ISSN 2406-1247
UDK 664.9:614.31: 637.5(05)

tehnologija mesa

meat technology

God. **56** **Br.** **1** **Beograd,** **2015**

Vol. **No.** **Belgrade,**

***Osnivač i izdavač* – FOUNDER AND PUBLISHER**
INSTITUT ZA HIGIJENU I TEHNOLOGIJU MESA, BEOGRAD
INSTITUTE OF MEAT HYGIENE AND TECHNOLOGY

ISSN 2406-1247
UDK: 664.9:614.31:637.5(05)

tehnologija mesa
naučni časopis

Tehnologija mesa God. 56 Br. 1 Str. 1–84 Beograd 2015

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U FINANSIRANJU ČASOPISA UČESTVUJE:
Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije

tehnologija mesa
Osnivač i izdavač: Institut za higijenu i tehnologiju mesa, Beograd

UDK: 637.524.035
ID: 216772876

Original scientific paper
Originalni naučni rad

The impact of starter culture on the pH and the content of lactic and volatile fatty acids in boiled-smoked sausages

Indzhelieva Dijana[1], Valkova-Jorgova Katja[2], Kuzelov Aco[3], Andronikov Darko[4]

A b s t r a c t: This paper reviews the effects of starter cultures with lactic acid bacteria as a factor of increasing quality and intensification of production processes in durable boiled – smoked sausages, type of Burgas. In this regard, the impact of starter cultures in the filling, their role in changing the pH value, the content of lactic acid and volatile fatty acids were tested. The two types of starter were used: pure cultures Bifidobacterium longum (B_2), and mixed cultures Bifidobacterium longum (B_2) and Lactobacillus plantarum (L_6), ratio 2 : 1. To test their impact in stimulating technological process we have produced 10 kg product model of boiled – smoked sausage durable type of Burgas. During the process of draining and drying, an increase in the number of lactobacilli and bifid bacteria was recorded. The test results show that the minimum pH value was detected in the period of intensive growth of microorganisms from the starter culture. pH values depend on the composition of starter cultures. Also, rapid decline of pH in the experimental samples with mixed starter cultures was recorded. Considering the amount of lactic acid, intense formation of lactic acid was detected in experimental samples, especially with mixed starter cultures. The results of the examination of the content of volatile fatty acids show that during the process of drying in all of the samples an increase in the value was observed, even a more intensive one in the test samples.

Keywords: *meat products, starter cultures, pH, volatile fatty acids.*

Introduction

One of the promising directions in the production of meat products is the use of biologically active substances, products of vital activity of beneficial microorganisms. Determined that, applied microorganisms in the form of starter cultures under the action of enzymes forming compounds which enhance the sensory characteristics of the meat products must be phenotypically and genotypically characterized, including technological, safety and probiotic features (*Ammor and Mayo,* 2007; *Arihara,* 2006; *Buckenhüskes,* 1993*; Demeyer and Toldra,* 2004).

One of the criteria in the selection of microorganisms for starter cultures is the degree of influence on the taste-aromatic characteristics of the finished product in terms of intensifying production technology. Most of lactic acid bacteria meet these criteria which are biological prerequisite for the formation properties of the sausages as a food product, and is also manifested as conserved factor (*Patarata et al.,* 2008; *Pennacchia et al.,* 2004; *Schillinger et al.,* 1996).

Lactic acid bacteria produce biochemical changes in the main components of the meat, accompanied by the formation of compounds that determine the taste, smell, texture; positive change in the physicochemical parameters of the filling; inhibiting the growth of harmful pathogenic microorganisms by the formation of different substances having antimicrobial activity (*Danielsen and Wind,* 2003; *Dellaglio et al.,* 1996).

The purpose of this work is to investigate the possibility of optimizing the production process of boiled-smoked sausages using starter cultures of lactic acid bacteria.

Materials and Methods

In the experimental work pure cultures *Lactobacillus plantarum* (L_6) and *Bifidobacterium longum* (B_2) were used. Strains were provided by a

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private laboratory for analysis and control of food – Burgas. *L.plantarum* (L_6) was isolated from raw-cured sausage „Lukanka", and *B. longum* (B_2) from yogurt, to which it was added as a probiotic. On the basis of previous studies it was found the two strains meet the criteria necessary for their use as starter cultures alone and as combination starters.

The two types of starter were used: pure cultures *Bifidobacterium longum* (B_2), and mixed cultures *Bifidobacterium longum* (B_2) and *Lactobacillus plantarum* (L_6), ratio 2 : 1. Activation of the dry bacterial preparation was done in skimmed milk, sterilized at 121°C for 13 min and cooled to 37°C. The amount of 0,1g was imported to 1l. The soured milk was left in a thermostat at 37°C, until the acidity 60–65°C and compression. Mixture was cooled to 5°C.

To study their impact on the course of the process, 10 kg model product of cooked smoked durable sausage „Burgas" was produced. For the preparation of 100 kg we used the following: beef meat first category 20 kg, veal meat first category 20 kg, pork lean meat first category 10 kg, semi-fatty pork meat second category 50 kg, NaCl 2.00 kg, sodium nitrite 0.005 kg, 0.050 kg ascorbic acid, sodium tripolyphosphate 0.100 kg.

Unlike traditional technology, according to which the aged bovine meat is minced in wolf machine and together with salting material is left to mature 2–3 days at 2–3°C, in the modelling product that was done for six hours in the process of drying under the influence of starter culture. First category of beef and veal meat were cutting-on the established technology for pratt for durable boiled – smoked sausages.

Then salting materials were added as well as pork lean meat (first and second category) cut in the particle size of 4 mm. Finally, the liquid starter culture was added in an amount of 5% with concentration of about 10 log (cfu/ml). The control sample without yeast was prepared. The produced sausage mass had to be filled tightly with hydraulic machine. The sausages were drained at 20°C for 6 hours and subjected to standard heat treatment including glowing, cooking, smoking. Drying was carried out at 20–25°C and a relative humidity (RV) of 75–80% and a decrease of water content determined according to the requirements of the standards. The number of lactobacilli and bifidobacteria in the filling was determined by plating respectively in MRS agar (Biokar Diagnostics, 089) and Bifidobacteria Selective Count Agar Base (BSC Propionate Agar Base).

The pH was measured using an automatic pH meter (model 2002 Microsyst, Crison), content of lactic acid was measured according to *AOAC* (1995). To establish the ability of test starter culture *L. plantarum* (L_6) to form the volatile fatty acids, a modified method *Halvarson* (1972) was used. The determination of the amount of free volatile fatty acids (VFA) was based on extraction from the test and control samples by steam distillation. The aqueous distillate was dried on a rotary evaporator. Subsequently, the methylation of the sample was determined

by the method of *Hartman and Lago* (1973), the amount of free volatile fatty acids by means of a gas chromatograph – Fractovap 2407 T, Karlo Erba, Italy.

Statistical data processing was performed by software STATPLUS 2009, including the two-way analysis of variance. To compare the average of the samples for multiple comparisons Duncan test was used for the likelihood of a statistically significant difference $p < 0.05$.

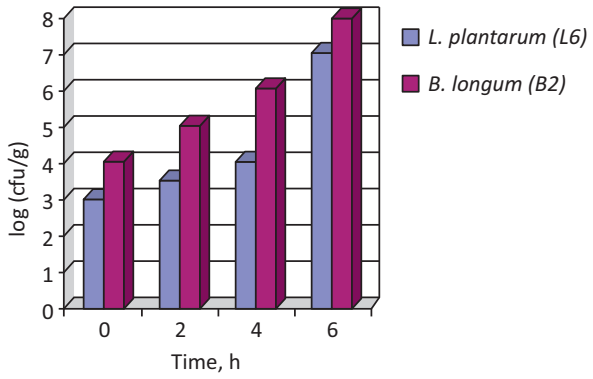
Results and Discussion

Microbiological analysis and starter culture growth

In the production of sausages biochemical and microbiological processes play important roles. Changes during these processes depend on the presence and growth of the beneficial bacteria. At present time sufficient material on use of lactic acid sticks of *L. plantarum* in the production of sausages and their positive role on the biochemical processes has been accumulated. Lactic acid bacteria initiate rapid acidification of the raw material. Recently, new starter cultures of lactic acid bacteria with an industrially important functionality are being developed. The latter can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages *(Leroy et al.,* 2006).

There is a lack of basic information about the use of bifidobacteria in sausage production. The study focusing on the use bifidobacteria in sausage production has a certain interest. Therefore, in the series of experiments the dynamics of a mixed starter cultures during the sausage production were studied (drying phase). These changes are presented in Figure 1.

Analysis of the results showed increase in the number of lactobacilli and bifidobacteria. After 6 hours the amount of cells of *B. longum* (B_2) was a 8 log (cfu/g), and on *L. plantarum* (L_6) – 7 log (cfu/g) [colony-forming units]. In the liquid starter culture microorganisms were in the phase of intensive growth, which contributed to rapid multiplication of cells in the sausage table at the beginning of draining. This resulted in a considerable shortening of the process. The results give a reason



Time/ Vreme, h	*L. plantarum* (L_6) log (cfu/g) ẋ= mean/sred. vrednost, Sd = standard deviation/standardna devijacija	*B. longum* (B_2) log (cfu/g) mean/sred.vrednost, Sd = standard deviation/standardna devijacija
0	3±0.27	4±0.45
2	3.5±0.68	5±0.12
4	4±0.17	6±0.96
6	7±0.85	8±0.74

Figure 1: Dynamics of growth of different starter culture microorganisms in the filling mass during the drying phase, n = 6

Figura 1: Dinamika rasta mikroorganizma iz različitih starter kultura u punjenu masu pri sušenju, n = 6

to assume that the sausage mass is a favourable environment for the development of microorganisms from the combined starter culture. Probably anaerobic conditions after filling, favour the intensive growth of *B. longum* (B_2).

Effect of starter culture on the change of pH value in the process of drying

The ripening process after filling has a major impact on the quality of the final product. A number of different microbial, chemical (tissue enzymes) and physical processes trigger a change in the pH value, dehydrate the sausage meat and produce the red color, aroma and texture of the sausage. The influence of lactic acid bacteria on the disintegration of glycogen and carbohydrate fermentation with lactic acid is typical process in the maturation of the filling mass. Objective indicator of the flow of lactic fermentation in the sausage mass is changing of pH value. For increasing the enzyme activity of the microorganisms, draining and drying are conducted at a temperature of 20°C *(Vuyst et al.,* 2008; *Toldra,* 2006; *Erkkilä et al.,* 2001).

The amount of lactic acid mainly depends on pH value and conditions for subsequent microbiological and biochemical processes. The obtained experimental results are presented in Figure 2.

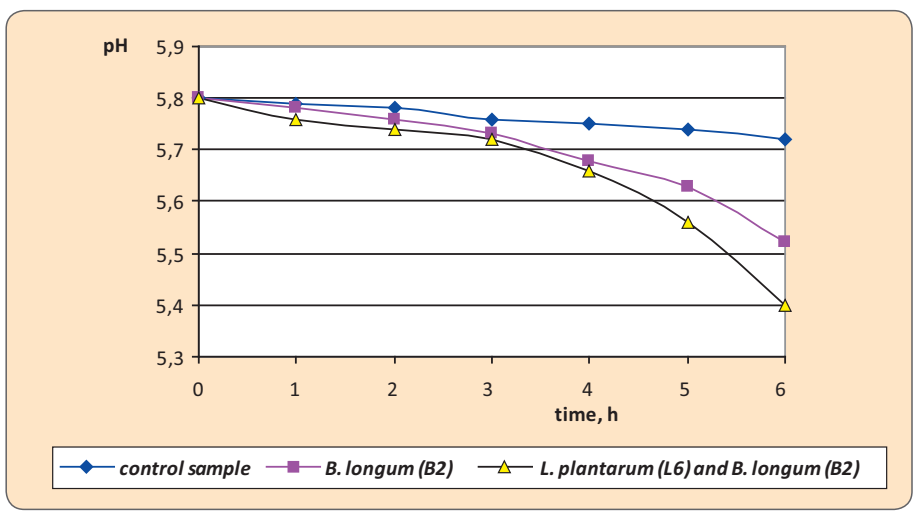


Figure 2. Influence of pH value and time on different starter cultures of the filling mass during the drying process

Figura 2. Uticaj pH vrednosti i vremena na različitih starter kultura na napunjenu masu pri sušenju

Legend/Legenda: Control sample/Kontrolni uzorak; B. Longum; L. Plantarum (L_6) and B. Longum (B_2)/B. Longum; L. Plantarum (L_6) i B. Longum (B_2)

From the results it can be concluded that the minimum pH value coincideed with the period of intensive growth of microorganisms in the starter culture. The results show that there were significant differences ($p < 0.05$) in pH values between the test samples and the control sample, which was produced without starter culture. Furthermore, pH values also depended on the composition of the starter culture. In the test sample with a combined starter culture lowering of pH to the optimum value of 5.4 was achieved within 6 hours. About at the same time pH value in the sample *B. longum* (B_2) was 5.52 while in control it was 5.72.

The pH of the control sample reached the value of 5.4 only just after 24 hours. In the process of drying the dynamics of change of pH has a critical significance. The change of pH value is characterized by two features: the rate of decrease of the pH value during the period of drying and the minimum pH value. If the pH value is not low enough, then the lactic acid fermentation is embarassed. This favors the development of pathogenic microflora.

The drastic reduction of pH below 5.3 is evidence of too intense process of lactic acid fermentation, which can cause a sour taste of the filling mass. Since they have low marginal acidity, bifidobacteria appear to be pH regulator in filling mass during drying process and prevent the emergence of an acid taste.

Effect of starter culture on the content of lactic acid and volatile fatty acids in the filling mass during drying process

One of the requirements for the starter cultures for the sausage is to produce substances which improve the organoleptic properties of the product. The accumulation in the medium and the production of non-volatile organic acids, in particular lactic acid, volatile fatty acids, amino acids, is associated with the formation of a specific odour and taste of sausages *(Pidcock et al.,* 2002; *Moyanos et al.,* 2008; *Ammor and Mayo,* 2007**).** In this regard was investigated changing the amount of lactic acid in the drying process. The results are presented in Figure 3.

The results obtained show a more intense formation of lactic acid in the test sample ($p < 0.05$). After 6 hours drying of lactic acid using a combined starter culture was 1250 mg/100 g *B. longum* (B_2) – 1100 mg/100 g, and in the control – only 710 mg/100 g. The reason for this probably is the influence of the starter culture on the rate of decomposition of glycogen in meat to lactic acid. Homofermentative lactic acid bacteria *L. plantarum* (L_6) ferment carbohydrates to about 95% lactic acid. One of the main products of the metabolism of bifidobacteria after fermentation of carbohydrates is also lactic acid. Formed lactic acid favourably affects the texture and the glue of the filling mass *(Vuyst et al., 2008; Toldra, 2006; Leroy et al., 2006).*

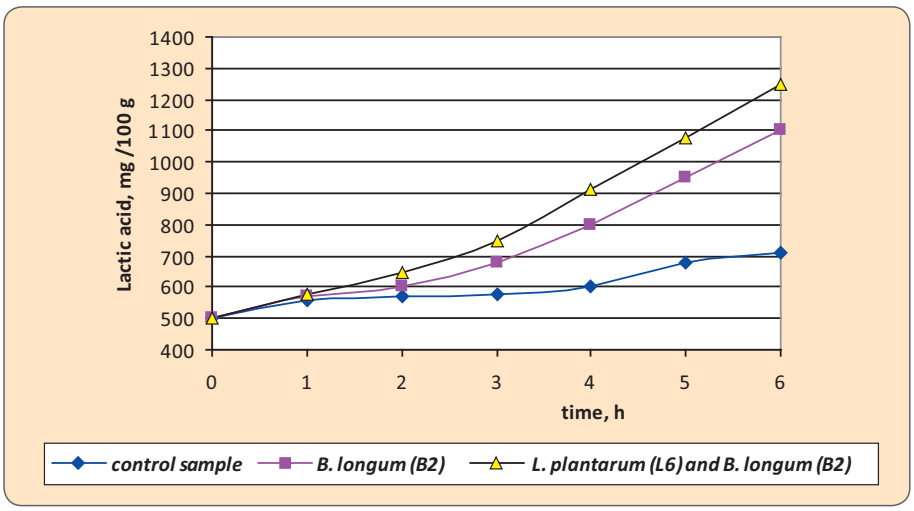


Figure 3. Effect of different starter cultures on the formation of lactic acid associated with time in the filling mass during the drying process

Figura 3. Efekat različitih starter kultura na formiranju mlećne kiseline povezano sa vremenom u punjenu masu pri sušenju

Legend/Legenda: Control sample/Kontrolni uzorak; B. Longum; L. Plantarum (L_6) and B. Longum (B_2)/B. Longum; L. Plantarum (L_6) i B. Longum (B_2)

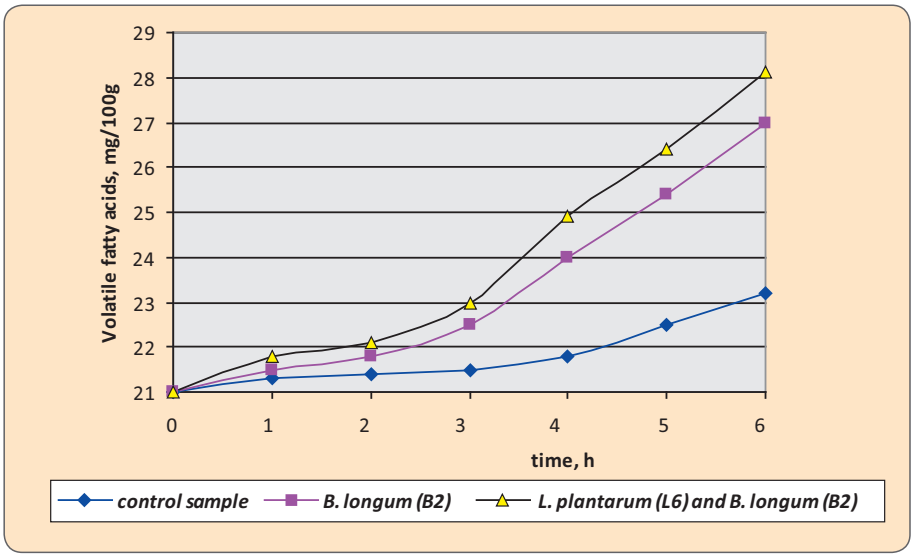


Figure 4. Effect of different starter cultures on the formation of volatile fatty acids associated with time in the filling mass during the drying process

Figura 4. Efekat različitih starter kultura na formiranju isparljivih masnih kiselina povezano sa vremenom u punjenu masu pri sušenju

Legend/Legenda: Control sample/Kontrolni uzorak; B. Longum; L. Plantarum (L_6) and B. Longum (B_2)/B. Longum; L. Plantarum (L_6) i B. Longum (B_2)

In the following series of tests, the characteristics of the dynamics of accumulation of volatile fatty acids during drying were examined. The results of this study are presented in Figure 4 and Table 1.

During the drying process, the values in all samples showed an increase in the volatile fatty acids content, while it was more intensive in the test samples ($p < 0.05$). This is probably due to the influence of the starter culture on the biochemical and physical-chemical processes associated with deamination of the amino acids, the oxidation of carbohydrates and carbonyl compounds. Moreover, the microorganisms involved in the starter culture are capable of producing volatile fatty acids. The quantitative results from the content of the individual volatile fatty acids are presented in the Table 1.

The values are averages of three measurements for a given indicator for the sample.

The constant accumulation of different volatile fatty acids in all the samples during the drying

Table 1: Content of volatile fatty acids in boiled-smoked sausages depending on the time and different starter cultures during the drying process, n = 3

Tabela 1: Sadržaj isparljivih masnih kiselina u kuvano-dimljenih kobasica u zavisnosti od vremena i različitih starter kultura u procesu sušenja, n = 3

Volatile fatty acids/ Isparljive masne kiseline mg/100 g	Duration of drying/Trajanje sušenja, h								
	L. plantarum (L_6) *B. longum* (B_2)			*B. longum* (B_2)			Control sample/ Kontrolni uzorak		
	2	4	6	2	4	6	2	4	6
Formic/Mravlja	4.42	9.20	13.90	3.62	7.80	12.60	2.20	6.60	9.80
Acetic/Sirćetna	18.04	23.80	32.58	16.02	21.55	28.81	15.7	20.00	24.45
Propionic/Propionska	2.72	5.30	6.02	2.60	4.43	5.16	2.45	4.32	4.73
Butyric/Buterna	0.42	0.814	1.41	0.46	0.739	1.162	0.453	0.715	1.17
Isovaleric/3-izovalerinska	0.081	0.102	5.20	0.066	0.892	4.94	0.056	0.825	3.582
Valeric/Valerinska	0.072	0.917	1.23	0.060	0.855	1.07	0.058	0.826	0.78
Capronic/Kapronska	0.074	0.84	2.62	0.075	0.713	2.41	0.072	0.968	1.17

process can be observed. But in experience, this process is accelerated and is determined by the starter culture and their production of volatile fatty acids.

The results show that the added combined starter culture in the filling mass was a essential factor for the formation of lactic acid and volatile fatty acids in the filling of the test samples ($p < 0.05$).

Conclusion

The experimental results justify the statement that the filling mass is a favorable environment for the development of starter cultures, which is a prerequisite for accelerating the biochemical processes in drying sausages and reduce production time. The content of lactic acid and volatile fatty acids increased significantly in the test pieces produced by the starter culture, especially in combination.This contributes helps to improves the sweet-taste one of the main sensory characteristics of the product. It should be noted that under the influence of imported starter culture a decrease of the pH during the initial process of drying was recorded. As it is known, this fact plays a crucial role in the specific formation and the colour of the meat products.

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Uticaj starter culture na pH i sadržaj mlečne i isparljivih kiselina u kuvano-dimljenim kobasicama

Indzhelieva Dijana, Valkova-Jorgova Katja, Kuzelov Aco, Andronikov Darko

R e z i m e: U ovom radu je istraživan uticaj starter kultura mlečnokiselinskih bakterija kao faktor povećanja kvaliteta i intenziviranje procesa proizvodnje u trajnim kuvano – dimljenim kobasicama, tipa Burgas. S tim u vezi je ispitan razvoj starter kultura u nadevu, njihovoj ulozi u promeni vrednosti pH, sadržaja mlečne kiseline i isparljivih masnih kiselina. U ovoj studiji smo koristili dve vrste starter kultura: a. monokultura Bifidobacterium longum (B_2) i b. kombinovanih kultura Bifidobacterium longum (B_2) i Lactobacillus plantarum (L_6),– u odnosu 2 : 1. Za ispitivanje njihovog uticaja na stimulaciju tehnološkog procesa proizveli smo 10 kg modelni proizvod na bareno-dimljenu trajnu kobasicu. U procesu ceđenja i sušenja zabeležio se porast broja laktobacila i bifidobakterija. Rezultati ispitivanja pH vrednosti pokazuju da minimalna vrednost pH je u periodu intenzivnog razvitka mikroorganizama iz starter kultura. Vrednosti pH zavise i od sastava starter kultura. Vrednosti pH brže opadaju u eksperimentalnim uzorcima sa mešanim starter kulturama. Ispitivanja u odnosu promena količine mlečne kiseline su pokazala intenzivnije formiranje mlečne kiseline u eksperimentalnim uzorcima posebno sa mešanim starter kulturama. U procesu sušenja u svim eksperimentalnim uzorcima se primećuje povećanje sadržaja isparljivih masnih kiselina, s tim što u opitnim uzorcima to povečanje je intenzivnije.

Ključne reči: *mesne prerađevine, starter kultura, pH, isparljive masne kiseline.*

Paper received: 24.03.2015.
Paper corrected: 23.07.2015.
Paper accepted: 24.07.2015.