Institute of Meat Hygiene and Technology
Belgrade-Serbia

PROCEEDINGS

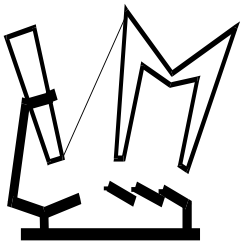
INTERNATIONAL
57th MEAT INDUSTRY CONFERENCE

MEAT AND MEAT PRODUCTS – PERSPECTIVES OF SUSTAINABLE PRODUCTION

Belgrade, June 10th-12th, 2013

ISBN 978-86-82547-07-5

INSTITUTE OF MEAT HYGIENE AND TECHNOLOGY – BELGRADE



PROCEEDINGS

International 57th Meat Industry Conference

MEAT AND MEAT PRODUCTS – PERSPECTIVES OF SUSTAINABLE PRODUCTION

Belgrade, 10th-12th June, 2013

Editorial Board/Reviewers

Schwägele Fredi, PhD – *Germany*
Jeney Zsigmond, PhD – *Hungary*
Tchernukha Irina Mikhailovna, PhD – *Russia*
Žlender Božidar, prof.– *Slovenia*
Steinhauser Ladislav, PhD – *Czech Republic*
Talone Regine, PhD – *France*
Vuković Ilija, prof. – *Serbia*
Baltić Ž. Milan, prof. – *Serbia*
Teodorović Vlado, prof. –*Serbia*
Radovanović Radomir, prof. –*Serbia*
Petrović Milica, prof. – *Serbia*
Ćirković Miroslav, prof.– *Serbia*
Matekalo-Sverak Vesna, PhD – *Serbia*
Spirić Aurelija, PhD – *Serbia*
Milićević Dragan, PhD – *Serbia*
Đinović-Stojanović Jasna, PhD – *Serbia*
Velebit Branko, PhD – *Serbia*
Estevez Mario, PhD – *Spain*
Jakovac-Strajn Breda, docent – *Slovenia*
Hengl Brigita, PhD – Croatia
Milijašević Milan, PhD – *Serbia*
Okanović Đorđe, PhD – *Serbia*
Vranić Danijela, PhD – *Serbia*
Parunović Nenad, PhD – *Serbia*
Borović Branka, PhD – *Serbia*

Organizing Committee

President Branko Velebit, PhD
Vice President Saša Prečanica
Members Danijela Šarčević, PhD
Živko Kragujević
Gordana Terzić
Igor Milošević
Ivana Branković-Lazić
Bojan Balaž
Andrea Končar

ISBN 978-86-82547-07-5

Number of copies/
200 electronic copes

PLENARY SESSION

POSTER SESSION

P-30

SOME CHARACTERISTICS OF FERMENTED SAUSAGES PRODUCED WITH COMMERCIAL PROBIOTIC *LACTOBACILLUS CASEI* LC01 AND POTENTIAL PROBIOTIC *LACTOBACILLUS PLANTARUM* 564 ISOLATED FROM SJENICA CHEESE

Dragan Vasilev[1], Zorica Radulović [2], Nemanja Mirković [2], Dušan Kekuš [2], Milica Petrušić[2], Nikola Čobanović[1]

[1] Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia
[2] Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

Abstract – This paper presents some important characteristics of fermented sausages produced with commercial probiotic strain *Lactobacillus casei* LC01 and potential probiotic *Lactobacillus plantarum* 564 isolated from autochthonous *Sjenički* cheese. The results show that at the end of ripening the pH-value of sausages produced with *Lb. pantarum* 564 was statisticaly lower (4,62-4,66) then the pH-value of sausages produced with *Lb. casei* LC 01 (4,75 - 4,79). There was no siginificant difference in a_w-value of experimental sausages. Both *Lb. plantarum* 564 and *Lb. casei* LC 01 strains showed a good potential to grow in fermented sausages, so the count of viable cells at the end of ripening was 7,8-8,0 log cfu/g and 8,5-8,8 log cfu/g respectively. The overal sensory quality of experimental sausages was highly evaluated: 4,8 (adjusted rating 94,4-95,3) for the sausages with *Lb. plantarum* 564 and 4,9 (adjusted rating 97,6-97,9) for the sausages with *Lb. casei* LC 01.

Key words – fermented sausages, functional food, probiotics.

I. INTRODUCTION

With the development of consumer awareness about the importance of healthy nutrition, functional food becomes even more significant. Besides the basic nutrients, functional food contains functional ingredient(s) that exercise positive effects on human health. One of the most important group of functional ingredients are certainly probiotics. Probiotics are live microorganisms that, after being ingested through food, may exercise health benefits on the host. In order to be selected and assigned as a probiotic, a microorganism must be a significant part of human intestinal flora, to be able to survive passage through the stomach and intestine, and possess the ability of adherence to intestinal epithelial cells [1]. Probiotic bacteria achieve positive effect on human health in several ways. They prevent gastro-intestinal infections, reduce cholesterol level in blood, have a positive effect on immune system and help in overcoming the lactose intolerance problem, and even more, it is also believed that they can prevent development of cancer [2, 3, 4]. Production of functional food is most developed by dairy and bakery products, but meat industry also develops products with functional properties. Considering the possibilities of application of probiotics in meat products, only fermented sausages can be used as probiotic carriers. It is explained by the fact that fermented sausages are not heat treated during production and that the bacteria from *Lactobacillus* genus naturally play important role in fermentation process. It is well known that a large number of *Lactobacillus* strains possess probiotic properties.

During the selection of a probiotic bacterial strain for applying in fermented sausages production it is of great importance to meet some requirements, such as to have the ability to survive the sausage production process as well as the conditions during storage in sufficient number to be able to achieve positive effects on human health. In order to be characterized as a probiotic food, a product must contain high

number of probiotic bacteria, which means at least 10^6 cfu / g [5]. In addition, there should be met the requirement not to alter the sensory properties of the product [3, 6].

One of the simpler methods of probiotic bacteria application in fermented sausages is to use commercial probiotic cultures, which are already used in dairy industry. The benefit of this approach is that the use of probiotics in dairy industry is already largely developed and the properties of probiotic bacteria originating from dairy products are mostly tested. However, some of probiotic bacteria which are used in dairy industry do not have the ability to grow and survive in fermented sausages, which is the case with the most commonly used strains such as *Lb. acidophilus* LA 5 and *Bifidobacterium lactis* BB 12 [7, 5]. The literature data [5, 7, 8], as well as the results of our own research [9] show that *Lb. casei* LC 01, a commercial probiotic starter culture which is used in dairy industry, successfully survives and grows in fermented sausages in high numbers so the sausage could be assigned as probiotic, without affecting the sensory properties of the product.

The harder approach is the use of probiotic bacteria isolated from fermented sausages. However, the bacteria that are naturally involved in the ripening of fermented sausages, with the exception of *Lactobacillus plantarum* strains, do not have probiotic significance. Vuyst et al. [10] reported that some strains of *Lb. plantarum*, which are isolated from fermented sausages, have better adherence ability to intestinal epithelial cells than strains of *Lb. paracasei* species, contributing to their stronger probiotic potential. However, Cebeci et al. [11] concluded that none of the *Lb. plantarum* strains isolated from fermented sausages has all the characteristics of a probiotic.They recommended that *Lb. plantarum* strains as probiotic candidates originated from fermented sausages should be used as mixtures of different strains to get all of the probiotic characteristics in one product. These authors also concluded that strains of *Lb. plantarum* originating from some fermented milk products (e.g. kefir and sourdough) have a better probiotic potential than the probiotic strains originating from fermented sausage.

Related to foregoing data, the aim of this study was to investigate the possibility of application of potential probiotic bacterial strain *Lb. plantarum* 564 isolated from autochthonous *Sjenički* cheese in production of functional fermented sausages. As a control product were used fermented sausages produced with a commercial probiotic strain *Lb. casei* LC 01, which is widely used in dairy industry and also proven to be successfully applied in the production of functional fermented sausages.

II. MATERIALS AND METHODS

Experimental sausages were produced according to two basic recipes, which in previous research provided products with the most acceptable sensory properties [9, 16]:

- Sausage A: lean beef 35 %, lean pork 40 %, pork backfat 22 %, inulin powder 2 % (Fibruline instant, Cosucra S.A., Belgium), pea fiber 1 % (Swelite, Cosucra S.A., Belgium).
- Sausage B: lean beef 35 %, lean pork 40 %, pork backfat 20 %, inulin suspension 4 % (Fibruline XL, Cosucra S.A., Belgium, inulin and water ration 1:1), pea fiber 1 % (Swelite, Cosucra S.A., Belgium).

Additions to 1 kg stuffing were identical for both recipes: 28.0 g curing salt with nitrite, 1.5 g dextrose, 4.0 g sucrose, 2.5 g grounded white pepper, 1.0 g peppercorns, 0.5 g cardamom, 4.0 g brandy with juniper, and 2.5 g omega-3 fatty acids preparation (Denomega Gat 100).

Inulin suspension for the Sausage B was prepared by homogenization of inulin and warm water (75^{o}C) in a meat bowl cutter in 1:1 ratio, followed by cooling and freezing (-12 ^{o}C).

From each of these recipes, two particular groups of sausages were produced. In the first group, as a starter culture was used a commercial probiotic strain *Lb. casei* LC 01 (Chr. Hansen, Denmark). For the second group, a potential probiotic culture *Lb. plantarum* 564 was used (from the collection of the Department of Technological Microbiology, Faculty of Agriculture, University of Belgrade), which was isolated from autochthonous *Sjenički* cheese and characterized as a potential probiotic [12]. In this way, four groups of experimental sausages were produced, as follows:

Sausage A with *Lb. casei* LC01
Sausage A with *Lb. plantarum* 564
Sausage B with *Lb. casei* LC01
Sausage B with *Lb. plantarum* 564

The stuffing preparation: frozen materials (pork meat, pork backfat and inulin suspension) were cut in a »meat block braker« and chilled beef meat was coarsely grinded. In a meat bowl cutter were firstly chopped frozen materials, followed by probiotic starter culture addition. After that, the chilled beef maet, sugars, inulin powder (recipe A), pea fiber, Denomega Gat 100 and grounded spices were added. The stuffing was comminuted to 2 mm particle size, and at the and of the process the peppercorn and curing salt were added, and finally properly blended. The stuffing was filled in collagen casings 60 mm diameter. Sausages were firstly tempered (drainage) up to 12 hours at room temperature, and then smoked at 20-24 ° C, casually during 3-4 days. Drying and ripening lasted another 15-17 days at 18-20 °C and relative humidity which decreased from 90 to 85 %. Total production process lasted 21 days.

Experimental sausages were examined using standard physico-chemical, bacteriological and sensory methods.

Physico-chemical methods: 1) pH-value was measured by means of a digital pH-meter WTW, model 521, with combined electrode (WTW-Wissenschaftlich-TechnischeWerkstäten GmbH, Weilheim, Germany). Water activity (a_w) was measured by means of an a_w-meter (GBX Scientific Instruments, Fa-St/1).

Bacteriological methods: 1) the count of cells of probiotic strain *Lb. casei* LC 01 in fermented sausages was examined on MRS agar (Merck) supplemented with *moxalactam* (Sigma-Aldrich Chemie, M-8158, Germany) in the amount of 112 µg mL^{-1}, at 37°C/72 hrs in anaerobic environment [5]. 2) The count of cells of potential probiotic strain *Lb. plantarum* 564 was examined on MRS agar (Merck) supplemented with *rifampicin* (Sigma-Aldrich Chemie, R-3501, Germany) in the ammount of 250 µg mL^{-1}, at 37°C/48 hrs in anaerobic environment. Rifampicin resistant strain *Lb. plantarum* 564 (Rif^r) was produced by cultivation of strain *Lb. plantarum* 564 in MRS broth (Merck, Germany) with increase of rifampicin concentration and incubation at 37°C /18 hrs [13].

Overall sensory quality was investigated by eight-member panel, following the method of adjusted five-point scale system [14]. Sensory characteristics of fermented sausages were evaluated by scores of 5 (excellent) to 1 (unacceptable), and the obtained scores were multiplied with corresponding coefficients of importance: appearance 2, cut surface appearance 4, color and sustainability of color 4, odor and taste 6 and texture 4.

The results were statistically analyzed by determining the average value, measure of variation and statistical significance.

III. RESULTS AND DISCUSSION

The results of examination of physico-chemical parameters of experimental sausages are shown in Table 1. The pH-value of experimental sausages produced with potential probiotic strain *Lb. plantarum* 564 was lower for more then 0.1 pH-unit than the pH-value of sausages produced with *Lb. casei* LC 01 strain, and this difference was statistically significant. Since *Lb. plantarum* strains do not show the ability to ferment inulin, but they possess a very good ability to ferment glucose and sucrose [15], the lower pH-values of sausages produced with *Lb. plantarum* 564 may be primarily a consequence of intensive fermentation of sucrose and dextrose which were added in experimental sausages.

Table 1 Physico-chemical parameters of fermented sausages

Parameter	Sausage A with *Lb. casei* LC01	Sausage A with *Lb. plantarum* 564	Sausage B with *Lb. casei* LC01	Sausage B with *Lb. plantarum* 564
pH	4.75[a]	4.62[b]	4.79[a]	4.66[b]
a_w	0.900	0.907	0.901	0.904

a,b = $p < 0.01$

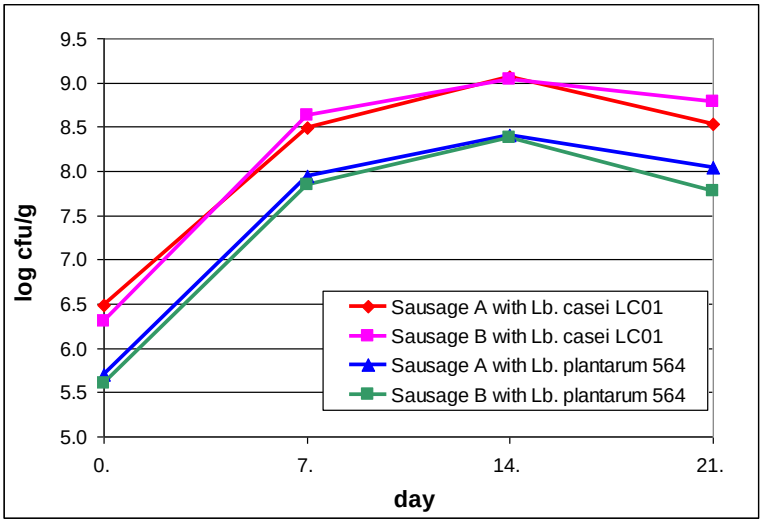


Figure 1 Count of probiotic bacterial cells in fermented sausages (log cfu/g)

The pH-value of sausages produced with 2 % inulin powder (recipe A) was lower than pH-value of sausages produced with 4 % inulin suspension (recipe B), which is in accordance with our previous findings [9,16], and was associated with the fermentation of inulin powder by *Lb. casei*, which had a good ability to ferment this prebiotic [15]. The water activity of all experimental sausages was almost the same (Table 1) and in accordance with a_w-values detected in fermented sausages containing dietary fibers and prebiotics [17, 18, 19] as well as the previous results of our own research [9, 16].

The results of *Lb.plantarum* 564 and *Lb. casei* LC01 probiotic strains viability examination in fermented sausages showed that the both strains had a good growing rate. The viable cells count of a potential probiotic strain *Lb. plantarum* 564 at the end of the sausage production was 7.8 - 8.0 log cfu / g (Fig. 1), which is sufficient for a product to be assigned as probiotic food [5]. These results support the claims that *Lb. plantarum* strains have a good ability to grow in fermented sausages in which they represent a part of natural microflora [10]. The viable cells count of a commercial probiotic strain *Lb. casei* LC 01 at the end of the sausage production was 8.5 - 8.8 log cfu / g (Fig. 1), which is in accordance with the literature data [7, 20] as well as the results of our own research [9, 21].

The examination of overall sensory quality of experimental sausages showed that all products were highly evaluated (Table 2), noting that the sausages produced with Lb. *plantarum* 564 had a slightly lower average score as well as the adjusted rating, regardless the basic recipe.

Table 2. Scores of some sensory properties of fermented sausages

Sensory property	Sausage A with *Lb. casei* LC01	Sausage A with *Lb. plantarum* 564	Sausage B with *Lb. casei* LC01	Sausage B with *Lb. plantarum* 564
Appearance	5.0	5.0	5.0	5.0
Cut surface appearance	4.9 ± 0,2	4.9 ± 0,2	4.9 ± 0.2	4.9 ± 0.2
Color and sustainability of color	4.9 ± 0,2	4.9 ± 0,2	4.9 ± 0.2	4.8 ± 0.3
Odor and taste	4.8 ± 0,3	4.4 ± 0,2	4.8 ± 0.3	4.4 ± 0.2
Texture	4.9 ± 0,2	4.9 ± 0,2	4.9 ± 0.2	4.8 ± 0.3
Average score *	4.90	4.83	4.92	4.78
Adjusted score **	97.6	95.3	97.9	94.4

*=maximal score is 5,00; **= maximal score is 100

Such results were obtained because the odor and taste of sausages produced with *Lb. plantarum* 564 was rated 4.4 and of sausages produced with *Lb. casei* LC 01 with a score 4.8, which influenced their overall sensory quality. The taste of sausages with *Lb. plantarum* 564 was slightly sour, which was supported by lower pH-value measured in these products (Table 1), and the flavor was slightly unspecific for fermented sausages. Other characteristics, such as appearance, cut surface appearance; color and sustainability of color as well as texture were highly evaluated in all experimental sausages, with no significant differences.

IV. CONCLUSION

When selecting an appropriate probiotic strain for the production of functional fermented sausages, the most important requirements for those strains are to be able to survive in sufficient number and not to alter the sensory properties of the product. Both *Lb. plantarum* 564 and *Lb. casei* LC 01 strains showed a good potential to grow in fermented sausages, being present in a higher number then the requested minimum for probiotic food. Even more, the use of these strains as starter cultures did not significantly affect the sensory properties of experimental fermented sausages, which were highly evaluated (4.8 and 4.9 respectively).

ACKNOWLEDGEMENTS

This paper resulted from the research projects numbered III 46009 and III 46010 funded by the Ministry of Science, Education and Technological Development of Republic of Serbia.

Authors are grateful to Professor dr Ilija Vuković for advice and help.

Corresponding author: vasilevd@vet.bg.ac.rs

REFERENCES

1. Isolauri, E., Salminen, S., Ouwehand, A.C.(2004). Probiotics. Best Practice and Research Clinical Gastroenterology18 (2):299-313.
2. Jimenez-Colmenero, F., Carballo, J., Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. Meat Science 59:5-13.
3. Holzapfel, W. H., Schillinger U. (2002). Introduction to pre- and probiotics. Food Research International 35:109-116.
4. Jones, P., Jew, S. (2007). Functional food development: concept to reality. Trends in Food Science and Technology 18:387-390.
5. Kröckel, L. (2006). Einsatz probiotischer Bakterien bei Fleischerzeugnissen. Mitteilungsblatt der Fleischforschung Kulmbach 45 (173):163-172.
6. Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fonden, R., Saarela, M. (2002). Technological challenges for future probiotic foods. International dairy Journal 12:173-182.
7. Bactoferm Bulletin (2003). Fermented, dry sausages produced with probiotic cultures. Bactoferm Bulletin 9:1-7.
8. Müller, W. D. (2006). Funktionelle Fleischerzeugnisse – Rohwürste. Mitteilungsblatt der Fleischforschung Kulmbach 45 (173):185-191.
9. Vasilev, D., Vuković, I., Saičić, S. (2011). Some quality parameters of functional fermented cooked and liver sausages. Meat technology 52 (1):141-153.

10. Vuyst, D.L., Falony, G., Leroy, F.(2008). Probiotics in fermented sausages. Meat Science 80:75-78.
11. Cebeci, A., Gürakan, C. (2003). Properties of potential probiotic Lactobacillus plantarum strains. Food Microbiology. 20:511-518.
12. Radulović, Z., Mirković, N., Bogovič-Matijašić, B., Petrušić, M., Petrović, T., Nedović, V.(2012). Quantification of viable spray-dried potential probiotic lactobacilli using real-time PCR. Archives of Biological Science 64 (4):1465-1472.
13. Bogovic Matijasic, B., Koman Rajsp, M., Perko, B., Rogelj, I. (2007). Inhibition of Clostridium tyrobutyricum in cheese by Lactobacillus gasseri. International Dairy Journal 17:157-166.
14. Radovanović, R., Popov-Raljić, J. (2001). Senzorna analiza prehrambenih proizvoda. Poljoprivredni fakultet, Beograd, i Tehnološki fakultet, Novi Sad.
15. Pennacchia, C., Vaughan, E.E., Villani, F.(2006). Potential probiotic *Lactobacillus* strains from fermented sausages: Further investigations on their probiotic properties. Meat Science 73:90-101
16. Vasilev, D., Saičić, S., Vasiljević, N. (2013). Qualität und Nährwert von mit Inulin und Erbsenfasern als Fettgewebe-Ersatzstoffe hergestellten Rohwürsten, Fleischwirtschaft 93(3):123-127.
17. Sieg, J. (2005). Produktideen noch nicht ausgeschopft, Ballaststoffe der zweiten Generation. Fleischwirtschaft 85 (7):57-58.
18. Eim, V. S., Simal, S., Rossello, C., Femenia, A.(2008). Effects of addition of carrot fibre on the ripening process of a dry fermented sausage (Sobrassada). Meat Science 80(2): 173-182.
19. Stajić, S., Perunović, M., Stanišić, N., Žujović, M., Živković, D. (2012). Sucuk (Turkisch-style dry-fermented sausage) quality as an influence of recipe formulation and inoculation of starter cultures. Journal of Food Processing and Preservation DOI: 10.1111/j.1745-4549.2012.00709.x
20. Troeger, K., Nitsch, P., Müller, WD, Munch, S. (2005). Kein Angriff auf Geschmack und Textur, Funktionelle Fleischerzeugnisse: Ein Beitrag zur gesunden Ernahrung. Fleischwirtschaft 85(7):54-56.
21. Vasilev, D. (2010). Ispitivanje činilaca od značaja za bezbednost i kvalitet fermentisanih kobasica proizvedenih kao funkcionalna hrana. Doktorska diesrtacija, Fakultet veterinarske medicine, Beograd.

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

637.5(082)
614.31:637.5(082)
664.9(082)

INTERNATIONAL Meat Industry Conference
Meat and Meat Products - Perspectives of
Sustainable Production (57 ; 2013 ; Beograd)
   Proceedings [Elektronski izvor] /
International 57th Meat Industry Conference
Meat and Meat Products - Perspectives of
Sustainable Production, Belgrade, 10th-12th
June, 2013 ; [organizer] Institute of Meat
Hygiene and Technology, Belgrade ; [editors
in chief Slobodan Lilić, Vesna Đorđević]. -
Belgrade : Institute of Meat Hygiene and
Technology, 2013 (Belgrade : Institute of
Meat Hygiene and Technology). - 1 elektronski
optički disk (CD-ROM) ; 12 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa
naslovnog ekrana. - Tiraž 200. -
Bibliografija uz svaki rad.

ISBN 978-86-82547-07-5
1. Institute of Meat Hygiene and Technology
(Beograd)
a) Meco - Зборници b) Месне прерађевине -
Зборници
COBISS.SR-ID 198874636