Development of A Scientific Study for Accessing the Criteria under Commission Regulation (EC) 2073/2005 on Traditional Slovak Sheep Cheese "Bryndza"

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Abstract

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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, a_w) 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

Commission Regulation (EC) 2073/2005 on microbiological criteria for food enables food producers to place the final products on the market even though the presence of pathogenic bacteria *Listeria monocytogenes* has been detected. According to this Regulation, the producer is obliged to develop a scientific study for each product before placing it on the market, demonstrating that the bacteria will not exceed the limit or that this kind of food disables the bacterial growth. The aim was to develop such a scientific study for the producer of the traditional Slovak sheep cheese "bryndza" because of the previous positive findings of *L. monocytogenes* in this type of food. The development of a scientific study with regard to *L. monocytogenes* is, despite its significance, only occasionally applied by the producers even though it would help them to place the final products on the market in spite be them containing listeria. It is important that the study should be conducted with respect to the specific characteristics of the product (particularly pH and a_w) and developed before the product was released to the market. Standard proceeding for the study development has been recommended by the EU Community Reference Laboratory for *Listeria monocytogenes* (EURL) in Paris (Aygun & Pehlivanlar 2006).

Listeria are bacteria which are very sensitive to some physico-chemical characteristics of food (a_w, pH, temperature) that influence their survival (Hokl *et al*. 1962; Doyle *et al*. 2001). Depending on these properties their behaviour changes in

different food matrices (El-Gazzar & Marth 1991; Gahan *et al*. 1996; Oyarzábal *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 (Blažková *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 1.7×10^7 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-

Table 1. The results of physico-chemical and microbiological analyses of "bryndza" cheese

Parameter	1st day	3rd day	5th day	7th day
a_w	0.95	0.96	0.96	0.95
	0.95	0.95	0.95	0.95
	0.95	0.95	0.95	0.95
pH	4.72	4.70	4.61	4.66
	4.69	4.65	4.84	4.73
	4.85	4.72	4.76	4.77
Detection of LM	positive	positive	positive	positive
Numbers of LM CFU/g / log CFU/g	18 / 1.26	< 10 / 0	< 10 / 0	64 / 1.80
	64 / 1.80	45 / 1.65	18 / 1.26	18 / 1.26
	18 / 1.26	55 / 1.74	27 / 1.43	36 / 1.56

LM – *Listeria monocytogenes*

tion of *L. monocytogenes* were performed. The results expected from the calculation should have been approximately 70 CFU/g. The results of the analyses are given in Table 1.

Growth potentials were calculated as follows:

Last day of cultivation	First day of cultivation
log = 1.80	log = 1.26
log = 1.26	log = 1.80
log = 1.56	log = 1.26

On the last (7th) day of cultivation, the mean value of logarithm was 1.56. On the first day of cultivation, the logarithm value was 1.26. As the difference between these values is 0.3, which is less than 0.5 (the value recommended by EURL), it means that the product does not support the growth of listeria.

The initial concentration was calculated using the following formula: 2 log – 0.3, which gives the result 50 CFU/g; final concentration (1 log + 0.3) gives the result 20 CFU/g.

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* (innoculation 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 physico-chemical 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.

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.

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