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Identification of lactic acid bacteria isolated from Serbian traditional fermented sausages Sremski and Lemeski kulen

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Abstract

The microbial composition of Serbian traditional fermented sausages, Sremski and Lemeski kulen, was studied. The dominant lactic acid bacteria in these products were *Lactobacillus* (77.1 and 54.3%, respectively) and *Leuconostoc* (20.0 and 22.9%, respectively). Lemeski kulen was characterized by a high percent of *Lactococcus* (20.0%). The most abundant *Lactobacillus* species in Sremski and Lemeski kulen was *Lb. brevis* (61.5 and 57.9%, respectively). Sremski kulen contained *Lb. pentosus* (11.5%) and *Lb. salivarius* (7.7%), in contrast to Lemeski kulen which contained *Lb. curvatus* ssp. *curvatus* (15.8%). *Lb. paracasei*, *Lb. plantarum* and *Lb. fermentum* were present in similar percentages in both products.

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Keywords: Sremski kulen; Lemeski kulen;microbiota; lactic acid bacteria

1. Introduction

Kulen is a dry fermented sausage produced from high-quality meat of mature pigs, seasoned with ground red paprika, stuffed into pork appendix or rectum, and which is preserved by smoking and drying followed by ripening.

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This type of sausage is traditionally produced in some areas of Pannonian Plain, (northern Serbia, western Croatia and southern Hungary)[1,2,3,4,5]. In Serbia, Kulen is produced in regions of Srem (Sremski kulen) and Backa (Lemeski kulen, Petrovská Klobása and other)[3,4,5,6] and according to Vukovic et al.[4], Danilovic et al.[6] and Lukic[7] these three types of kulen have protected designation of origin.

Traditional fermented sausages are recognized by their desirable sensory characteristics which are influenced by the activity of indigenous microbiota during fermentation and ripening[8]. Vukovic et al.[2,3,4,5] report that during the ripening of kulen, the number of lactic acid bacteria (LAB) increases, while enterococci and micrococci survive although their number decreases. Spoilage bacteria, *Pseudomonadaceae* and *Enterobacteriaceae* die out and pathogen bacteria such as *Salmonella* species and *Listeria monocytogenes* become not detectable. Similar changes of microbiota in traditional fermented sausages are reported by Papamanoli et al.[8], Drosinos et al.[9] and Comi et al.[10].

The composition of LAB in traditional fermented sausages has been studied in many countries. The predominant LAB species in traditional fermented sausages from Greece, Hungary and Italy are the genus *Lactobacillus*, followed by *Pediococcus* and sporadically, *Leuconostoc, Lactococcus* and *Weissella.* The majority of the lactobacilli belong to the species *Lactobacillus sakei*, *L. plantarum, L. curvatus* subsp. *curvatus* and *L. buchneri*[8,9,10,11]. In Serbia, the characterization of LAB to date, has been conducted only in Petrovská Klobása[6] and in Sremska kobasica[11]. It is important to mention that Sremska kobasica is stuffed into pig small intestine and is a different product from Sremski kulen[3]. Danilovic et al.[6] found that LAB in Petrovská Klobása were comprised of 59-79% *L. sakei*, 6-21% *Ln. mesenteroides* and up to 35% *Pediococcus pentosaceus*. Borovic et al.[11] concluded that in Sremska kobasica the dominant lactobacilli were *L. delbrueckii ssp. delbrueckii* (26.0%), *L. curvatus* (13.3%) and *L. plantarum* (10.0%). Of other LAB, *Pediococcus pentosaceus* comprised 10.0%, *Leuconostoc* strains 8.6% and *Lactococcus lactis* ssp. *lactis* 2.0%.

As there are no literature data about the characterization of the indigenous microbiota isolated from Sremski kulen and Lemeski kulen, the aim of this study was to identify representative LAB from these products.

2. Materials and methods

Ten samples of Sremski kulen, as well as Lemeski kulen (20 in total) were obtained from different small producers. A 25g sample from each sausage was homogenized with 225ml of sterile 0.1% (w/v) saline peptone water in a stomacher, from which serial decimal dilutions were prepared and inoculated on appropriate agar plates: LAB in de Man, Rogosa, Sharpe agar (MRS, Merck, Germany) at 30°C for 72h; total aerobic plate count in Plate count agar (PCA, Merck, Germany) at 30°C for 72h; *Micrococcaceae* on Mannitol salt agar (MSA, Merck , Germany) at 30°C for 72h; enterococci on Kanamycin esculin azide agar (KEA, Merck, Germany) at 37°C for 48h; *Enterobacteriaceae* on Brilliant green agar (Lab M Limited, United Kingdom) at 37°C for 24h; *Pseudomonadaceae* on Pseudomonas agar (Lab M Limited, United Kingdom) at 30°C for 48h. The presence of *Salmonella* spp. and *Listeria monocytogenes* was investigated according to the standard methods ISO 6579 and ISO 11290-1 respectively. Identification of LAB was performed on colonies chosen from MRS agar. For each sausage sample, 3-4 morphologically different colonies were collected from a representative dilution MRS agar plate, so a total of 35 isolates from Sremski kulen and 35 from Lemeski kulen were prepared for further investigation. All isolates were Gram-positive and catalase-negative. The species identification was conducted by API 50 CH test (BioMerieux, France) and the results interpreted by the ApiWeb™ identification software.

3. Results and discussion

The results of the investigation of microbiota composition in Sremski and Lemeski kulen (Table 1) show that the most dominant microorganisms were LAB, followed by *Micrococcaceae* and enterococci, without the presence of spoilage and pathogen bacteria, which is a similar patern reported by Vukovic et al.[2,3,4,5]. Lemeski kulen contained more LAB then Sremski kulen but this difference was not statistically significant. Statistically significant differences were observed in total aerobic plate count and *Micrococcaceae* count, which were higher in Lemeski kulen. This could be explaned by the greater quantities of ground red paprika (3-4%) in Lemeski kulen than in Sremski kulen (1.0-1.5 %)[5], as it is known that spices are one of the main vectors transferring microorganisms to meat products[12]. The presence of micrococci is desirable because of their contribution to aroma and color

development, as well as their antioxidative effect[13]. Also, enterococci play a significant role in aroma development in fermented sausages[14].

Table 1.Microbiota of Sremski and Lemeski kulen (log cfu/g).

Microorganism	Sremski kulen ($\bar{x} \pm SD$)	Lemeški kulen ($\bar{x} \pm SD$)
Lactic acid bacteria	6.94 ± 0.8	7.18 ± 1.2
Total aerobic plate count	4.42 ± 0.5	5.40* ± 0.5
Micrococcaceae	3.53 ± 0.7	5.00* ± 0.5
Enterococcaceae	2.94 ± 1.3	2.56 ± 1.5
Enterobacteriaceae	< 1	< 1
Pseudomonadaceae	< 1	< 1
Salmonella spp. in 25g	not detected	not detected
Listeria monocytogenes in 25g	not detected	not detected

*$p < 0.01$

The proportion of LAB genera in Sremski and Lemeski kulen is shown in Fig. 1. In both products, *Lactobacillus* was the most common genus, which is typical for fermented sausages[2,3,4,5,6,8,9,10,11].The percentage of *Leuconostoc* genus was quite similar in both products (20.0 and 22.9%), but in Sremski kulen, we identified *Ln. mesenteroides* and in Lemeski kulen, two species of *Leuconostoc*: *Ln. mesenteroides* (17.2%) and *Ln. lactis* (5.7%).

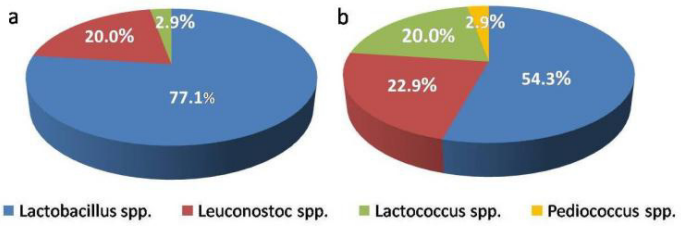

Fig. 1. Lactic acid bacteria isolated from (a) Sremski kulen; (b) Lemeski kulen.

Danilovic et al.[6] also detected *Ln. mesenteroides* in Petrovská Klobása, and which accounted for up to 21% of all LAB, but Borovic et al.[11] found *Ln. mesenteroides* in much lower levels (8.6 %) in Sremska kobasica. Unlike products from Serbia, in sausages from Greece, Italy and Hungary, *Leuconostoc* spp. accounted for 1.3%, 2.0% and 4.8% of isolated LAB, respectively. The main difference in LAB composition between Sremski and Lemeski kulen, was the proportion of *Lactococcus* spp. (2.9% and 20.0%, respectively), although all isolates were identified as *Lactococcus lactis ssp. lactis*. Borovic et al.[11] reported the presence of *Lc. lactis ssp. lactis* in Sremska kobasica, and which accounted for 2.0% of LAB, which was similar to our results in Sremski kulen. Interestingly, our results also showed that among LAB in Lemeski kulen, *Pediococcus pentosaceus* accounted for 2.9%, and this bacterium was not detected in Sremski kulen. On the contrary, literature data show that in Sremska kobasica, *P. pentosaceus* accounted for10%[11]and in Petrovská Klobása up to 35%[6] of LAB.

The composition of *Lactobacillus* species in Sremski and Lemeski kulen is shown in Fig. 2. In both products, the most abundant species was *Lactobacillus brevis* (61.5 and 57.9% respectively). However, the main difference was in the second most abundant species: Sremski kulen contained *Lb. pentosus* (11.5%) and *Lb. salivarius* (7.7%), in contrast to Lemeski kulen which contained *Lb. curvatus* ssp. *curvatus* (15.8%). Both products contained *Lb. paracasei* ssp. *paracasei*, *Lb. plantarum* and *Lb. fermentum* in similar percentages. According to literature data, the main *Lactobacillus* species in other Serbian traditional fermented sausages were *Lb. sakei* (59-79%) in Petrovská Klobása and *Lb. delbrueckii ssp. delbrueckii* (26.0%) in Sremska kobasica. As for traditional fermented sausages from Hungary, Italy and Greece, the most abundant LAB detected was *Lb. sakei* but in different proportions,

accounting for 70%, 48.8% and 23.6-42.8% of the total, respectively.

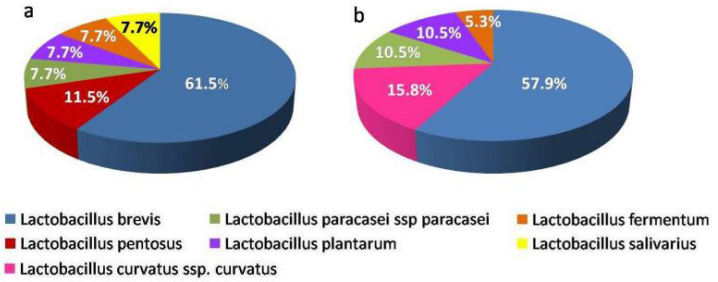

Fig. 2. *Lactobacillus* species isolated from (a) Sremski kulen; (b) Lemeski kulen.

4. Conclusion

The composition of microbiota of Sremski and Lemeski kulen was typical for fermeted sausages. The dominant LAB in both products were *Lactobacillus* (77.1 and 54.3%, respectively), followed by *Leuconostoc* spp (20.0 and 22.9% respectively). Lemeski kulen was characterized by a high percent of *Lactococcus* spp. (20.0%). The most abundant *Lactobacillus* species in Sremski and Lemeski kulen was *Lb. brevis* (61.5 and 57.9% respectively). Sremski kulen contained *Lb. pentosus* (11.5%) as well as *Lb. salivarius* (7.7%), in contrast to Lemeski kulen which contained *Lb. curvatus* ssp. *curvatus* (15.8%). *Lb. paracasei* ssp. *paracasei*, *Lb. plantarum* and *Lb. fermentum* were present in similar percentages in both products.

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