brett, Short, & McLauchlin, 1998). These reports highlighted the importance of cross-contamination of processed foods from environmental sources. Cheese is one RTE type of food that has been associated with foodborne listeriosis (Donnelly, 2001). *Listeria* is widely disseminated in the rural environment and consequently cheese may be contaminated at any stage from farm to table. Presently in Portugal, there is no surveillance for *L. monocytogenes* infections and consequently, there is no reported human listeriosis associated with food consumption.

The type of cheese analysed in this study is a traditional Portuguese cheese made from raw ewe's milk coagulated, at 28–30 °C, with added salt (NaCl) and plant rennet (cardoon flower), without deliberate addition of any starter culture. The curd is manually worked, molded and pressed at room temperature (20 °C) and finally is bandaged with a strap of clean cotton cloth. Attached to the side of each cheese is a casein label (Passport® casein marks, DSM Food Specialties, Delft, Netherland) containing a number that identifies the daily production. The cheeses are taken to the first maturation room (6–10 °C) where ripening is carried out on wooden shelves for 15–21 days. At the end of this first stage of ripening, the cheeses are moved to the second maturation room (10–14 °C) where they are kept also on wooden shelves until the end (30–120 days). From the first day of production, cheeses are turned upside down daily. Usually after one week a viscous smear spontaneously appears on the surface of the cheese. Cheeses are then washed with water, generally with brushes, and bandaged again with

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doi:10.1016/j.foodcont.2006.08.002

new clean cotton straps. This procedure is performed, on average, four and two times during the first and the second stage, respectively (Rodrigues et al., 2000).

As protection against transmission of infectious diseases, a minimum age of at least 60 days, at not less than 35 °F (1.7 °C), is required in the USA since 1949, for cheese made from raw milk (Anonymous, 1950). For this type of cheese, the Portuguese legal directive stands that it cannot be commercialized before 30 days of ripening, although it is generally put on the market with a maturation period of approximately 42 days (semi-soft cheese with 61–69% moisture, on a fat free base, and 45–60% fat, reported to dry matter). Sometimes the ripening period is extended to get a trade mark of aged cheese (at least 120 days of maturation). In this case the resulting semi-hard to hard cheese has on the same basis a moisture content of 49–56% and more than 60% fat (Anonymous, 2002). Previous studies of the pH variation of this type of cheese during ripening showed that the pH of raw milk ($6.69 \pm .16$) in the coagulation vat is statistically equal to the pH of fresh cheese ($6.62 \pm .23$). Then the pH significantly decreases within the seven days thereafter (to $4.90 \pm .51$) and the following weeks are characterized by a stabilization of the pH ($4.78 \pm .46$ after 15 days, and $4.84 \pm .69$ after 30 days (Macedo, Malcata, & Oliveira, 1993). Costa, Barr, Margalho, Nabais, & Pereira (1996) also reported that in this type of cheese, the pH value stabilizes after 18 days of cheese maturation.

In this study we aimed to investigate the *in loco* evolution of the numbers of *L. monocytogenes* in naturally contaminated raw ewe's milk cheese, during 120 days of ripening.

2. Materials and methods

2.1. Dairy farmhouses background

Two farmhouses (A and B) with their own flocks and dairies were selected for this research. Farmhouse A had a flock with 60 sheep and the flock from farmhouse B had 240 sheep. Milking was done mechanically in farmhouse A, and manually in farmhouse B. At each dairy one batch of cheese consisted of cheeses produced daily with the milk obtained from the evening milking of the day before, mixed with the milk from the morning milking of the day of cheese production. The two dairies were selected from a group previously surveyed by our team, which showed recognised problems regarding this pathogenic bacterium. There were no incidents of listeriosis in the flocks, to our knowledge, during the surveyed periods. The bulk milk that was in dairy A the source of the analysed batch of cheeses was negative for the presence of *L. monocytogenes*. In dairy B, the bulk milk from which the cheeses were made was positive for the presence of *L. monocytogenes*, though the level of the pathogen in the milk was not ascertained. The existence of *L. monocytogenes* in cheeses from both dairies led to the interdiction of their trade and to their quarantine for the purpose of this study. The maturation of the reported batches of cheese proceeded with cleaning and sanitation procedures followed as usual in these dairies.

2.2. Sampling

Two batches of cheese, one from each farmhouse (A and B), were analysed in this study. The batch consisted in farmhouse A of 20 cheeses weighing from 500 g to 750 g, and in farmhouse B the batch was formed by 16 cheeses weighting about 1000 g each. In each batch four sampling times within the ripening period, were considered (7, 42, 60 and 120 days). Five or four cheeses, for dairy A and B, respectively, were considered at each time and each cheese was taken as a sample. The cheeses were transported to the laboratory under refrigerated conditions (4–8 °C) and analysed in less than 24 h. An aliquot of 25 g of cheese was removed by cutting radially and vertically three nearly equidistant wedges, which included approximately equal amounts of material from the inner and outer parts of the cheese. The remaining amount of cheese was destroyed after removing the cheese aliquots for analysis.

2.3. Enumeration and detection of *L. monocytogenes*

At each ripening time enumeration of *L. monocytogenes* was performed basically according to EN ISO/11290-2 (Anonymous, 2004) as follows: from each cheese the 25 g aliquot was blended for 2 min, in a Stomacher 400 (IUL, Barcelona), with 225 ml of LEB (Listeria Enrichment Broth, Oxoid). Portions of 0.2 ml of the homogenised or of the appropriately diluted samples were surface plated on each of five replicate plates of ALOA (Ottaviani & Agosti, 1997) (AES Laboratoire, Bruz, France) and PALCAM agar (Merck, Darmstadt, Germany), respectively. Plates were incubated aerobically at 37 °C for 24–48 h. Colonies presumed to be *Listeria* spp. were further confirmed as described in Leite et al. (2006). The counts of *L. monocytogenes* on each out of five plates of two different culture media, respectively, were summed (five replicate plates × 0.2 ml of a 1:10 dilution).

In order to continue with the enrichment steps the sample in LEB was homogenised with the suggested supplements (SR141, Oxoid, Hampshire, UK) and detection of *L. monocytogenes* was performed, according to the vertical standard IDF143A:1995 (Anonymous, 1995). Two *Listeria* reference strains obtained from CECT (Colleción Española de Cultivos Tipo) were used in this study as positive and negative controls, respectively: *L. monocytogenes* CECT4032 (=NCTC11994) (serovar 4b) and *Listeria innocua* CECT910^T (=NCTC11288) (serovar 6a).

2.4. Data analysis

ANOVA of the bacterial counts were carried out using LSD *post-hoc* multiple comparison tests using the program