Microbial and physicochemical succession in fermented sausages produced with bacteriocinogenic culture of *Lactobacillus sakei* and semi-purified bacteriocin mesenterocin Y

Nevijo Zdolec a,*, Mirza Hadžiosmanović a, Lidija Kozacinski a, Željka Cvrtila a, Ivana Filipović a, Mario Škrivanko b, Kristina Leskovar c

a Department of Hygiene and Technology of Foodstuffs of Animal Origin, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

b Croatian Veterinary Institute, Regional Veterinary Laboratory Vinkovci, Josipa Kozarca 24, 32100 Vinkovci, Croatia

c Veterinary Station Vrbovec, Kolodvorska 68, 10340 Vrbovec, Croatia

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Abstract

The influence of the bacteriocinogenic culture *Lactobacillus sakei* (10⁵/g) and semi-purified bacteriocin mesenterocin Y (2560 AU/kg) on the safety and quality of traditional Croatian fermented sausages was investigated. The addition of *Lb. sakei* and/or mesenterocin Y reduced microbial counts (*P* < 0.05) in the final products. After 28 days of ripening, coagulase-negative cocci decreased 1.5–2.0 log, yeasts 1.2–1.4 log and enterococci 1.7–2.7 log. In the case of the addition of *Lb. sakei*, the lactic acid bacteria count was significantly (*P* < 0.05) higher at day 7 of ripening, and was accompanied by a lower pH and a higher amount of lactic acid (*P* < 0.05). In the final product the amount of acetic acid was significantly lower. More intensive proteolysis and an increase in ammonia content were found at the beginning of fermentation, and in the second phase of ripening in the control samples, respectively. The free fatty acid concentration was significantly lower during the entire ripening process compared to the control (*P* < 0.05). Semi-purified mesenterocin Y did not affect the sensory properties of the sausages, whilst the addition of *Lb. sakei* enhanced them.

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1. Introduction

During the ripening of dry fermented sausages, biochemical, microbiological and sensorial changes take place closely related to the activity of dominant microorganisms in the filling. Lactic acid bacteria (LAB) and coagulase-negative cocci (CNC) are the most active indigenous microorganisms; first in acidification process, second in denitrification, lipolysis and proteolysis (Hammes & Hertel, 1998; Lücke, 2000). In addition, some types of fermented sausages are characterized by a stable population of yeasts, moulds or enterococci (Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). However, the microbial flora of naturally fermented sausages could contain undesirable microorganisms such as heterofermentative bacteria, biogenic amine producers and pathogens. Their presence and activity in fermented sausages may be suppressed by the introduction of a hygienically acceptable starter culture, which contributes to faster acidification, denitrification and standardization of the sensorial properties of the final products (Leroy, Verluyten, & De Vuyst, 2006).

The protective effect of LAB starter cultures is manifested in relation to pathogenic and spoilage bacteria through the antimicrobial properties of their metabolites,
such as organic acids, hydrogen peroxide and bacteriocins (Ammor & Mayo, 2007; Leistner & Gorris, 1995; Lücke, 2000). Numerous studies confirm the usefulness of bacteriocinogenic LAB in the production of fermented sausages based on enhanced reduction of inoculated pathogens, e.g. Listeria monocytogenes (Benkerroum et al., 2005; Drosinos et al., 2006; Työppönen (née Erkkilä), Markkula, Petäjä, Suikko, & Mattila-Sandholm, 2003; Zdolec et al., 2007). Moreover, from a hygienic and technological point of view, it is important to determine their influence on natural microbial flora, and physicochemical and sensorial changes during the maturation of the sausages.

In this study, non-indigenous bacteriocinogenic Lactobacillus sakei and semi-purified mesenterocin Y were used in the production of traditional Croatian fermented sausages to investigate their possible contribution to upgrading product quality and safety. Sakacin P producing strain I151 was shown to be a potential protective culture in the same type of sausage inoculated with L. monocytogenes (Zdolec et al., 2007). The antilisterial activity of Leuconostoc mesenteroides E131 was also observed in laboratory tests (Drosinos, Mataragas, & Metaxopoulos, 2006), but because of the hetero-fermentative nature of the strain, only its bacteriocin was included in the present study.

2. Materials and methods

2.1. Starter and bacteriocin preparation

A sakacin-producing strain of Lb. sakei I151 was isolated from traditional Italian fermented sausages (Urso, Cocolin, & Comi, 2004). The culture was grown in MRS broth at 30 °C for 24 h and sub-cultured twice (1% inoculum). One liter of active culture was centrifuged at 2000g for 5 min at 4 °C and the cells were re-suspended in sterile saline water (50 ml; 10⁷ cells/ml).

Leuconostoc mesenteroides E131, previously isolated from Greek fermented sausages (Drosinos et al., 2005), was used for bacteriocin purification. The strain was grown in MRS broth at 30 °C for 24 h, sub-cultured twice (1% inoculum) and 21 of active culture was centrifuged at 10,000g for 30 min at 4 °C. The supernatant was separated and pH was adjusted to 6.5 with 10 M NaOH. After neutralization, the supernatant was placed in a beaker at 4 °C and 484.54 g/l of ammonium sulfate (10 g each 3 min) was added for bacteriocin precipitation. Stirring was continued for 18 h at 4 °C and pellets of precipitates were collected and dissolved in 25 ml of 0.05 M sodium phosphate buffer (pH 7). This phase was repeated, and the final solution of semi-purified bacteriocin was prepared (50 ml) reaching the final concentration of 5120 AU/ml (Xiraphi, Georgalaki, Mataragas, Tsakalidou, & Drosinos, 2005). Bacteriocin activity was determined according to Barefoot and Klaenhammer (1983) using L. monocytogenes NCTC 10527 as the indicator organism.

2.2. Sausage production

Sausages were produced using lean pork (60%), beef meat (10%), pork back fat (24%), salt with 0.5% NaNO₂ (2.5%), dextrose (0.5%) and ground black pepper, minced red pepper and garlic (3%). Frozen meat and fat (−15 °C) were pre-tempered to −2 °C before use. After grinding to 12 mm in diameter, other ingredients were added separately. Meat and fat were further cut into pieces 2 mm in diameter under continuous mixing and homogenization of the mixture. Eighty kilograms of filling was prepared and divided into 4 equal parts. The first one was filled immediately (control sausages) into porcine casings (32–34 mm in diameter). The second part was inoculated with Lb. sakei reaching a final cell number of 10⁷/g, while 10 ml of bacteriocin solution was added to the third part reaching a final concentration of 2560 AU/kg. Lastly, the fourth part was produced, using both the starter culture and semi-purified bacteriocin. After vacuum stuffing, sausages were allowed to equilibrate at room temperature (12 h at 20 °C and 95% relative humidity), then cold smoked for 48 h at the same temperature and 85–90% RH. Finally, the sausages were ripened until day 28. During ripening, the temperature and relative humidity were gradually reduced to 16–18 °C and 75% RH, respectively. Experimental sausage production was repeated three times.

2.3. Microbiological analyses

Three sausages from each batch (n = 3) were sampled at day 0, 3, 4, 7, 14 and 28 of ripening. For microbiological analysis, 25 g of the test sample was taken aseptically, diluted in 225 ml of salt peptone water and homogenized for 2 min (Stomacher 400 Circulator, Seward, UK). After serial dilution, appropriate dilution samples (1 or 0.1 ml) were poured or spread on agar plates. Total viable count (TVC) was determined on Plate Count Agar (PCA, bioMérieux, Marcy l’Etoile, France), incubated at 30 °C for 24 h. LAB count was performed on MRS agar (bioMérieux) overlaid with 5 ml of the same media and incubated at 30 °C for 48–72 h; coagulase-negative cocci (CNC) on Manitol Salt Agar (MSA, bioMérieux), incubated at 30 °C for 48 h; enterobacteria-E. coli in Coli-ID agar (bioMérieux), incubated at 37 °C for 48 h; enterococci on kanamycin esculin azide agar (Merck, Darmstadt, Germany), incubated at 37 °C for 48 h; Staphylococcus aureus on Baird–Parker agar (BP, Merck), incubated at 37 °C for 48 h, using Bactident Coagulase (Merck) and SlideX Staph Plus agglutination test (bioMérieux) for confirmation; yeasts and moulds on oxytetracycline–glucose yeast extract agar (Oxoid, Basingstoke, Hampshire, England), incubated at 25 °C for 72–120 h; Pseudomonas on cetrimide agar (bioMérieux), incubated at 25 °C for 48 h. After counting, means and standard deviations were calculated. For the isolation of Salmonella and L. monocytogenes ISO 6579:2002 and ISO 11290-1:1996 methods were used, respectively.
2.4. Physicochemical analyses

Physicochemical analyses during ripening included measurement of pH, moisture, NaCl, nitrites, ammonia, lactic acid, acetic acid, free fatty acids (% of oleic acid) and proteolysis index (NPN/PN). For the pH measurement, 10 g of test samples were diluted in 90 ml of distilled water. After the homogenization, pH values were determined with digital pH-meter (WTW, Germany). Water content, salt, nitrites, free fatty acids contents and proteolysis index were determined according to AOAC methodology (2002). Amounts of ammonia, lactic and acetic acids were measured by means of Megazyme diagnostic kits K-AMIA 01/05, K-DLATE 01/05 and K-ACET 10/04 according to the manufacturer’s instructions (Megazyme International Ireland Ltd., Ireland).

2.5. Sensory evaluation

A panel of 10 persons evaluated the sensory properties of the fermented sausages. The panel consisted of trained staff from the Department of Hygiene and Technology of Animal Foodstuffs, Veterinary Faculty, University of Zagreb, with experience in sensorial evaluation of foodstuffs of animal origin. Each parameter in sensorial analysis was evaluated by means of a scale from 1 to 10, using a line 10 cm long. The panellists had to mark the line on the basis of the intensity perceived (left end (1) – unacceptable; right end (10) – optimum). Prior to tasting, colour, cut surface, coherence between fat and muscle tissue and smell (as well as off odour appearance) were evaluated. The products were tasted and graded for rancidity, fat quality, acidity, juiciness, tenderness and overall flavour. In addition, the sausages were graded for after-taste 10 min after tasting. Finally, the panellists graded their overall impression of the sensorial evaluation. After the evaluation, mean values were calculated for each parameter.

2.6. Vacuum packaging

At the end of the manufacturing process, the four groups of sausages (control, with Lb. sakei, with mesenterocin Y and with both ingredients) were sliced, vacuum packaged and stored at 4 °C for 90 days. Once a week TVC, LAB count and the presence of Salmonella spp, L. monocytogenes, E. coli O157:H7 (according to ISO 16564:2001), generic E. coli and S. aureus were determined, as well as pH values.

2.7. Statistical analysis

Statistical analysis was performed with the Statistica 7.1 program (StatSoft Inc., Tulsa, USA). Differences between the control sausages and sausages with bacteriocinogenic Lb. sakei and/or mesenterocin Y with regard to microbiological and physicochemical parameters during ripening phases were tested by ANOVA ($P < 0.05$).

3. Results

3.1. Microbial succession of the sausages produced with bacteriocinogenic Lb. sakei and/or semi-purified mesenterocin Y

Results of microbiological analyses of sausages produced with or without bacteriocinogenic culture of Lb. sakei and/or semi-purified mesenterocin Y are presented in Figs. 1–5. The total viable count was significantly ($P < 0.05$) lower in control sausages during all ripening phases (Fig. 1). The addition of Lb. sakei increased the LAB count significantly in the first days of fermentation, resulting in a higher number in the final product (Fig. 2). The coagulase-negative cocci (CNC) population increased continuously during ripening only in the control sausages, while in the other three experimental groups it grew only slightly until the 4th day. In the final products, a significantly lower ($P < 0.05$) CNC count was present.
with the use of starter and/or bacteriocin (Fig. 3). Enterococci increased during the fermentation and ripening of the control sausages (1.53 log increase). However, sausages with \textit{Lb. sakei} or mesenterocin Y were characterized by significantly ($P < 0.05$) lower numbers of enterococci at the end of maturation (2.0 and 1.74 logs less, respectively). The largest reduction in enterococci was observed in sausages with both starter and bacteriocin (2.76 log lower than in control, $P < 0.05$) (Fig. 4). Addition of \textit{Lb. sakei} and/or mesenterocin Y reduced the population of yeasts by 1.2–1.4 log (Fig. 5). Enterobacteria were found in all experimental sausages only at the beginning of fermentation, in low numbers (<3 log cfu/g). \textit{S. aureus} was present (>2 logs) in the control sausages and in sausages with \textit{Lb. sakei} and/or mesenterocin Y until days 7 and 4, respectively. \textit{Pseudomonas} spp., \textit{Salmonella} spp. and \textit{L. monocytogenes} were not isolated from the sausages at any stage of the process.

3.2. Physicochemical succession of the sausages produced with bacteriocinogenic \textit{Lb. sakei} and/or semi-purified mesenterocin Y

The results of the physicochemical analyses of the sausages are reported in Tables 1 and 2. Within the first days of fermentation significantly ($P < 0.05$) lower pH values were found in sausages inoculated with \textit{Lb. sakei}. There was no difference between the control sausages and sausages with mesenterocin Y ($P > 0.05$). At the end of production, sausages with starter and/or bacteriocin did not differ significantly in terms of the pH value, while in the control samples the pH was significantly ($P < 0.05$) lower. The rapid decrease of pH in early fermentation was accompanied by an intensive increase in lactic acid. However, lactic acid content was significantly ($P < 0.05$) higher in the groups of sausages with \textit{Lb. sakei} added (Table 1). Acetic acid increased slightly during the first days of fermentation, and on day 7 there were no differences between the sausages ($P > 0.05$). Towards the end of ripening a significant increase in acetic acid concentration was observed in the control (Table 1). The sausages produced with \textit{Lb. sakei} had the lowest moisture and the highest NaCl content at the end of maturation (data not shown). Nitrites decreased to below 10 mg/kg until the 7th day in all sausages (not shown). Nitrites decreased more intensively with the addition of \textit{Lb. sakei}, probably due to stronger acidification. A significantly higher proteolysis index in early fermentation was seen in sausages with \textit{Lb. sakei}, and in the further maturation period in the control ($P < 0.05$). Within the first 7 days of ripening, a significantly ($P < 0.05$) higher ammonia concentration was found in sausages with \textit{Lb. sakei}. At the same time, the ammonia concentration remained constant or increased slightly in the control sausages and sausages with mesenterocin Y. The opposite trend was observed in the second phase of ripening (14–28 day), i.e. a more intensive increase of ammonia content was found in the control sausages and sausages with mesenterocin Y. The free fatty

![Fig. 3. Coagulase-negative cocci count (log cfu/g – X ± SD) during the ripening of sausages produced with bacteriocinogenic culture \textit{Lb. sakei} and/or bacteriocin mesenterocin Y.](image)

![Fig. 4. Enterococci count (log cfu/g – X ± SD) during the ripening of sausages produced with bacteriocinogenic culture \textit{Lb. sakei} and/or bacteriocin mesenterocin Y.](image)

![Fig. 5. Yeast count (log cfu/g – X ± SD) during the ripening of sausages produced with bacteriocinogenic culture \textit{Lb. sakei} and/or bacteriocin mesenterocin Y.](image)
acid (FFA) content increased during the ripening in all sausages. However, higher FFA content was found in the control sausages in all phases of ripening (Table 2).

### 3.3. Sensory evaluation

The results of sensory analyses of control and sausages with starter and/or bacteriocin are presented in Fig. 6. Sensory evaluation of the final products showed that the addition of semi-purified mesenterocin Y did not have a negative impact on the sensory properties of the sausages, i.e. the traditional features remained recognizable. Furthermore, control sausages were evaluated as inferior to sausages produced with the bacteriocinogenic culture of *Lb. sakei* alone or combined with mesenterocin Y. Improvement of sensory parameters, mainly acidity, juiciness and tenderness, could arise from microbiological and physicochemical processes such as enhanced acidification and proteolysis as a result of *Lb. sakei* activity.

### 3.4. Shelf-life of products

During storage (90 days, 4 °C) of sliced vacuum packaged sausages, TVC increased and LAB decreased in all groups. Towards the 8th week, the highest TVCs were observed in sausages with *Lb. sakei* and in combination with mesenterocin Y (8.72 and 8.64 log cfu/g; 8th week, respectively). From week 9 to the end of storage TVC

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**Table 1**

Changes in pH, lactic and acetic acids during the ripening of sausages produced with bacteriocinogenic culture *Lb. sakei* and/or bacteriocin mesenterocin Y

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
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<tbody>
<tr>
<td><strong>pH</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>5.89 ± 0.04*</td>
<td>5.28 ± 0.06a</td>
<td>5.24 ± 0.06*</td>
<td>5.14 ± 0.03a</td>
<td>5.13 ± 0.04*</td>
<td>5.23 ± 0.02a</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151</td>
<td>5.77 ± 0.02b</td>
<td>5.17 ± 0.05b</td>
<td>5.16 ± 0.05b</td>
<td>5.10 ± 0.03bc</td>
<td>5.17 ± 0.05b</td>
<td>5.28 ± 0.02b</td>
</tr>
<tr>
<td>Mesenterocin Y</td>
<td>5.89 ± 0.02a</td>
<td>5.29 ± 0.04a</td>
<td>5.25 ± 0.03a</td>
<td>5.18 ± 0.02acd</td>
<td>5.24 ± 0.03b</td>
<td>5.30 ± 0.03bcd</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151 + Mesenterocin Y</td>
<td>5.81 ± 0.04b</td>
<td>5.19 ± 0.04b</td>
<td>5.17 ± 0.03b</td>
<td>5.19 ± 0.03d</td>
<td>5.24 ± 0.02b</td>
<td>5.33 ± 0.01cd</td>
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**Lactic acid (g/100 g)**

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<tbody>
<tr>
<td>Control</td>
<td>0.10 ± 0.01*</td>
<td>3.07 ± 0.02c</td>
<td>n.p</td>
<td>3.08 ± 0.01a</td>
<td>3.49 ± 0.03a</td>
<td>5.14 ± 0.03*</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151</td>
<td>0.13 ± 0.01ab</td>
<td>3.62 ± 0.02ab</td>
<td>n.p</td>
<td>2.69 ± 0.02b</td>
<td>4.99 ± 0.02b</td>
<td>5.16 ± 0.03*</td>
</tr>
<tr>
<td>Mesenterocin Y</td>
<td>0.10 ± 0.01c</td>
<td>3.09 ± 0.02c</td>
<td>n.p</td>
<td>3.92 ± 0.02c</td>
<td>3.53 ± 0.01i</td>
<td>4.87 ± 0.01b</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151 + Mesenterocin Y</td>
<td>0.32 ± 0.01ad</td>
<td>4.38 ± 0.01ad</td>
<td>n.p</td>
<td>4.76 ± 0.01d</td>
<td>4.28 ± 0.01d</td>
<td>4.81 ± 0.02c</td>
</tr>
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**Acetic acid (g/100 g)**

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<tbody>
<tr>
<td>Control</td>
<td>4.56 ± 0.02a*</td>
<td>6.40 ± 0.01a</td>
<td>n.p</td>
<td>6.20 ± 0.02a</td>
<td>6.86 ± 0.01a</td>
<td>8.20 ± 0.01a</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151</td>
<td>3.23 ± 0.02b</td>
<td>5.07 ± 0.02b</td>
<td>n.p</td>
<td>4.42 ± 0.01b</td>
<td>7.06 ± 0.02b</td>
<td>8.14 ± 0.01b</td>
</tr>
<tr>
<td>Mesenterocin Y</td>
<td>3.31 ± 0.01c</td>
<td>4.85 ± 0.01c</td>
<td>n.p</td>
<td>6.05 ± 0.03c</td>
<td>6.94 ± 0.01i</td>
<td>8.10 ± 0.01c</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151 + Mesenterocin Y</td>
<td>4.22 ± 0.02d</td>
<td>6.69 ± 0.01d</td>
<td>n.p</td>
<td>6.71 ± 0.01d</td>
<td>7.93 ± 0.01d</td>
<td>8.04 ± 0.02d</td>
</tr>
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</table>

Values are expressed as mean ± standard deviation.

n.p: not performed.

* The differences between the values with the same letters (in columns) are not statistically significant at *P* = 0.05.

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**Table 2**

Changes in proteolysis index, ammonia and free fatty acids during the ripening of sausages produced with bacteriocinogenic culture *Lb. sakei* and/or bacteriocin mesenterocin Y

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
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<tbody>
<tr>
<td><strong>Proteolysis index</strong> (NPN/PN)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.03 ± 0.06*</td>
<td>7.15 ± 0.07a</td>
<td>7.06 ± 0.06a</td>
<td>6.88 ± 0.03a</td>
<td>6.74 ± 0.07a</td>
<td>6.47 ± 0.06</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151</td>
<td>7.12 ± 0.10b</td>
<td>7.41 ± 0.06b</td>
<td>7.31 ± 0.08b</td>
<td>7.03 ± 0.07b</td>
<td>6.53 ± 0.05bc</td>
<td>6.46 ± 0.03</td>
</tr>
<tr>
<td>Mesenterocin Y</td>
<td>7.05 ± 0.05</td>
<td>7.31 ± 0.06c</td>
<td>7.11 ± 0.09a</td>
<td>6.80 ± 0.06c</td>
<td>6.57 ± 0.05b</td>
<td>6.31 ± 0.08a</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151 + Mesenterocin Y</td>
<td>7.07 ± 0.03</td>
<td>7.43 ± 0.04b</td>
<td>7.26 ± 0.03b</td>
<td>7.03 ± 0.03b</td>
<td>6.49 ± 0.05c</td>
<td>6.40 ± 0.03</td>
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**Ammonia (g/100 g)**

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<tbody>
<tr>
<td>Control</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.01a</td>
<td>n.p</td>
<td>0.04 ± 0.01a</td>
<td>0.23 ± 0.02a</td>
<td>0.27 ± 0.01ad</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151</td>
<td>0.06 ± 0.01*</td>
<td>0.18 ± 0.01b</td>
<td>n.p</td>
<td>0.18 ± 0.02b</td>
<td>0.21 ± 0.01i</td>
<td>0.24 ± 0.02bcd</td>
</tr>
<tr>
<td>Mesenterocin Y</td>
<td>0.00 ± 0.00</td>
<td>0.06 ± 0.01c</td>
<td>n.p</td>
<td>0.08 ± 0.01c</td>
<td>0.19 ± 0.01b</td>
<td>0.22 ± 0.01bc</td>
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<tr>
<td><em>Lb. sakei</em> I151 + Mesenterocin Y</td>
<td>0.00 ± 0.00</td>
<td>0.16 ± 0.01d</td>
<td>n.p</td>
<td>0.19 ± 0.01b</td>
<td>0.21 ± 0.01i</td>
<td>0.26 ± 0.01abcd</td>
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**FFA (% of oleic acid)**

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<tbody>
<tr>
<td>Control</td>
<td>0.80 ± 0.01*</td>
<td>0.81 ± 0.01a</td>
<td>0.83 ± 0.01a</td>
<td>0.86 ± 0.01a</td>
<td>0.87 ± 0.01a</td>
<td>0.88 ± 0.01a</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151</td>
<td>0.67 ± 0.03b</td>
<td>0.76 ± 0.02b</td>
<td>0.80 ± 0.01</td>
<td>0.81 ± 0.01</td>
<td>0.82 ± 0.01</td>
<td>0.83 ± 0.01</td>
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<tr>
<td>Mesenterocin Y</td>
<td>0.77 ± 0.04a</td>
<td>0.80 ± 0.01c</td>
<td>0.81 ± 0.01</td>
<td>0.82 ± 0.02</td>
<td>0.83 ± 0.01</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td><em>Lb. sakei</em> I151 + Mesenterocin Y</td>
<td>0.71 ± 0.01b</td>
<td>0.77 ± 0.01b</td>
<td>0.79 ± 0.02</td>
<td>0.80 ± 0.02</td>
<td>0.82 ± 0.02</td>
<td>0.83 ± 0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.

n.p: not performed.

* The differences between the values with the same letters (in columns) are not statistically significant at *P* = 0.05.
increased more rapidly in the control sausages, and resulted in significantly higher numbers in the final product (9.42 log cfu/g; \( P < 0.05 \)). During the whole storage period, the LAB count was higher in sausages with \( L. \) sakei added (7–8.5 log cfu/g). The LAB count started to decrease after the 3rd week in all groups, but more intensively in the control sausages and sausages with mesenterocin Y. At the end of storage (13th week) the most numerous LAB populations were present in sausages with \( L. \) sakei alone or in combination with mesenterocin Y (>7 log cfu/g). All samples were free of \( S. \) aureus, \( L. \) monocytogenes, \( E. \) coli O157:H7 and \( S. \) aureus. The pH value of vacuum packaged sausages constantly increased, starting at the second week of storage (data not shown). However, the increase was more evident in the control sausages and sausages with mesenterocin Y. At the end of storage, the pH was highest in the control sausages (5.90; \( P < 0.05 \)).

4. Discussion

The good adaptation of \( L. \) sakei to meat substrate resulted in a significant increase of LAB count and TVC, that was also found in sausages with both starter and bacteriocin. The colonization capability of the strain used in our study was confirmed by Urso, Rantsiou, Cantoni, Comi, and Cocolin (2006) who reported that >70% of LAB isolates collected during all ripening phases of Italian fermented sausage production were bacteriocinogenic \( L. \) sakei. Moreover, Urso et al. (2006) found a significant decrease in TVC compared to traditionally produced sausages. Comparison of our data and the results of Urso et al. (2006) is only partially possible, since, although the same strain was used, there were important differences between the sausages – sausage composition, duration of ripening (Italian 45 days, ours 28), the technique applied (e.g. smoking in our case), initial microbial flora, etc. In our study, the highest increase of CNC count was found in the control sausages during the last phase of ripening; whereas in the other three groups of sausages, the CNC count remained at the initial level. These results suggest the poor competitiveness of CNC due to intensive growth of lactic acid bacteria, as reported by Samelis et al. (1998). According to Giraffa (2002) enterococci can survive and grow during fermentation of meat and dairy products, especially in foods without the use of competitive starter cultures. Our results confirm this observation, since enterococci grew continuously only in the control sausages. Urso et al. (2006) also found a reduction in the enterococci count, which was not the case with traditional production without a starter culture (Comi et al., 2005). In our experiment the greatest reduction of enterococci was in the combined activity of \( L. \) sakei and mesenterocin Y. The addition of \( L. \) sakei and/or mesenterocin Y resulted in significantly \( (P < 0.05) \) lower yeast counts between the 3rd and 28th days of ripening. Urso et al. (2006) found nearly the same yeast count (4.5 log cfu/g) using bacteriocin-producing \( L. \) sakei, with a decreasing trend towards the end of ripening. Enterobacteria were only present in all groups of sausages at the beginning of fermentation, which is a regular finding, due to their well-known sensitivity to acidic environments (Adams & Nicolaides, 1997). As with enterobacteria, \( S. \) aureus was reduced below the detection limit in the early phase of fermentation. Schillinger and Lücke (1989) reported that a rapid drop in pH to below 5.3 is important for the reduction of \( S. \) aureus.

During the ripening of the control sausages, we found the usual pH trend (Kozacˇinski et al., 2006; Zdolec, Kozaˇciniski, Hadziosmanovi´c, Cvrtila, & Filipovi´c, 2007), which is manifested as a rapid decrease during the first days of fermentation and a slight increase towards the end of ripening. However, the addition of \( L. \) sakei and its combination with mesenterocin Y caused stronger acidification and significant increases of lactic acid \( (P < 0.05) \), which is in direct correlation with the differences found in the LAB count. Slower increase of acetic acid found in sausages with \( L. \) sakei and/or mesenterocin Y could be due to the suppression of the hetero-fermentative microbial flora. The presence of hetero-fermentative microorganisms in fermented sausages is also undesirable due to the production of \( CO_2 \), diacetyl and alcohol (Axellson, 1998). The most intensive proteolysis, found during early fermentation in sausages with \( L. \) sakei added, could be due to the proteolytic activity of the culture (Fadda et al., 1999; Montel, Seronine, Talon, & Hébraud, 1995) or faster activation of tissue proteases because of the pH decrease (Demeyer, 1995). That intensity of proteolysis with the use of \( L. \) sakei was reflected in the high ammonia content during the first days of fermentation. However, in the second part of ripening, proteolysis was less but the ammonia content increased significantly in the control sausages, which could be related to the evident increase of CNC and yeasts (Figs. 3 and 5), known as main proteolytic organisms in fer-

![Fig. 6. Sensory evaluation of the sausages produced with bacteriocinogenic culture \( L. \) sakei and/or bacteriocin mesenterocin Y and the controls (average of three batches, \( n = 9 \)). *Grade scale 1–10.](image-url)
mented sausages (Hammes & Hertel, 1998). The higher FFA content in the control sausages could also be attributed to the high CNC and yeasts counts (Hadzˇiosmanovi´c, 1978).

In conclusion, the results indicate the positive effect of a bacteriocinogenic culture of Lb. sakei on the overall quality and safety of traditional Croatian fermented sausages. It was shown that combination with bacteriocin mesenterocin Y did not affect the activity of the culture. Implementation of semi-purified mesenterocin Y without Lb. sakei I151 may be used to reduce the growth of enterococci, coagulase-negative cocci and yeasts. The most evident effect of the sakacin-produced culture was a decrease in enterococci, coagulase-negative cocci and yeasts during ripening, and enhanced reduction of enterobacteria and pathogenic staphylococci. The colonization of the strain was manifested in the increased LAB count, more rapid acidification and the significantly higher amount of lactic acid. The sensory profile of the sausages was also improved, particularly their acidity, tenderness and juiciness. Bearing in mind the results of the previous study on the antilisterial capacity of the cultured sausages, the present results, Lb. sakei I151 may be used as a starter culture in the upgrading of traditional fermented sausages.

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References


