MICROBIAL CHANGES DURING RIPENING OF FERMENTED HORSEMEAT SAUSAGES

Alagić[1], D., L. Kozačinski[2], I. Filipović[2], N. Zdolec[2], M. Hadžiosmanović[2], B. Njari[2], Z. Kozačinski[3], S. Uhitil[4]

SUMMARY

In this paper changes in microflora occurring during ripening of horsemeat sausages were monitored. Three sausages were sampled on day 0, and again on days 14, 28 and 42 of ripening. During fermentation, in the sausage microflora prevailed lactic acid bacteria (6-8 $\log_{10}$ cfu g^{-1}), but there were also micrococci (2-3 $\log_{10}$ cfu g^{-1}), and yeasts and fungi (3-4 $\log_{10}$ cfu g^{-1}). Significant drop in pH value was recorded during the first 14 days of fermentation (5.82 on day 0, and 5.02 on day 14). That, and other possible antimicrobial mechanisms of LAB, led to the expected reduction of microorganism counts or elimination of enterobacteria, E. coli, pathogenic staphylococci and sulphite-reducing clostridia.

Key words: *horsemeat sausages, microflora*

INTRODUCTION

As it is known from literature data, horsemeat is rich in proteins (19.8 % on average), but low in fat content (6.6 % on average). Horsemeat proteins have a high content of both lysine and threonine (1.57 and 0.84 g/100 g), and a low content of tryptophan (0.15 g). In comparison with other types of meat, it is a good source of P, Fe, Zn, Cu and Mg (Badiani et al., 1997). According to Täufel et al. (1993), horsemeat is finely textured and bright red in young animals and dark red with bluish tinge in older animals. It is characterised by hardness and sweetish taste owing to high ratio of glycogen compared to meat of other animals. From the nutritional standpoint, horsemeat is readily digested in comparison with beef, and has a very low content of cholesterol in comparison with beef and pork, and is therefore recommended as dietary food. It is also recommended for the nutrition of children, sportsmen and persons affected with anaemia (Täufel et al., 1993). Horsemeat products do not make a standard range of products in meat industry. Horsemeat sausages are rarely available on the market, mostly only as regional specialities, produced in the artisan meat processing plants, including also fermented sausages made of horsemeat. Fermentation process has been known from ancient times as a successful mode of storage and prolongation of shelf life of food. During the fermentation, ripening and drying processes of fermented sausages, a large number of complex microbiological, biochemical and physicochemical processes occur. All of them influence the quality and safety of food products (Hadžiosmanović et al. 2005). Fermented horsemeat products have lower levels of saturated fatty acids compared to those made of beef or pork, with pH value being about 5.92, and a_w 0.94 (PALEARI et al., 2003).

The aim of this study was to monitor the changes in microflora occurring during ripening of horsemeat sausages.

MATERIAL AND METHODS

Sausages were made of horsemeat (75 %), firm, pork back fat (25 %) and additional ingredients (salt, pepper, paprika, garlic, and nitrite salt). After filling into artificial casings of 55 mm in diameter, and drying on sticks, the sausages were cold smoked for 2-3 days, and then left to ripen in the ripening chamber till day 42. Three sausages were sampled on day 0, and again on days 14, 28 and 42. Samples of horsemeat and pork back fat were also collected at the start of production (0 day). All samples (three of each raw material and sausage) were subjected to microbiological and physicochemical analyses. Microbiological analysis included the determination of counts of aerobic mesophilic bacteria, lactic acid bacteria, micro-

[1] **Damir Alagić, MSc, DVM, College of Agriculture at Križevci; Milislava Demerca 1, 48260 Križevci, contact e-mail: dalagic@vguk.hr**

[2] **Lidija Kozačinski, DSc, associate professor; Ivana Filipović, DVM, junior research assistant; Nevijo Zdolec, DSc, junior researcher - senior assistant; Mirza Hadžiosmanović, DSc, full professor; Bela Njari, DSc, full professor; Institute of Foodstuff Hygiene and Technology, Veterinary Faculty, University of Zagreb, Heinzelova 55, Zagreb**

[3] **Zvonimir Kozačinski, MSc, DVM, Veterinary health station Velika Gorica, Sisačka bb, Velika Gorica**

[4] **Sunčica Uhitil, DSc, Veterinary health station Zagreb, Heinzelova 68, 10000 Zagreb**

Table 1. Microorganisms count in raw material (log_{10} cfu g^{-1})

	Horsemeat	Pork fat tissue
Enterobacteriaceae	<2	<2
E.coli	<2	<2
Micrococcus spp	2,71 ± 0,01	3,17± 0,15
LAB	3,36 ± 0,02	<2
AB	6,27 ± 0,01	5,93 ± 0,00
Enterococcus spp	3,68 ± 0,01	3,25 ± 0,01
Pseudomonas spp	4,4 ± 0,02	4,28 ± 0,03
Yeast and moulds	4,17 ± 0,02	3,70 ± 0,02
Sulphite-reducing bacteria	<1	<1
S.aureus	<2	<2

cocci, *Staphylococcus aureus*, enterobacteria, *Escherichia coli*, yeasts and moulds, enterococci, *Pseudomonas* spp, sulphite-reducing clostridia, and identification of the presence of *Listeria monocytogenes* and *Salmonella* species. Physicochemical analysis included the determination of water and salt content and pH value that was also determined in raw material.

For microbiological analysis, 25 g of sample were diluted with 225 ml of saline peptone water and homogenised in stomacher to obtain basic dilution. Further decimal dilutions were prepared from the basic dilution, and then an appropriate decimal dilution was used for inoculation in agar or was poured on agar plates. Aerobic mesophilic bacteria were determined on the Plate Count agar (PCA, BioMerieux, France) at 30˚C /72 h; LAB count on MRS agar (Merck, Germany) at 30˚C/ 48-72 h; micrococci on Manitol Salt agar (MSA, BioMerieux) at 30ºC/ 48 h; enterobacteria on Violet Red Bile Glucose agar (VRBG, Oxoid, England) at 37ºC/48 h; *E. coli* on Coli-ID agar (BioMerieux) at 37ºC/48 h; enterococci on Kanamycin Aesculin Azide agar (Merck) at 37ºC/48 h; *S. aureus* on Baird-Parker agar (BP, Merck) at 37ºC/48 h; yeasts and moulds on oxytetracycline-glucose yeasts extract agar (Oxoid) at 25ºC/72-120 h, *Pseudomonas* spp. on cetrimide agar (BioMerieux) at 25ºC/48 h, and sulphite-reducing clostridia on Sulphite Polymyxin Sulphadiazine agar (SPS, Merck), anaerobically at 37ºC/72 hours. The method HRN ISO 6579:2003 was used for determination of the presence of *Salmonella* spp. in 25 g of sample, and the method HRN ISO 11290-1 for *Listeria monocytogenes.* The pH values were determined in meat extract by digital pH-meter (Hanna instruments, Romania). Water and salt content were determined during the sausage ripening process by AOAC (2002) methods.

RESULTS AND DISCUSSION

Results of microbiological analyses are presented in Tables 1 and 2. Total viable count (TVC) in horsemeat exceeded the limit value according to the Croatian microbiological standards for meat cuts. During ripening, TVC in sausages ranged between 7 and 8 log_{10}cfu g^{-1} and then gradually decreased to 6 log_{10}cfu g^{-1} in the last stage of ripening, on day 42. LAB count significantly increased during ripening, from the initial low (4 log_{10}cfu g^{-1}) to high (7-8 log_{10}cfu g^{-1}) on day 14, 28 respectively. LAB count at the end of ripening period amounted to 6 log_{10}cfu g^{-1}. High counts of yeasts and moulds were found at the beginning of ripening (4 log_{10}cfu g^{-1}), and at the end of ripening process were by 1 log_{10} lower. Such high number of yeasts is probably due to high yeast count recorded in raw material (Table 1). Besides LAB and coagulase-negative cocci (*Staphylococcus* spp. and *Micrococcus* spp.), yeasts and moulds play an important role in sausage ripening (Hutkins, 2006). Yeasts contribute to sensory traits of fermented sausages, and of other meat products, due to their lipolytic and proteolytic activity (Hammes and Hertel, 1998). The same applies to coagulase-negative cocci (Hadžiosmanović et al., 1979). Populations of micrococci and enterococci were also reduced from 4 to 3 log_{10}cfu g^{-1} during ripening. Population of enterococci often varies in different types of fermented sausages, and depends primarily on hygienic quality of the raw material used (Hugas et al. 2003; Drosinos et al., 2005; Comi et al., 2005; Kozačinski et al. 2006). *Pseudomonas* species were found in horsemeat and pork fat tissue and also in the sausage stuffing on day 0 (4 log_{10}cfu g^{-1}), but not during fermentation of sausages. *S. aureus* was not found in pork fat tissue and horsemeat, however, its count in sausages was 2 log_{10}cfu g^{-1}. From day 14 till the end of fermentation, *S. aureus* was not isolated. Enterobacteria and *E.coli* were found on day 0 of fermentation (2-3 log_{10}cfu g^{-1}), but later their counts dropped below the limit of detection. Sulphite-reducing clostridia were present throughout the ripening period, although their count dropped from 3 log_{10}cfu g^{-1} to 1 log_{10}cfu g^{-1}. As sulphite-reducing clostridia were not detected in meat and pork back fat, their subsequent presence in sausages may be attributed to contamination of spices, which is consistent with results of the study carried out by Fujisawa et al. (2004), in which clostridia had been found in 47% of analysed spice samples. *Salmonella* spp. and *Listeria monocytogenes* were not isolated from any sample collected during the ripening period.

Decrease in the number of pathogenic microorganisms in the sausage stuffing may be attributed mostly to both the acidification and drying process. Principal function of

Table 2. Microorganisms count in sausages (log_{10} cfu g^{-1})

	Day 0	Day 14	Day 28	Day 42
Enterobacteriaceae	3,00 ± 0,41	<1	<1	<1
E. coli	2,23 ± 0,15	<2	<2	<2
Micrococcus spp	4,62 ± 0,14	4,68 ± 0,03	4,33 ± 0,02	3,79 ± 0,06
LAB	4,05 ± 0,07	8,53 ± 0,03	7,95 ± 0,04	6,22 ± 0,06
AB	7,01 ± 0,11	8,60± 0,06	8,23 ± 0,20	6,67 ± 0,02
Enterococci	4,64 ± 0,02	3,71 ± 0,10	3,79 ± 0,06	3,49 ± 0,04
Pseudomonas spp	4,84 ± 0,05	<2	<2	<2
Yeast and moulds	4,62 ± 0,14	4,32 ± 0,15	3,63 ± 0,17	3,45 ± 0,12
Sulphite-reducing bacteria	3,16 ± 0,24	2,38 ± 0,07	1,43 ± 0,26	1,70 ± 0,18
S.aureus	1,35 ± 1,01	<2	<2	<2

LAB in meat fermentation is rapid lowering of the stuffing pH value that ensures: product safety by inactivation of pathogens, product stability and prolongation of shelf life, and creation of biochemical conditions needed for obtaining new sensory traits of products (Lűcke, 2000).

The recorded pH value was 5.63 in horsemeat, 5.9 in fat tissue, respectively, while the initial pH of sausages was 5.82. The lowest pH value was recorded on day 14 of ripening (5.07), and then it slowly increased and exceeded the initial value on day 42 (6.34). The LAB count was in correlation with pH value, since it was the highest on day 14, when the lowest pH value was recorded and significantly lower on day 42, when pH value of sausages was high. Salt content increased with decrease of water content. Water content dropped from the initial 59% to only 17% in final product. That extremely low water content may be associated with long period of ripening, since the organoleptic examination of sausages revealed excessive dryness and toughness. Shortening of the ripening period to 36 days was recommended.

CONCLUSION

During fermentation, in the sausage microflora prevailed LAB (6-8 log_{10} cfu g^{-1}), but there were also micrococci (2-3 log_{10} cfu g^{-1}), and yeasts and fungi (3-4 log_{10} cfu g^{-1}). Significant drop in pH value was recorded during the first 14 days of fermentation (5.82 on day 0, and 5.02 on day 14). That, and other possible antimicrobial mechanisms of LAB, led to the expected reduction of microorganism counts or elimination of enterobacteria, *E. coli*, pathogenic staphylococci and sulphite-reducing clostridia.

ZUSSAMENFASSUNG

MIKROBIOLOGISCHE ÄNDERUNGEN WÄHREND DER REIFE DER FERMENTIERTEN WÜRSTE AUS PFERDEFLEISCH

In der Arbeit wurden die Änderungen während der Reife der fermentierten Würste aus Pferdefleisch beobachtet. Je drei Würste wurden am O., 14., 28. und 42. Reifetag gemustert. Während der Fermentation waren Bakterien der Milchsäure dominante Mikroflora der Würste (6-8 log_{10} cfu g^{-1}). Bei der Fermentation waren auch die Mikrokokken beteiligt , deren Zahl sich um 2-3 log_{10} cfu g^{-1} drehte; sowie auch Hefe und Pilze (3-4 log_{10} cfu g^{-1}). In den ersten 14 Fermentationstagen verminderte sich bedeutend der pH Wert (5,82 am 0.Tag, 5,02 am 14. Tag), was neben möglichen anderen antimikroben Mechanismen LAB mit erwarteter Verminderung oder Elimination der Enterobakterien, E. Coli, pathogenen Staphylokokken und sulfidreduzierenden Clostridien resultierte.

Schlüsselwörter: *Würste aus Pferdefleisch, mikrobiologische Änderungen*

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UPGRADING THE SAFETY AND QUALITY OF TRADITIONAL FERMENTED SAUSAGES

Zdolec[1], N., M. Hadžiosmanović[1], L. Kozačinski[1], Ž. Cvrtila Fleck[1], I. Filipović[1], K. Leskovar[2], P. Popelka[3], S. Marcinčak[3]

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 Cvrtila 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**