**Listeria monocytogenes** presence during fermentation, drying and storage of Petrovská klobása sausage

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**Abstract.** The majority of human listeriosis cases appear to be caused by consumption of ready-to-eat (RTE) foods contaminated at the time of consumption with high levels of **Listeria monocytogenes**. Although strategies to prevent growth of **L. monocytogenes** in RTE products are critical for reducing the incidence of human listeriosis, this pathogen is highly difficult to control in fermented sausage processing environments due to its high tolerance to low pH and high salt concentration. The aims of the present study were to investigate the occurrence, presence and elimination of **L. monocytogenes** in Petrovská klobása sausage during processing, fermentation, drying and storage. **L. monocytogenes**, which was detected at the beginning of the production cycle, disappeared before day 30. The pathogen decline was much faster in those sausages which were dried in controlled, industrial conditions than in those dried applying the traditional, household technique.

1. Introduction

Dry fermented sausages are characterized by their relatively longer shelf-life, which is brought about by the production of lactic acid in the fermentation process (pH<4.5-5) and low water activity (<0.90) of the final product [1]. Traditionally, fermented sausages are made using lactic acid bacteria (LAB) and Gram-positive catalase positive cocci, in particular coagulase-negative staphylococci naturally present in the meat, or with the inoculation of starter cultures at the chopping step. The mixture is then filled into natural or artificial casings, left to ferment and then dried [2].

Petrovská klobása is a traditional and autochthonous fermented pork meat product, which is a part of gastronomic heritage of Slovaks in Vojvodina, and which is produced in a traditional way in rural households in the Municipality of Bački Petrovac. In rural households, this sausage is at the end of November and during December. Petrovská klobása is made by mixing partly cooled (chilled for cca 4 h after slaughter) or cold (chilled for cca 24 h after slaughter) medium-chopped lean pork and fat (up to 10 mm) with addition of powdered red hot spicy paprika, salt, crushed garlic, caraway and sugar. A well-mixed filling, which is prepared within 15-30 minutes by using a unique technique of manual mixing with kneading and overturning, is stuffed into natural casings consisting of the rear part of pig intestines (rectum), forming units 35-45 cm long and 4.5-5.0 cm in diameter. After stuffing, the sausages are left to drain for a while and then they are smoked by a cold process for about 10-15 days with pauses, using specific kinds of wood (cherry wood in particular). When the smoking process is finished, the sausage is kept in a dry and well-ventilated place to dry and ripen, until it achieves an optimum quality, which takes about four months [3,4].
Fermented sausages contaminated with *L. monocytogenes* have rarely been implicated in critical listeriosis outbreaks [5]. Insufficiently dried sausages can have water activity levels close to 0.92-0.94 [6], and *L. monocytogenes* is able to survive during sausage fermentation, overcoming the hurdles encountered during the manufacturing process. In general, the contamination levels at the end of ripening are always lower than 100 CFU/g [7], because *L. monocytogenes* cannot compete with the prevailing LAB. Only without competitive microflora is *L. monocytogenes* able to multiply and reach high levels of contamination (higher than 1000 CFU/g), representing a major public health concern [8-10].

The aims of the present study were to investigate the occurrence, presence and elimination of *L. monocytogenes* in Petrovská klobása sausage during processing, fermentation, drying and storage in natural and artificial casings, and in household and industrial conditions.

2. Materials and Methods

2.1. Preparation of Petrovská klobása

Petrovská klobása dry fermented sausages were manufactured from a mixture of lean minced pork (80%) and pig fat (20%) obtained from carcasses of Large White cross breed animals. Meat was either hot (4 h-chilled meat) or cooled (24 h-chilled meat). After grinding the meat and the fat to a size of about 10 mm (with adjustable plate holder diameter set), raw materials were mixed with seasonings (red hot paprika powder, salt, raw garlic paste, caraway and sucrose) for about 10 min. The seasoned batter was immediately stuffed into collagen casings (two types of collagen casings – one artificial, and one natural pig intestine 500 mm long and 55 mm in diameter), and raw sausages were entirely processed in a traditional smoking/drying room or under industrial conditions during 120 days, then stored until 270 days.

2.2. Samples

Samples produced from the hot meat in a traditional way in the household – natural casing (A1) and A2 (artificial casing), samples produced from the cooled meat, smoked and dried in a household – natural casing (B1), samples produced from the cooled meat, smoked and dried in a household – artificial casing (B2), samples produced from the cooled meat, smoked and dried at the processing plant – natural casing (B3) and samples produced from the cooled meat, smoked and dried at the processing plant – artificial casing (B4) were taken before stuffing (at day 0) and during processing (on days 2, 4, 6, 9, 12, 15, 30, 45, 60, 90, 120, 150, 210 and 270).

2.3. Microbiological methods

Each sample was tested according to SRPS ISO 11290-1 and 2.

3. Results and Discussion

Results of testing are presented in figures 1, 2 and 3. The sausages contained detectable low levels of *L. monocytogenes*, which occurred naturally in the sausage mixture at the start of the study.
Figure 1. Decline of *Listeria monocytogenes* during smoking, fermentation, drying and storage of Petrovská klobása in groups A1 (hot meat/natural casing/household production) and A2 (hot meat/artificial casing/household production).

![Graph showing decline of Listeria monocytogenes in A1 and A2 groups.]

Figure 2. Decline of *Listeria monocytogenes* during smoking, fermentation, drying and storage of Petrovská klobása in groups B1 (cooled meat/natural casing/household production) and B2 (cooled meat/artificial casing/household production).

![Graph showing decline of Listeria monocytogenes in B1 and B2 groups.]

Figure 3. Decline of *Listeria monocytogenes* during smoking, fermentation, drying and storage of Petrovská klobása in groups B3 (cooled meat/natural casing/industrial production) and B4 (cooled meat/artificial casing/industrial production).

![Graph showing decline of Listeria monocytogenes in B3 and B4 groups.]

The presence of *L. monocytogenes* in A1 (figure 1) was detected on days 0, 2, 4, 6 and 12, and in A2 (figure 1) on days 0, 2 and 4. In B1 and B2 sausages, *L. monocytogenes* was found up to 15 days (figure 2). In B3 and B4 sausages (figure 3), the presence of *L. monocytogenes* disappeared much faster in sausages which were dried in controlled, industrial conditions than in those dried applying the traditional household technique. The results are in accordance with the results obtained by [11,12,13]. Generally, the declines in pathogen presence can be explained by the fact that the preservatives and protective microbiota have a significant impact on the survival and growth of *L. monocytogenes*. The organism is inhibited in fermented sausages by sequential steps: the “hurdle technology” concept includes several sequential hurdles, essential at different stages of the fermentation or ripening process [14]. These include lowering of pH by fermenting sugars to mainly lactic acid, lowering of water activity by salting, drying by evaporating water, inhibiting growth of aerobic bacteria by creating an anaerobic environment, inhibiting microbial growth by addition of nitrate or nitrite, and inhibiting surface growth by smoking or by addition of specific moulds. Together, these hurdles generally lead to a shelf-stable product [15]. These hurdles are essential in different steps of the fermentation or ripening process and lead to stable and safe final products [16].
4. Conclusion
According to the results, *L. monocytogenes*, which was detected at the beginning of the production cycle, disappeared before day 30. The pathogen decline was much faster in those sausages which were dried in controlled, industrial conditions than in those dried applying the traditional, household technique. Petrovská klobása sausage may be contaminated by *L. monocytogenes* at several stages. The raw materials may be contaminated from the slaughterhouse environment, during the production process or by contact with contaminated unprocessed raw materials, unclean surfaces or people or in the post-processing stages. In order to prevent growth of *L. monocytogenes* in dry fermented sausages, good manufacturing practices, correct sampling schemes, adequate cleaning and disinfection procedures and HACCP principles have to be applied. The use adequate hurdles can minimize the potential for growth of *L. monocytogenes*.

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