INFLUENCE OF BACTERIOCINS ON CHARACTERISTICS AND SAFETY OF TRADITIONALLY FERMENTED SAUSAGES

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SUMMARY

Semi-purified bacteriocins produced by Lactobacillus sakei I154 (sakacin) and Leuconostoc mesenteroides E131 (leucocin) were implemented in the production of traditional Bosnian, Hungarian, Serbian and Croatian fermented sausages together with Listeria monocytogenes. Furthermore, leucocin and Lactobacillus sakei I151 were added to the sausages in order to investigate their influence to the natural microbial flora and physicochemical characteristics of traditional products. Leucocin possessed better antilisterial activity than sakacin. With addition of Lb. sakei I151 and leucocin the typical sensory characteristics of the traditional products remained recognizable, while the safety of sausages was improved due progressive elimination of undesirable microbial flora.

Key words: *Lactobacillus sakei, leucocin, sakacin, safety, quality*

INTRODUCTION

Use of lactic acid bacteria as starter cultures has directed researches towards the discovery of bacteriocinogenic strains. Among bacteriocinogenic lactic acid bacteria, some species of the genus *Lactobacillus* occupy an important place, such as *Lb. sakei* (bacteriocin sakacin), *Lb. curvatus* (curvacin), *Lb. plantarum* (plantaricin), *Lb. acidophilus* (lactacin, acidocin) and *Lb. bavaricus* (bavaricin). In addition to lactobacilli, bacteriocins are also produced by *Pediococcus* spp. (bacteriocin pediocin), *Leuconostoc* spp. (leucocin), *Enterococcus* spp. (enterocin), *Lactococcus* spp. (lacticin, nisin), and *Carnobacterium* spp. (carnocin) (Zdolec et al., 2005)

The most widely used bacteriocin in food systems is nisin, the only bacteriocin registered as additive in food industry in over 50 countries of the world (Federal Register, 1988). Other bacteriocins and their possible application as food preservatives are investigating intensively. Bacteriocins can be applied in meat systems by adding crude, purified or semi-purified or by adding of bacteriocinogenic strains (O' Sullivan et al., 2002). Several studies reported a desirable antilisterial effectiveness of semi-purified or purified bacteriocins in dry fermented sausages (Laukova et al., 1999; Annanou et al., 2005).

Antimicrobial activity of individual strains and semi-purified bacteriocins against different pathogenic and spoilage microorganisms has been confirmed under laboratory conditions, and efforts have been made to obtain identical results in practice, i.e. in the production of fermented sausages and other products of excellent hygienic quality and safety. The aim of our work was to determine the efficacy of bacteriocins from *Lactobacillus sakei* I154 (sakacin) and *Leuconostoc mesenteroides* E131 (leucocin) as well as protective culture of *Lactobacillus sakei* I151 in upgrading of quality and safety of Bosnian, Hungarian, Serbian and Croatian traditionally fermented sausages.

MATERIALS AND METHODS

The filling mixtures were manufactured in local meat factories according to a standard practice

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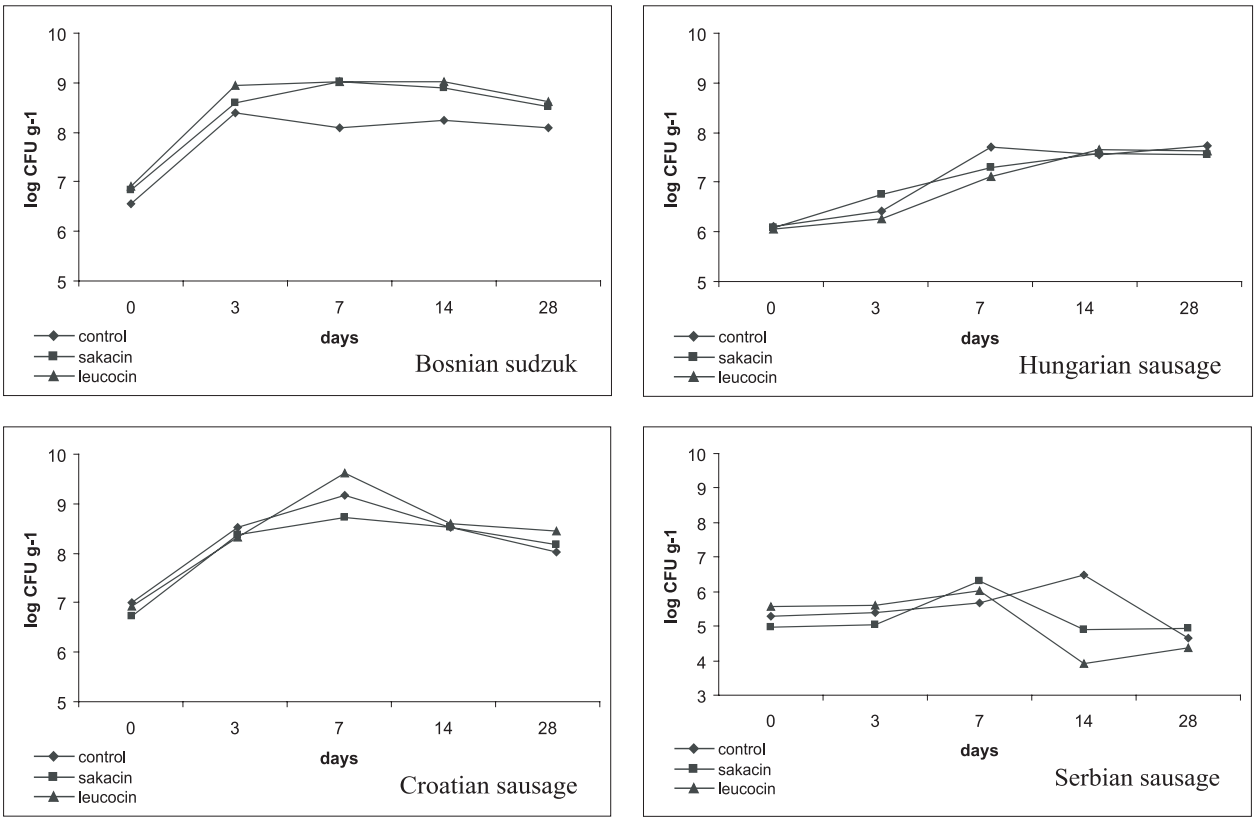
▼ **Figure 1.** TVC during fermentation and ripening process of fermented sausages with or without semi-purified bacteriocins (average of 3 fermentations)

▼ **Graf 1.** Ukupni broj bakterija tijekom fermentacije i zrenja fermentiranih kobasica sa ili bez dodatka polu-pročišćenih bakteriocina (srednja vrijednost 3 fermentacije)



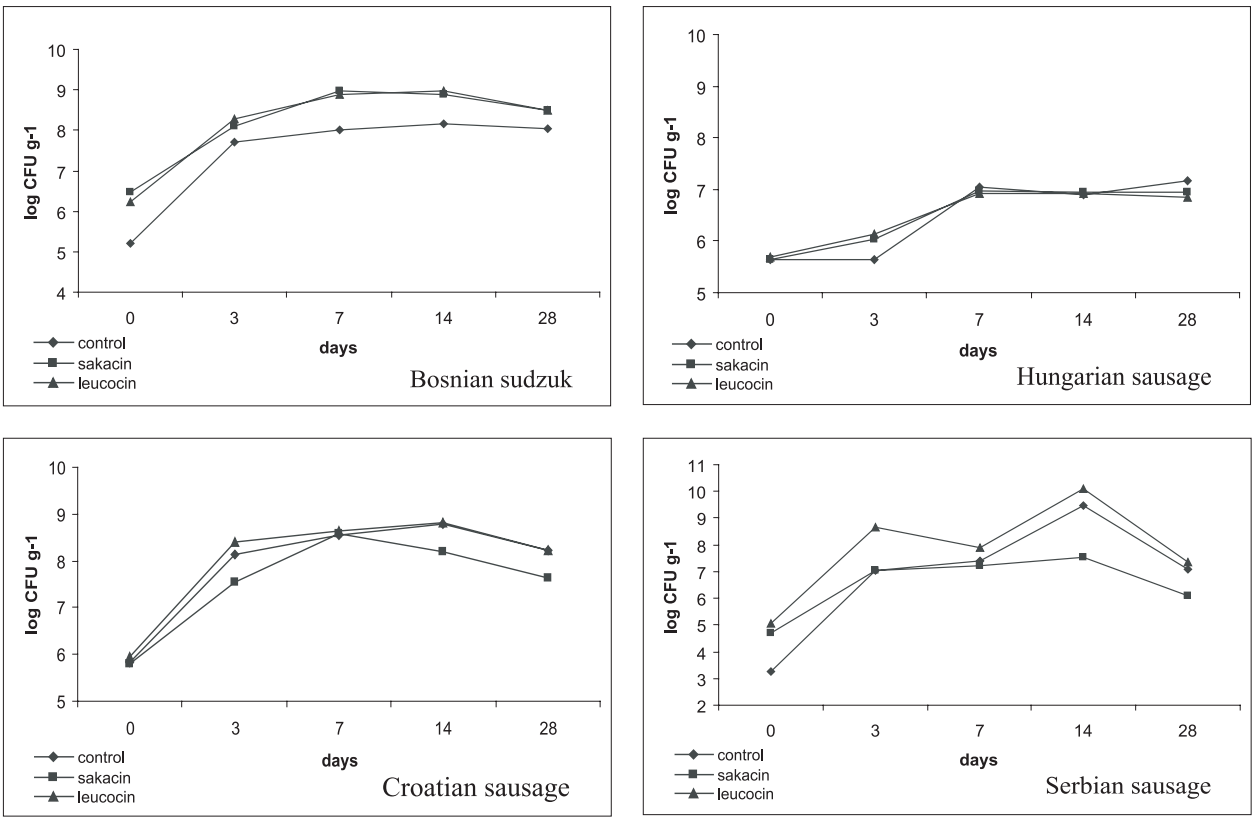
applied there. In order to prevent contamination of industrial facilities with *L. monocytogenes*, the filling mixture was sent to laboratory where its inoculation with the pathogen and semi-purified bacteriocins was performed. The final number of *L. monocytogenes* in the filling mixture was 10^4 – 10^5 cells/g. After inoculation with the pathogen, the mixture was divided into three equal parts - one part was used to produce control sausages (inoculated only with *L. monocytogenes*), while each of the other two parts, in addition to the pathogen inoculation, were inoculated with semi-purified bacteriocins of *Lactobacillus sakei* I154 and *Leuconostoc mesenteroides* E131 (1280 AU/kg). After the inoculation sausages were stuffed into the casings and returned to the industry. Three batches of sausages were used for the experiments. Samples were taken from each batch at 0, 3, 7, 14 and 28 days after formulation and subjected to physicochemical (pH) and microbiological analysis (total viable count, lactic acid bacteria and *L. monocytogenes*).

In the second experiment, semi-purified bacteriocin produced by *Leuconostoc mesenteroides* E131 was implemented in the production of traditionally fermented sausages in order to evaluate the influence on sensorial characteristics of sausages. In sensorial analysis a panel of 10 persons was created in each country. In a 10 degrees scale the panelists had to grade the produced sausage for tenderness, juiciness, aroma (smell-taste), acidity, and overall acceptability.

RESULTS AND DISCUSSION

With addition of semi-purified bacteriocins some differences were found in changes of TVC (figure 1) and LAB count (figure 2) between investigated sau-

▼ **Figure 2.** LAB count during fermentation and ripening process of fermented sausages with or without semi-purified bacteriocins (average of 3 fermentations)
▼ **Graf 2.** Ukupni broj bakterija mliječne kiseline tijekom fermentacije i zrenja fermentiranih kobasica sa ili bez dodatka polu-pročišćenih bakteriocina (srednja vrijednost 3 fermentacije)



sages. Only in Serbian sausage the TVC strongly decreased during ripening phase. Addition of bacteriocins didn't influence evidently to the LAB count, except in Serbian and Croatian investigation where it was lower when sakacin was used. Ananou et al. (2003) also reported that the presence of bacteriocin had a moderate negative effect on total lactic acid bacteria in a model meat sausage system.

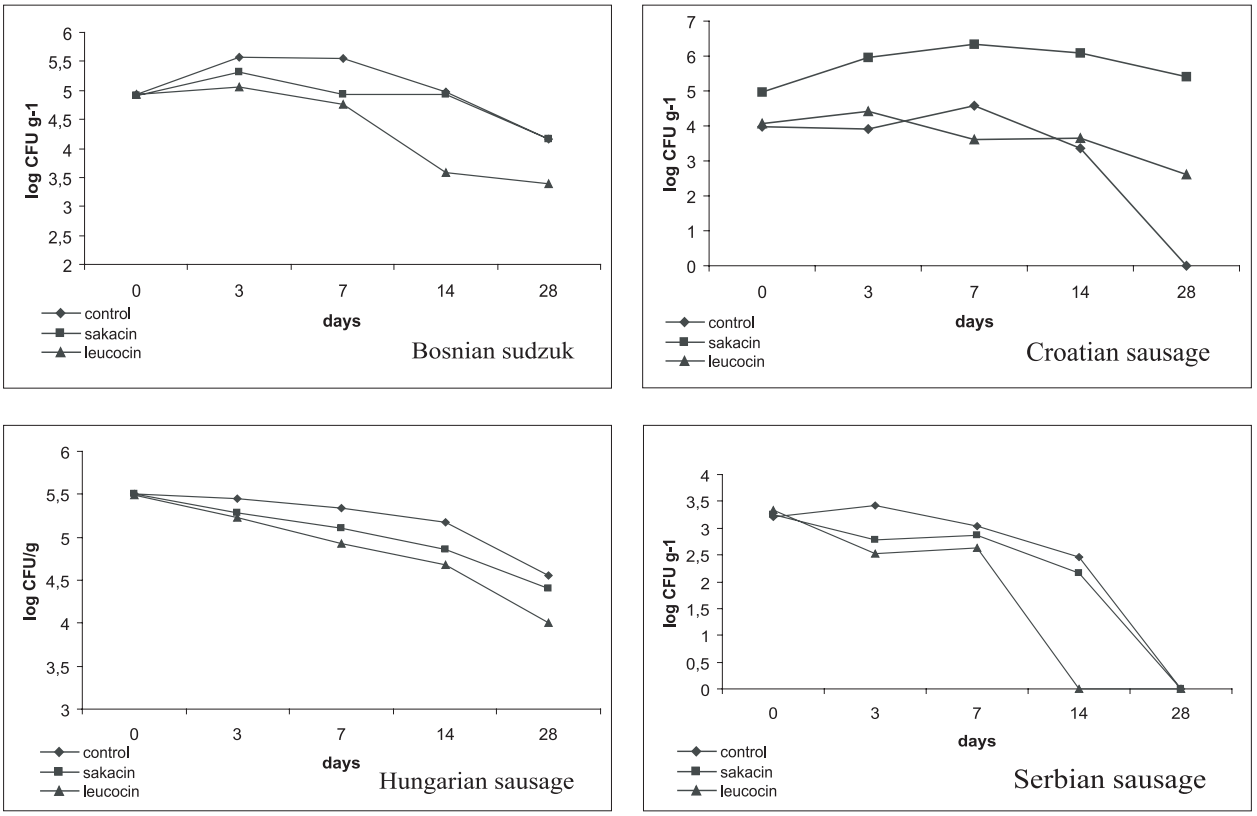
With addition of bacteriocins number of *L. monocytogenes* decreased during fermentation. In all experimental sausages leucocin showed better antilisterial effect than sakacin (figure 3). The highest reduction of Listeria population was found in Serbian sausage with leucocin added within 14 days of investigation. Using of sakacin didn't show satisfied antilisterial activity. Evident antilisterial activity of bacteriocins of *Leuconostoc* genus are reported also from other different sources (Harris et al., 1989; Harding and Shaw, 1990; Héchard et al., 1992; Mataragas et al., 2003; Bjørn Buddeet al., 2003). Several bacteriocins-producing *Leuconostoc* strains have been isolated from meat and all strains produce bacteriocins that are active against *L. monocytogenes* and other lactic acid bacteria of concern in meat spoilage (Hastings et al., 1994).

Using of semi-purified bacteriocins had no influence to the changes of pH in all investigated sausages (Figure 4), but they influenced to the decreasing of pH in comparison to the control sausages.

Sensorial evaluation of control and inoculated sausages showed that addition of bacteriocin didn't change the specific sensory properties of traditional fermented sausages (Figure 5). The typical sensory characteristics of the traditional products remained recognizable, which is probably the main factor for possibility of implementation of bacterio-

▼ **Figure 3.** Decrease of Listeria monocytogenes in fermented sausages with or without semi-purified bacteriocins (average of 3 fermentations)

▼ **Graf 3.** Smanjenje broja bakteirje L.isteria monocytogenes u fermentiranim kobasicama sa ili bez dodatka polu-pročišćenih bakteriocina (srednja vrijednost 3 fermentacije)



cin producing strains or bacteriocins in dry sausage manufacturing.

CONCLUSION

The addition of bacteriocins to fermented sausages reduced the number of viable *L. monocytogenes* cells even to a level below the detection limit. Bacteriocin produced by *Leuconostoc mesenteroides* E131 (leukocin) possessed a better antilisterial activity than bacteriocin of *Lb. sakei* 1154 (sakacin) in all experimental sausages. In addition, leucocin doesn't change specific properties of traditionally fermented sausages, so it could be used as safe protective ingredient.

ACKNOWLEDGMENTS

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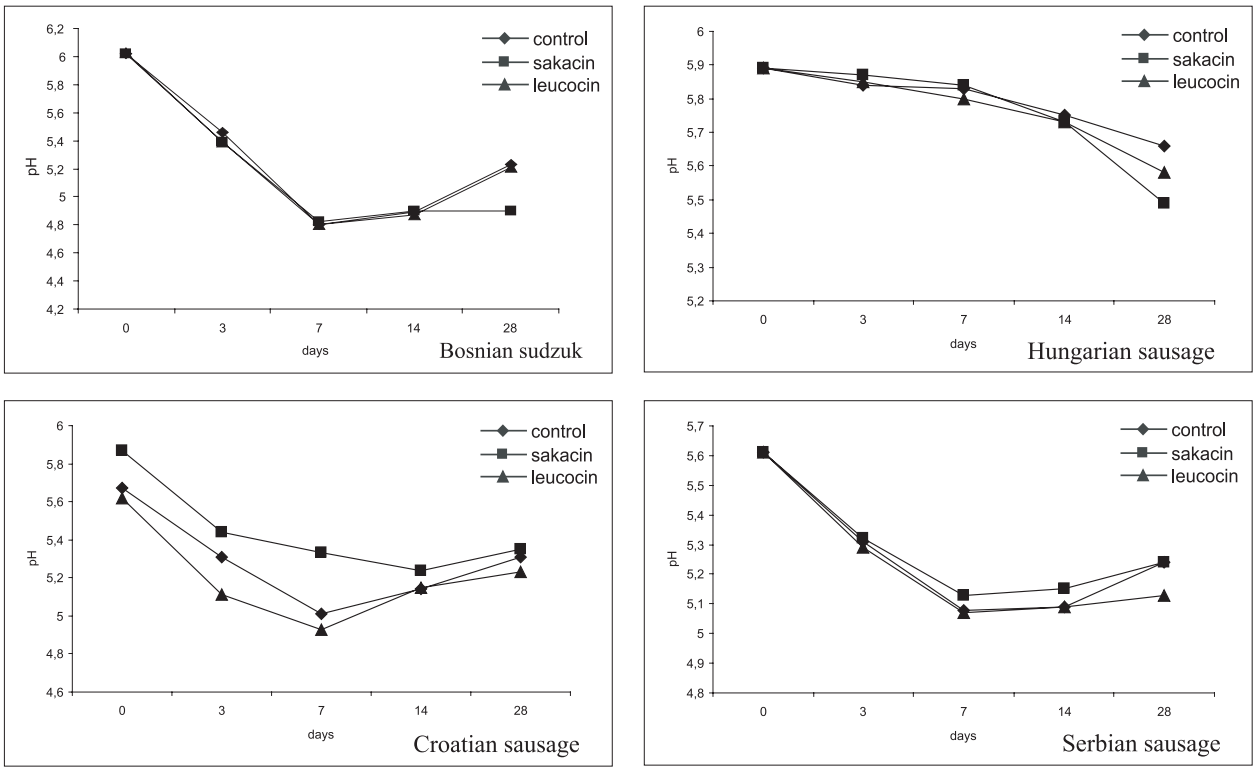
PROŠIRENI SAŽETAK

UTJECAJ BAKTERIOCINA NA KAKVOĆU I SIGURNOST TRADICIONALNO FERMENTIRANIH KOBASICA

UVOD

Otkako se bakterije mliječne kiseline koriste kao starter-kulture, istraživanja su usmjerena na pronalaženje bakteriocinogenih sojeva. Među bakteriocinogenim bakterijama mliječne kiseline značajno mjesto zauzimaju vrste roda Lactobacillus, i to L. sakei (bakteriocin sakacin), L. curvatus (curvacin), L. plantarum (plantaricin), L. acidophilus (lactacin, acidocin) i L. bavaricus (bavaricin). Osim laktobacila bakteriocine stvaraju i Pediococcus spp. (bakteriocin pediocin), Leuconostoc spp. (leucocin), Enterococcus spp. (enterocin), Lactococcus spp. (lacticin, nizin), Carnobacterium spp. (carnocin).

▼ **Figure 4.** pH of fermented sausages with or without semi-purified bacteriocins (average of 3 fermentations)
▼ **Graf 4.** pH fermentiranih kobasica sa ili bez dodatka polu-pročišćenih bakteriocina (srednja vrijednost 3 fermentacije)



Cilj ovog rada bio je utvrditi učinkovitost bakteriocina soja Lactobacillus sakei I154 (sakacin) i Leuconostoc mesenteroides E131 (leucocin) kao i zaštitne kulture Lactobacillus sakei I151 u poboljšanju sigurnosti i kakvoće tradicionalno fermentiranih bosanskih, mađarskih, srpskih i hrvatskih kobasica.

MATERIJAL I METODE

Nadjev kobasica inokuliran je s L. monocytogenes (10^4-10^5 CFU/g) te jednim od polu-pročišćenih bakteriocina (sakacin i leucocin; aktivnost 1280 AU/kg). Kobasice su uzorkovane 0., 3., 7., 14. i 28. dana zrenja, a određivan je ukupni broj bakterija, L. monocytogenes, bakterija mliječne kiseline te pH. Nadalje, u drugom dijelu istraživanja nadjev je inokuliran zaštitnom kulturom Lb. sakei I151 i/ili bakteriocinom Ln. mesenteroides E131 radi ocjene utjecaja na senzorna svojstva kobasica.

REZULTATI I DISKUSIJA

Dodatak bakteriocina utjecao je na smanjenje ukupnog broja bakterija samo u srijemskoj kobasici. Broj bakterija mliječne kiseline nije se značajno smanjio, osim u srijemskoj i hrvatskoj kobasici, uz dodatak sakacina. U svim istraživanjima utvrđeno je da bakteriocini nisu utjecali na promjene pH nadjeva. Uspoređujući rezultate inhibicije L. monocytogenes, evidentno bolji učinak postignut je dodavanjem bakteriocina Ln. mesenteroides E131.

Primjenom Lb. sakei I151 samog ili u kombinaciji s leucocinom specifična senzorna svojstva kobasica ostala su očuvana.

ZAKLJUČAK

Bacteriocin Ln. mesenteroides E131 (leucocin) postiže bolji antimikrobni učinak u fermentiranim kobasicama od bakteriocina Lb. sakei I154 (sakacin). Dodavanjem bakteriocina nije utvrđen negativan utjecaj na senzorna svojstva kobasica.

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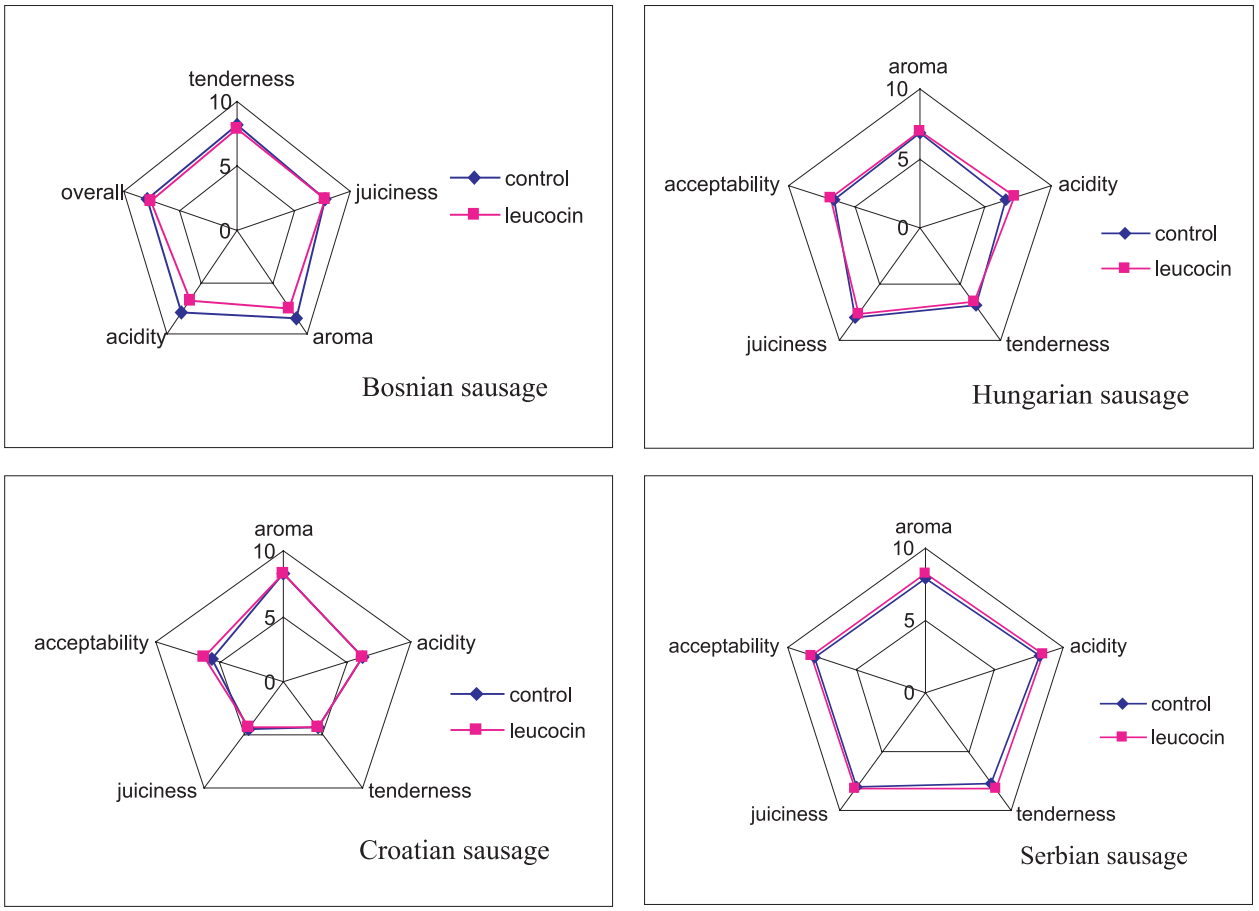
Figure 5. Sensory evaluation of fermented sausages with or without bacteriocin
Graf 5. Sentorska ocjena fermentiranih kobasica sa ili bez bakteriocina



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