
LENKA CABANOVÁ¹, OLGA ŠKUNTOVÁ¹, DANIELA MATISOVÁ¹ and MONIKA PIPOVÁ²

¹State Veterinary and Food Institute, Dolny Kubin, Slovakia; ²Department of Food Hygiene and Food Technology, University of Veterinary Medicine, Košice, Slovak Republic

Abstract


A scientific shelf-life study for Listeria monocytogenes in the typical Slovak cheese “bryndza” was performed in accordance with the requirements of the Commission Regulation (EC) 2073/2005. Based on the previous positive findings of L. monocytogenes in the final products, the producer decided to perform laboratory tests, the results of which would allow him a different evaluation of these positive results. Both the physico-chemical (pH, aw) and microbiological examinations of “bryndza” cheese stored at 5.8–6.2°C were performed every two days till the end of the product shelf-life (7 days). Microbiological analyses were performed after artificial contamination of the final product with a mixture of three L. monocytogenes strains. The growth potential of L. monocytogenes was calculated as the difference in the counts of this bacterium between the last day and the first day of the test. The Slovak traditional “bryndza” cheese has been found not to support the growth of L. monocytogenes. Thus, the counts of L. monocytogenes must not exceed 50 CFU/g at the beginning and 20 CFU/g at the end of the product shelf-life in order to ensure its safety for the consumer.

Keywords: Listeria monocytogenes; traditional cheese; Slovakia
different food matrices (El-Gazzar & Marth 1991; Gahan et al. 1996; Oyarzabal et al. 2003, Cataldo et al. 2007). Most of the strains can multiply within the pH range 5.6 to 9.6 while optimum pH value for the growth of L. monocytogenes is 7.0 to 7.5 (Blázková et al. 2005). With decreasing pH in the food below 4.4, this kind of product is considered to disable further multiplying of the listeria present (Phan-Thanh et al. 2000).

Foodborne listeriosis from milk and dairy products represents almost half of all the listeriosis cases reported in Europe (Lundén et al. 2004). Most of them are associated with the consumption of raw milk or dairy products made from unpasteurised milk (Buazzi et al. 1992; Casadeo et al. 1998; Aygun & Pehlivanlar 2006). Boyer et al. (2009) found that lactic acid bacteria present in food could exist competitively in the combination with listeria. In our case, a study was developed for the traditional Slovak cheese “bryndza” which can be prepared from unpasteurised sheep cheese or from mixtures of pasteurised cow cheese and unpasteurised sheep cheese. The final product does not undergo any further heat treatment, thus no inactivation occurs of the microorganisms present. The quality of the raw material is therefore of a great importance for the production.

The scientific shelf-life study for Listeria monocytogenes can be performed in two different ways, either as a durability study on naturally contaminated products or as a challenge test in artificially contaminated samples (which was the case of “bryndza” cheese). Based on the value of the growth potential, maximum counts of L. monocytogenes at the beginning as well as at the end of the product shelf-life were calculated in this study in order to ensure the safety of the product.

**MATERIAL AND METHOD**

**Preparation of artificially contaminated cheese samples.** Cheese “bryndza” which, based on the previous microbial analyses, did not contain any listeria, was examined. The shelf-life of this product was 7 days. Eight subsamples were prepared, four of them were prepared for microbial and four of them for physico-chemical analyses. The innoculum was a mixture of two Listeria monocytogenes (CCM 5576 and CCM 4699) reference strains and a third Listeria monocytogenes strain which had been previously isolated from this type of product, and was artificially added to the samples for microbial examination. The suspension of 0.2 McFarland turbidity was prepared from the strains, representing $1.7 \times 10^7$ bacteria in 1 ml (7.2 log). Samples of 10 g were weighed into sterile bags and artificially contaminated with 29 μl of $10^{-3}$ dilution of the suspension prepared. The samples intended for physico-chemical analyses were treated in a similar way but instead of the bacterial suspension, the same volume of saline solution was added. In order to ensure the cold chain interruption, the samples were left at room temperature for 1 hour. The contaminated samples were stored at 5.8–6.2°C till the end of their shelf-life (7 days).

**Physico-chemical analysis.** The pH value was determined potenciometrically using the SevenGo pH meter SG2 (Mettler Toledo GmbH, Schwerzenbach, Switzerland), and the water activity ($a_w$) was measured using Novasina aw Sprint TH-500 (Axair Novasina, Pfäffikon, Switzerland).

**Microbiological analysis.** The detection and enumeration of L. monocytogenes were performed according to the valid standard procedures (STN EN ISO 11290-1 and STN EN ISO 11290-2). For the detection, 25 g of the sample was mixed with 225 ml of half-Frazer broth (Merck, Darmstadt, Germany). After incubation at 30°C for 24 h, 0.1 ml of the suspension was transferred into 10 ml of Frazer broth and incubated at 37°C for 48 hours. Both broths were then streaked onto the surface of two selective solid media (OCLA, PALCAM, Oxoid, UK). The count of L. monocytogenes was determined in the basic dilution prepared from 10 g of the sample and 90 ml of saline solution. 1 ml of the suspension was further inoculated onto solid OCLA agar (Oxoid, Basingstoke, UK) and cultivated at 37°C for 48 hours.

**Evaluation of the results.** After the final analyses, the differences between the values of the last and first days of testing (growth potential) were calculated according to the Guidance document of the EURL (Guidance document... 2008).

**RESULTS AND DISCUSSION**

Each sample consisted of three subsamples since “bryndza” is considered to be a foodstuff with a heterogeneous character whose properties may change depending on the season. In parallel, microbial analyses for the detection and enumera-
Interpretation of results

From the results obtained, it is clear that “bryndza” cheese is the product which, based on its characteristics (pH, $a_w$) and the value of the growth potential, is not able to support the growth of L. monocytogenes.

The results showed that L. monocytogenes could occur in the final product but must not exceed 50 CFU/g at the beginning of the production, or not more than 20 CFU/g at the end of the shelf-life. The results are very important mainly from the sampling point of view in the production plant where, in spite of the positive findings, the product can be considered as acceptable after meeting the calculated limits.

Different scientific studies were developed abroad but in most cases were focused on the ability of Listeria to survive in the final products depending on their characteristics without the final enumeration of the acceptable Listeria numbers. Similar studies were given to yoghurts (Cottin et al. 1990) but also to the traditional soft cheese and the control of the expiration date and Listeria survival in this kind of cheese in Greece (Mataragas et al. 2008). The final products were contaminated with a mixture of five strains of Listeria monocytogenes (inoculation cca 6 log CFU/g) and the samples were stored at 5°C, 10°C, 15°C, and 20°C. Different models of predictive microbiology were used to evaluate the results obtained. These showed that the survival of the pathogen depended on the temperature, and that the bacterial cells survived at lower temperatures for a longer period. The study underlined the importance of predictive microbiology as a useful tool for real estimation and control of listeria in foods which pose a risk for the consumers.

Cataldo et al. (2007) checked the characteristics of L. monocytogenes and its survival in traditional soft cheese of Italian type depending on the physicochemical features. The samples were stored after artificial contamination at 4°C. The Listeria survival and acidity tolerance observed during cool storage were probably related to the intrinsic acid and saline features of the soft cheeses analysed. Italian soft cheeses tested may represent a potential hazard for the recovery of acid-adapted L. monocytogenes cells with enhanced ability to adhere to inert surfaces and/or to penetrate host cells.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st day</th>
<th>3rd day</th>
<th>5th day</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_w$</td>
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<td>0.96</td>
<td>0.96</td>
<td>0.95</td>
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<tr>
<td>pH</td>
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<td>4.70</td>
<td>4.61</td>
<td>4.66</td>
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<tr>
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<td>positive</td>
<td>positive</td>
<td>positive</td>
</tr>
<tr>
<td>Numbers of LM CFU/g / log CFU/g</td>
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<td>&lt; 10 / 0</td>
<td>&lt; 10 / 0</td>
<td>64 / 1.80</td>
</tr>
<tr>
<td>of LM CFU/g / log CFU/g</td>
<td>64 / 1.80</td>
<td>45 / 1.65</td>
<td>18 / 1.26</td>
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<tr>
<td></td>
<td>18 / 1.26</td>
<td>55 / 1.74</td>
<td>27 / 1.43</td>
<td>36 / 1.56</td>
</tr>
</tbody>
</table>

LM – Listeria monocytogenes
Cottage cheese was studied in England by Hicks and Lund (2008). The cheese sample was taken, within 24 h inoculated with *Listeria* F6861, and stored at 4°C, 8°C, or 12°C for 14 days. As pH, acidity, and lactic acid content varied, 3 different doses were used for the analyses. There was no increase in listeria enumeration, instead a decrease occurred while the degree of the decline differed and was the lowest in the product with the highest pH and the lowest content of lactic acid. Rogga et al. (2005) analysed fresh Greek cheese stored at 4 and 12°C. Concerning the low pH, it was estimated that at the end of the shelf-life listeria will neither survive nor exceed the legal 100 CFU/g.

**References**


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**Corresponding author:**

Dr. Lenka Cabanová, Štátnej veterinárný a potravinový ústav, Odbor hygieny potravin, Jánoškova 1611/58, 02 601 Dolny Kubín, Slovak Republic
tel.: + 421 435 837 111, e-mail: cabanova@svpudk.sk, cabanova@orangemail.sk