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UPGRADING THE SAFETY AND QUALITY OF TRADITIONAL FERMENTED SAUSAGES

Zdolec¹, N., M. Hadžiosmanović¹, L. Kozačinski¹, Ž. Cvrtić¹, I. Filipović¹, K. Leskovar², P. Popelka³, S. Marcinčak³

ABSTRACT

*The aim of this study was to investigate the influence of non-indigenous bacteriocin-producing culture of *Lb. sakei* on safety and quality of traditional Croatian fermented sausage. Two experiments were carried out; first to evaluate the effect of the culture on artificially inoculated population of *Listeria monocytogenes*, and the second to characterize the sausages produced with *Lb. sakei* regarding microbiological, chemical and sensorial properties. *L. monocytogenes* count was significantly reduced ($P < 0.05$) during all phases of ripening. Sakacin-producing culture decreased the microbial count in final products, mainly enterococci, coagulase negative cocci and yeasts ($P < 0.05$). The sensory properties of sausages were improved, mainly acidity, tenderness and juiciness.*

Key words: safety, quality, fermented sausages

INTRODUCTION

Implementation of lactic acid bacteria (LAB) as starter cultures in fermented sausages is well established and

standardized procedure introduced few decades ago. Nowadays, the researches are focused to introduce new functional starters, including those able to synthesize bacteriocins. Within the LAB group, the most suitable candidates for functional starters seems to be bacteriocinogenic strains of *Lactobacillus (Lb.) sakei* and *Lactobacillus (Lb.) curvatus* as predominant species during the natural sausage fermentation (Hammes, 1990; Drosinos et al., 2005; Comi et al., 2005). Functionality of bacteriocin-producing LAB has been mainly demonstrated thorough antilisterial activity in different types of fermented sausages (Gasparik-Reichardt et al., 2006; Drosinos et al., 2006; Zdolec, 2007a). Moreover, bacteriocin-producing lactobacilli have been shown as technologically acceptable cultures in production of fermented sausages (Hadžiosmanović et al., 2005; Urso et al., 2006). The objective of this study was the characterization of traditional Croatian fermented sausage produced with non-indigenous bacteriocin-producing *Lb. sakei*. The protective effect of culture was tested towards the inoculated population of *L. monocytogenes*.

¹ Nevijo Zdolec, DSc, junior researcher - senior assistant; Mirza Hadžiosmanović, DSc, full professor; Lidija Kozačinski, DSc, associate professor; Željka Cvrtić Fleck, DSc, assistant professor; Ivana Filipović, DVM, junior research assistant; Institute of Foodstuff Hygiene and Technology, Veterinary Faculty, University of Zagreb, Heinzelova 55, Zagreb; contact e-mail: nzdolec@vef.hr

² Kristina Leskovar, DVM, Veterinary health station Vrbovec, Kolodvorska 68, 10340 Vrbovec

³ Peter Popelka, DSc; Slavomir Marcinčak, DSc; Institute of Foodstuff Hygiene and Technology, The University of Veterinary Medicine, Komenského 73, 041 81 Košice, The Slovak Republic

MATERIAL AND METHODS

Experiment I

Sausages were produced in local meat industry according to standard procedure (Kozačinski et al., 2006). *Lactobacillus sakei* I151 used in this research was previously isolated from traditional Italian fermented sausages (Urso et al., 2004). The strain was grown in MRS broth (30 °C for 24 hours with triple sub-cultivation (1% inoculums)), centrifuged at 2000xg for 15 minutes, and re-suspended in 50 ml of sterile saline water. For the purpose of first part of study, the culture of *Listeria monocytogenes* NCTC 10527 was prepared as described and inoculated into sausage mixture reaching final number of 5 log₁₀ CFU/g. The mixture was divided, and the second part was additionally inoculated with *Lb. sakei* reaching also 5 log₁₀ CFU/g in the final mixture. Control sausage and experimental one were than vacuum stuffed and fermented for 28 days in controlled conditions (Zdolec et al., 2007b). The production of sausages was performed three times. Three sausages were sampled at days 0, 3, 7, 14 and 28 and subjected to pH measurement and analysis for total viable count (TVC), lactic acid bacteria (LAB) and *L. monocytogenes* count using standard plating microbiological methods.

Experiment II

In the second part of study, culture of *Lb. sakei* I151 was added to sausage mixture (5 log₁₀ CFU/g) in order to compare with controls regarding microbiological, physicochemical and sensorial succession during the ripening (Zdolec et al., 2008; in press). The sampling was done at days 0, 3, 4, 7, 14 and 28 of ripening. Microbiological parameters included TVC, LAB, enterococci, coagulase-negative cocci, yeasts, *Staphylococcus aureus*, enterobacteria, *Pseudomonas* spp., *Salmonella* spp. and *L. monocytogenes*. Physicochemical analyses included measurement of pH, moisture, NaCl, nitrites, ammonia, lactic acid, acetic acid, free fatty acids (% of oleic acid) and proteolysis index (NPN/PN) with the use AOAC methods and Megazyme diagnostic kits. At the end of manufacturing process the sausages were sensory evaluated for color, cut surface, coherence, smell, rancidity, fat quality, acidity, juiciness, tenderness, overall flavour, after taste

and overall impression.

Statistical analysis

Statistical analysis was performed with Statistica 7.1 (StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Experiment I

During the fermentation phase, TVC was significantly lower in control (p<0.05). In the final products no differences in TVC and LAB between sausages existed. Lactic acid bacteria count rapidly increased towards 3rd day of fermentation in both groups of sausages. Significantly higher numbers were found using protective culture at day 0 and 7. Results showed in table 1 indicate the stronger decrease of *Listeria* until 14th day of ripening in the presence of *Lb. sakei*.

Survival and growth of *L. monocytogenes* in fermented sausages largely depends on the sausage type, environmental conditions during fermentation, starter cultures used and adaptability of pathogen to meat substrate (Encinas et al., 1999; Thévenot et al. 2005). Within the concept of hurdle technology, the use of bacteriocinogenic LAB cultures has shown to be an additional protective factor against *L. monocytogenes* (Foegeding et al., 1992; Hugas et al., 1997; Lahti et al., 2001; Dicks et al., 2004). In our study, presence of *Lb. sakei* in sausages matrix caused significant decrease in *L. monocytogenes* count within the first half of process. Since there were no significant differences in pH values between sausages (data not shown), it could be assumed that decrease of *Listeria* was enhanced by sakacin activity. In connection, Cocolin et al. (2005) showed that expression of sakacin in Italian fermented sausages produced with *Lb. sakei* is the highest during early phase of ripening. The reduction of pathogen beneath detection limit toward the end of manufacturing process (28th day) in control sausages confirms the effectiveness of natural ripening process in an assurance of product's safety, regardless the high initial number of pathogen (Zdolec et al., 2005, 2007c).

Experiment II

Bacteriocinogenic *Lb. sakei* (10⁵/g) significantly (P<0.05)

increased TVC and LAB count. Ability of the *Lb. sakei* to colonize sausage matrix during ripening was reported by Urso et al. (2006) in Italian Friuli traditional sausage. Using *Lb. sakei*, CNC count remained at the initial numbers (3.5 -3.7 log CFU/g) which wasn't the case in con-

▼ **Table 1.** *L. monocytogenes* count (log₁₀ CFU/g – X ± SD) during the ripening of sausages produced with or without protective culture of *Lb. sakei*

	Day of ripening				
	0	3	7	14	28
Control	4,94 ± 0,18	5,37 ± 0,41	4,77 ± 0,15 ^{a*}	4,65 ± 0,57 ^a	<2
Sausages with <i>Lb. sakei</i> I151	4,82 ± 0,25	5,40 ± 0,38	3,99 ± 0,20 ^b	2,55 ± 0,21 ^b	<2

* mean values with different letters in columns are significantly different (P<0.05)

trol (2 logs higher in final products) ($P < 0.05$). Similarly to CNC count, enterococci increased in control sausages constantly during the manufacturing process (1.53 log increase). Sausages with *Lb. sakei* were characterized in significantly lower number (2 logs) at the end of production ($P < 0.05$). Urso et al. (2006) have also found decrease of enterococci in presence of *Lb. sakei*, which wasn't the case in traditional production without starter culture (Comi et al., 2005). Regarding yeasts, implementation of *Lb. sakei* resulted in 1.43 log lower numbers in final products. Enterobacteria were found in both sausages just in the beginning of fermentation in low numbers (< 3 log CFU/g). *S. aureus* was present above detection limit (2 logs) in control sausages till the day 7, while in sausages with *Lb. sakei* till the day 4. *Pseudomonas* spp., *Salmonella* spp. and *L. monocytogenes* were not isolated from sausages from beginning to the end of manufacturing process. Within first days of fermentation significantly lower ($P < 0.05$) pH values were found in sausage groups with *Lb. sakei* implemented, accompanied with significant increase of lactic acid concentration ($P < 0.05$). Towards the end of ripening significant increase of acetic acid was found in control. It could be assumed that implementation of culture suppressed the hetero-fermentative microbial flora which is responsible for occurrence of higher amount of acetic acid. Presence of hetero-fermentative microorganisms in fermented sausages is undesirable also due production of CO_2 , diacetyl and alcohol (Axellson, 1998). Significantly higher proteolysis index and ammonia content were determined in early fermentation with the presence of *Lb. sakei*, while in further maturation period in control ($P < 0.05$). Higher FFA content was found in control sausages in all ripening phases which could be attributed to higher count of CNC and yeasts, known as important factors of lipolitic changes in fermented sausages (Hadžiosmanović, 1978). Improving 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. In conclusion, results obtained implicate the positive effect of bacteriocinogenic culture of *Lb. sakei* on total quality of fermented sausages as well as the safety which also arise from results obtained in challenge study of antilisterial activity of culture during the ripening of traditional Croatian fermented sausage.

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ZUSAMMENFASSUNG

VERBESSERUNG VON SICHERHEIT UND QUALITÄT DER TRADITIONELLEN FERMENTIERTEN WÜRSTE

Das Ziel dieser Arbeit war den Einfluss der bacteriocinogenen Kultur *Lactobacillus sakei* auf Sicherheit und Qualität der traditionellen fermentierten Würste zu erforschen. Die Forschung ist in zwei Teilen durchgeführt worden; im ersten Teil wurde die Wirkung der Kultur auf inoculierte Population der Bakterie *Listeria monocytogenes* geprüft, im zweiten Teil wurde die Forschung zwecks Einsicht in die Wirkung der Kultur auf mikrobiologische, physikalisch-chemische und sensorische Charakteristiken der Würste vorgenommen. Die Zahl der *Listeria monocytogenes* wurde in allen Reifenphasen vermindert ($P < 0,05$). Durch die Anwendung der bacteriocinogenen Kultur wurde auch die Zahl der Enterokokken, Koagulase der negativen Kokken und Hefe im Fertigprodukt ($P < 0,05$) gesenkt. Die Resultate zeigten auch die Verbesserung der sensorischen Eigenschaften der Würste in Bezug auf das Aussehen, den Geschmack, den Geruch und die Konsistenz.

Schlüsselwörter: Sicherheit, Qualität, fermentierte Würste

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QUALITY OF SLAVONIAN HAMS ON THE THIRD NATIONAL HAM FESTIVAL

Senčić¹, Đ., Danijela Butko¹

SUMMARY

Slavonian ham is a traditional Croatian cured meat product. There were 19 hams registered for evaluation, produced by different and mostly commercial producers. Except for organoleptic (sensory) characteristics, the pH-value of ham meat (*M. semimembranosus*) has been determined by the pH-meter Mettler Toledo, as well

as meat color parameters ("L" and "a" value), which have been determined by the chromo meter Minolta CR-410. Evaluation results indicate the variability of quality of Slavonian ham. High and significant correlations have been determined among sensory characteristics of ham (appearance, cross-section appearance, consistency, smell, taste), both mutually and with average grade of

¹ Đuro Senčić, DSc, full professor; Danijela Butko, assistant; Department of Animal Sciences, Faculty of Agriculture Osijek, Trg Sv.Trojstva 3, 31000 Osijek, Contact e-mail: dsencic@pfos.hr