The analysis of the behaviour of Listeria monocytogenes in fresh cheeses with various spices during storage

Adriana Lobacz, Justyna Zulewska, Jaroslaw Kowalik
1 – University of Warmia and Mazury in Olsztyn (Poland), Faculty of Food Sciences, Department of Dairy Science and Quality Management

Abstract

The aim of this work was to evaluate the growth possibilities of Listeria monocytogenes in the artificially contaminated fresh cheese during storage. Three variants of acid homogenized commercial fresh cheeses: mixed herbs, red pepper and garlic-pepper were studied. The lowest value of the specific growth rate of L. monocytogenes in the fresh cheeses was reported for red pepper variant. The highest value of this parameter was determined for the fresh cheese with pepper-garlic. The use of spices in the production of fresh unripened cheeses influence the viability and growth of bacteria L. monocytogenes during storage at the temperature range 3-15 ºC.

Key words: Listeria monocytogenes, fresh cheese, predictive microbiology, natural preservatives

1. Introduction

Listeria monocytogenes is bacteria that can be found in food products. The consumption of the bacteria present in food may result in listeriosis, which is especially dangerous for people with low immunity, pregnant women, children and elderly people. L. monocytogenes grows at chilling storage conditions even at high concentration of NaCl (up to 12%) [4]. Relatively anaerobic rods of Listeria can grow at temperatures from -1,5 to 45 ºC, in the range of pH from 4,4 to 9,6 [9]. There is a growing number of listeriosis in European Union, the number of cases increased from 1425 to 1763 in years 2008 and 2013, respectively [3]. Among food products with high risk of L. monocytogenes occurrence soft cheeses and highly processed food should be mentioned. Dairy products are good substrates for L. monocytogenes growth, as they contain proteins, lactose and trace elements. The growth of Listeria in such products can be limited by use of food preservatives. However, the consumers often seek the products without any chemical additives, and the food producers tries to address such consumers’ needs by launching new or improved food products that are preserved by use of natural substances rather than synthetic one. Some herbs and spices that are used in food production are potentially antagonistic against L. monocytogenes [8].

To answer the question: will the undesirable bacteria grow in the food product, it is necessary to carry out a storage study in wide range of temperatures. The results (of studies carried out using the food samples purposely contaminated with pathogens) can be further analyzed by predictive microbiology. Predictive microbiology and mathematical models provide reliable description of microorganism behavior in food. Adequately validated models can be applied for quantitative estimation of microbial risk at real time [5].

The aim of present work was to evaluate the growth possibilities of Listeria monocytogenes in the artificially contaminated fresh cheeses during storage at the following temperature: 3, 6, 9, 12 and 15 ºC. Three flavor variants (mixed herbs, red pepper and garlic-pepper) of acid, homogenized commercial fresh cheeses were studied.

2. Material and Methods

2.1. Materials

The „Gusto” cheese was used in the studies with three different coatings: mixed herbs, red pepper and garlic-pepper. “Gusto” cheese is made of natural tvarog cheese (acid fermentation fresh cheese), cream, stabilizer (gelatin), dietary fiber, locust bean gum and NaCl. The cheese contains ca. 16% of fat and ca. 64% of water; its pH is the range 4,8-4,9. It can be described by delicate, clean, slightly creamy flavor. It is a popular cheese in central Poland with firm and homogenous structure and white color.

2.2. Inoculation and storage

The three strains of Listeria monocytogenes (strains ATTC: 0232S, 0737S, 0398S; Microbiologics, St Cloud, USA) were used to contaminate the samples.
In order to contaminate the products, fresh cheeses with three flavor variants (mixed herbs, red pepper and garlic-pepper) were divided into 25 g samples and an adequate dilution of *L. monocytogenes* culture was added to obtain the final concentration at 2 log cfu/g of cheese. The contaminated fresh cheese samples were stored in incubators equipped with cooling mode (ICP, Memmert, Germany) at 3 °C, 6 °C, 9 °C, 12 °C and 15 °C until the final curve of logarithmic growth of *L. monocytogenes* was obtained. The experiment was carried out in three replicates. In a precise time frame depended on storage temperature, the number of *L. monocytogenes* was determined with chromocult agar medium with a selective additive for *Listeria* (according Ottaviani and Agosti) (Merck, Millipore, Warsaw, Poland).

2.3. Mathematical Analysis

For primary modelling, the predictive growth models of Baranyi and Roberts’ were used [1] from www.combase.cc.

The square root model described by Ratkowsky, polynomial model and Arrhenius models were used within secondary modelling [6, 10].

The mathematical validation of secondary models included a calculation of the determination coefficient (R^2) as well as bias (Bf) and accuracy (Af) factors. The Bf factor describes the distribution of the observed values with reference to the line of equality, whereas the Af factor indicates the average distances between the observed values and the line of equality [7]. The mathematical analyses were performed with MS Excel software (Microsoft, Warsaw, Poland).

3. Results and discussion

The growth of *Listeria monocytogenes* in fresh cheeses with various spices: mixed herbs, red pepper and garlic-pepper, at the temperature range 3-15°C was shown on the Figures 1-3.

The highest level of *L. monocytogenes* cells was determined for “Gusto” cheese with garlic-pepper coating, and the lowest with red pepper coating, independently of storage temperature.

Figure 1. The observed growth of *Listeria monocytogenes* in fresh cheeses with red pepper at incubation temperatures of 3°C, 6°C, 9°C, 12°C and 15 °C.
The application of primary models available in the online program DMFit (www.combase.cc) resulted in calculation of specific growth rates (GR) of *Listeria monocytogenes* in studied cheeses (Table 1). The fastest GR of *L. monocytogenes* was determined for garlic-pepper cheese for almost whole temperature range 3-15°C, except 12°C, and the slowest for red pepper cheese. It was noted that the growth rate of bacteria increased as the temperature of storage increased.

Table 1. The Growth Rate (GR) coefficients for *L. monocytogenes* in fresh cheeses with various spices.

<table>
<thead>
<tr>
<th>Temperature [°C]</th>
<th>Red pepper</th>
<th>Garlic pepper</th>
<th>Mixed herbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.00059</td>
<td>0.00207</td>
<td>0.00164</td>
</tr>
</tbody>
</table>
The application of secondary models provides the possibility to predict the growth rate of *Listeria* depending on the storage temperature. Among the models used: the square root model described by Ratkowsky, polynomial model and Arrhenius models, the best values of determination coefficient ($R^2$) were calculated with polynomial model. 0,98, 0,99 and 0,88 for red pepper, mixed herbs and garlic-pepper cheeses, respectively. The prediction of a model is better when the value of $R^2$ approximates one [2]. The $A_f$ (accuracy factor) and $B_f$ (bias factor) values compare prediction of the polynomial models regardless of the applied primary model. The following values of $A_f$ and $B_f$ were calculated with polynomial models: 1,1874 and 1,0299, 1,0877 and 1,0071; 1,5231 and 1,1936, for red pepper, mixed herbs, and garlic-pepper cheese, respectively.

The value of the $A_f$ coefficient at 1 indicates ideal prediction of the model. The $A_f$ values within 1.3-1.5 range are also accepted as correct predictions. The $A_f$ and $B_f$ coefficients demonstrated that the predictions of a polynomial model based on GRs calculated with the Baranyi and Roberts model are within the acceptable limits [2]. The value of $B_f$ in the 0.9-1.05 range proves the ideal conformity between the predicted and the observed values. $B_f$ at 0.7-0.9 and 1.05-1.15 is regarded as acceptable [7].

Depending on the spices used as a coating, the difference in *Listeria monocytogenes* growth was observed for analyzed cheeses. Red pepper cheese was characterized by the lowest growth of this pathogen, however, the better conditions for growth were noted for garlic-pepper cheese.

The literature study showed that there is a number of research papers on the use of natural substances inhibiting the growth of *Listeria*, i.e. allspice, extracts of oregano, cloves, and mountain cranberry [8]. The rosemary (*Rosmarinus officinalis*) oil also demonstrates antibacterial properties. A phenolic substances of plant origin (thymol and carvacrol) inhibit the growth of *Listeria* at the concentration 0.5-1%. A free alcohol, named borneol, extracted from sage and rosemary, also showed the antimicrobial properties at the concentration 0.02-0.05% [11].

4. Conclusions

Fresh cheeses containing different spices constituted a good matrix for the *Listeria monocytogenes* growth. The lowest value of the specific growth rate of *L. monocytogenes* in the studied fresh cheeses was reported in case of red pepper spice. The highest value of this parameter was obtained for the fresh cheese with the addition of pepper-garlic spice. The goodness-of-fit of primary models was checked by calculation of the R-square parameter. Performed mathematical validation of the elaborated secondary models revealed the best accuracy of the polynomial model.

The use of different spices in the production of fresh cheeses may benefit in terms of safety of food production and distribution, contributing at the same time to limited use of food preservatives. Moreover, different natural additives used in food production may function as natural preservatives, which makes a strong element of marketing strategy.

5. References
