

**GOCE DELCEV UNIVERSITY - STIP
FACULTY OF AGRICULTURE**



JOURNAL OF AGRICULTURE AND PLANT SCIENCES

YEAR 2017

VOLUME 15, Number1/2

**GOCE DELCEV UNIVERSITY - STIP, REPUBLIC OF MACEDONIA
FACULTY OF AGRICULTURE**

UDC 63(058)
ISSN 2545-4447 print
ISSN 2545-4455 on line



**Journal of Agriculture and Plant Sciences, JAPS, Vol 15
Successor of the Yearbook of Faculty of Agriculture of GDU, Vol 14**

YEAR 2017

VOLUME XV, Number 1/2

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THE INFLUENCE OF STARTER CULTURE OF LACTIC- ACID BACTERIA AND BIFIDO BACTERIA OVER THE SANITARY- HYGENIC, SENSOR AND PHYSICAL – CHEMICAL INDICATORS ON THE RE – BOILED – SMOKED DURABLE SAUSAGE

Dijana Indzhelieva¹, Katja Velkova-Jorgova², Darko Andronikov³, Aco Kuzelov⁴

¹University "Prof. dr. A. Zlatarov", College of Tourism, Prof. Yakimov, Burgas, Republic of Bulgaria,

²Faculty of Food Technology, University of Food Technologies -
Bulevar Marica 23, 3200 Plovdiv, R of Bulgaria,

³Faculty of Technology, Goce Delcev University, 2000 Stip, Republic of Macedonia,

⁴Faculty of Agriculture, Goce Delcev University, 2000 Stip, Republic of Macedonia,

dindjelieva@abv.bg; katiavjorgova@gmail.com; darko.andronikov@ugd.edu.mk; aco.kuzelov@ugd.edu.mk

Abstract

The objective of this research was to study the influence of the lactic-acid bacteria and bifid bacteria over the growth of the sanitary indicator bacteria *E.coli* during the process of draining of the boiled-smoked durable sausage. The influence of these starter cultures was also examined over the physical, chemical and sensor indicator of the final product. Two types of starter cultures were used for the experiment: monoculture *B. longum* (B_2) and combined culture in relation *B. longum* (B_2) : *L. plantarum* (L_2) - 2 : 1. For the study of their influence over the technological process and the qualitative features of the product, 10 kg sample of boiled-smoked durable sausage "Burgas" was produced. The obtained results are the basis for conclusion that the starter cultures repress the development of the *E.coli* from the beginning of the production of the boiled-smoked sausage and increase the sanitary-hygienic indicators of the final product. They also contribute to the formation of the sensor characteristics of the sausage and speed the reaching of the standard values of the physical chemical indicators.

Key words: meat products, starter cultures, lactic acid bacteria, bifidobacteria

INTRODUCTION

In the modern meat industry, the use of starter cultures is increasingly important. The artificially created starter cultures have additional qualities than those of the more classical species, as it helps to optimize the technological processes in the production of meat products and to produce smoother, safer and healthier products (Dellaglio et al., 1996; Demeyer et al., 2004; Toldra 2006). One of the most commonly used microorganisms as starter cultures are lactic acid bacteria. Their basic function is to achieve a rapid reduction in the pH of the bulk mass, which favours: 1) the safety of the product by deactivating the pathogens; 2) the stability and shelf life of the product by inhibiting adverse changes caused by micro-organisms that spoil the product; 3) creates biochemical conditions for achieving new sensory qualities of meat products by modifying the raw materials (Ammor & Mayo 2007.; Arihara 2006; Buckenhüskes 1993). At the

moment, there are sufficient materials for the use of *Lactobacillus plantarum* in the production of sausages and their positive role on the leaks of biochemical processes (Erkkilä et al., 2001; Leroy et al., 2006; atarata et al., 2008; Pennacchia et al.,2004). Relatively little data exist about the use of bifidobacteria in the meat industry Indzhelieva (2015). Therefore, the examination of the effect of using bifidobacteria in the production of sausages is of a particular interest. The literature data and our previous research (Indzhelieva et al.,2014; Indzhelieva et al., 2015; Pidcock et al., 2002; Velkova – Jorgova et al.,2014; Vayst et al., 2008), had found that bifidobacteria develops successfully, both independently and in combination with lactic acid bacteria, in the bulk mass of sausages; and produce lactic and volatile fatty acids. Their metabolic products have high reducing properties that help to form and stabilize the colour of the sausage with a relatively low concentration of sodium nitrite.

These properties of bifidobacteria reveal a serious prospect of their use as starter cultures in the meat industry.

Beside the useful, the raw meat also contains various types of harmful organisms such as *E.coli*, *B. proteus*, *Salmonella*, *Cl. botulinum* and others. Literature data testify to the important role of starter cultures in regulating the breeding of pathogenic microorganisms in sausage (Danielsen et al., 2003; Rivera-Espinoza & Gallardo-Navarro 2010; Ruiz et al., 2008). The antimicrobial activity of some strains of lactic acid bacteria can be used as an alternative to chemical preservatives in the production of meat products, preventing

microbial contamination and at the same time intriguing the consumer for a new attractive product (Schillinger et al., 1996).

In the bulk mass of sausages, the development of starter cultures and pathogenic microorganisms flows simultaneously with direct contact of bacterial cells.

The objective of this study is to examine the influence of the lactic acid bacteria and the bifid bacteria over the growth of the sanitary indicator bacteria of *E.coli* in the process of draining the boiled-smoked durable sausage. Also, the effect of these starter cultures over the physical, chemical and sensor characteristic of the final product is studied.

MATERIAL AND METHODS

In the experimental work, the clean cultures *Lactobacillus plantarum* (*L.*) and *Bifidobacterium longum* (*B.*) are used. The seeds are obtained from the private licensed laboratory for analyses and food control - Burgas. Based upon the previous studies it was determined that both seeds fulfil the necessary criteria for their usage both as independent cultures and combined.

For this research two types of starter cultures were used: monoculture *B. longum* (*B.*) and combined culture in relation *B. longum* (*B.*): *L. plantarum* (*L.*) - 2 : 1. The activation of the dry bacteria concoction is made in oil-freed and sterilized milk on 121°C for 13 minutes, which is then cooled to the temperature of 37°C. The starter is obtained in quantity 0,1g on 1l. The soured milk remains in the thermostat on 37°C in order to reach the level of acidity of 60-65°C as well as thickening. The concoction on cooled to 5°C.

In order to study their impact on the flow of the technological process and the quality of the product, a 10 kg sample was produced - a product of cooked-smoked permanent sausage "Burgas". The raw materials for 100 kg are: beef first category 20, veal meat first category 20, pork loose meat 10, pork half-fat meat 0.050, cooking salt 2.000, sodium nitrite 0.005, ascorbic acid 0.050, sodium tripolyphosphate 0.100. In contrast to the traditional technology, according to which the meat of large live animals is grinding on the wolf machine and together with the salting materials, it is left to

mature for 2 - 3 days at 2 – 3°C, in the sample product it is achieved for 6 hours in the process of drying under the influence of the starter culture. Beef and veal are prepared according to the technology for obtaining prawns for durable cooked-smoked sausages. The salting additives, pork loose and semi-fat meat are added until they are decanted with a particle size of 4 mm. At the end, the activated liquid starter culture is added in a quantity of 5% of the weight and with a concentration of 10 logs (cfu/ml). Also a control test without a starter culture is prepared. The finished bulk mass is filled on a hydraulic machine firmly, so as not to allow inside the charge table to have air voids. After that the sausages are crushed and dried at 22°C for 6 hours. The number of lactobacilli and bifidobacteria in the filling was determined by plating respectively in MRS agar (Biocar diagnostics 089) and Bifidobacteria Selective Count agar base (BSC Propionate Agar Base the filled and dried sausages and are subjected to standard heat treatment, which includes heating, cooking and smoking. Drying is carried out at 15 – 25°C and relative humidity of 75-80% up to reduce of water content according to the requirements of the standards.

A sensory analysis of 7 members, pre-selected and trained (Patarate et al., 2008) was performed to determine the sensory properties of the product. The assessors evaluated the features of the outer appearance, colour of the cross-section, aroma, taste, consistency and

general grade in each test by a hedonic scale from 1 (low intensity) to 9 (intense). The sessions were carried out at a sensory assessment laboratory equipped with individual assessment cabins for marks and equal lighting conditions at a temperature of 20 °C. Each tester received 3 sausages (\pm 15 grams). Each sample was marked with a random three-digit number. Drinking water and unsalted bread were also provided

for cleansing the mouth between tastings.

The determination of the lactic acid bacteria was made according to ISO 13721 [12]. The determination of *E. coli* was made according to ISO 6391:1997 [13]. The determination of bifido bacteria was made according to ISO 29981:2010(en) [14]. Data were transformed into log₁₀ cfu/g before comparison of means.

RESULTS AND DISCUSSION

The results from the study of the influence of the starter cultures over the growth of *E. coli*

in the process of draining the sample of boiled-smoked sausage are given in Table 1.

Table 1. The change in the quantity of *E. coli* in the bulk mass of the boiled-smoked sausage in the process of draining.

Time for draining, h	Content of bacteria in the bulk mass, log cfu/g						
	Контрола		<i>B. longum</i> (B_2)		<i>B. longum</i> (B_2): <i>L. plantarum</i> (L_6) 2:1		
	MKB*	<i>E. coli</i> .	BFB*	<i>E. coli</i>	MKB*	BFB*	<i>E. coli</i>
0	3.01	5.47	4.90	5.22	5.22	4.49	5.20
2	3.52	5.03	6.72	5.01	6.25	6.26	4.46
4	3.91	4.86	7.50	3.72	7.10	7.32	3.34
6	4.68	4.02	8.46	2.31	7.62	8.80	1.05

MKB* - number of lactic acid bacteria in the bulk mass; BFB* - number of bifid bacteria in the bulk mass

From the obtained results, it can be seen that during the whole process of extraction during the experiment tests there is an intensive development of *L. plantarum* (L_6) and *V. longum* (B_2). The development of the beneficial microflora is also accompanied by the destruction of *E. coli*. At the end of the extraction, the amount of *E. coli* in tests with a combined starter culture is barely 1.05 log cfu/g, with *C. longum* (B_2) - 2.31 log cfu/g, and in the control sample - 4.02 log cfu/g. The reason of the antagonistic effect of the naturally occurring cultures is probably due to the accumulation of compounds with antibiotic action, the change in the physical chemistry conditions in the bulk mass of the sausage (pH, oxide-reductive potential), and also the competition in relation to the energy substrate. In this way, the development of the microflora from starter cultures prevents the growth of *E. coli* in the very early stages of the production of boiled-smoked sausage and increases the sanitary-hygienic indicators of the finished product. *Salmonella spp.* and *L. Monocytogenes* were not found in the boiled-

smoked sausages from the experiment tests in 25 g of the final products.

As a result of our previous research, it is shown that the weight of the sausage is a favourable environment for the development of the combined starter culture, with an optimum starter culture of 5%. It has been established that starter cultures with bifidobacteria have a significant influence on the speed and dynamics of the pH change, the accumulation of volatile fatty acids and free amino acids in the bulk mass of the sausage during the period of extraction (Indzelieva et al., 2015; Indzelieva 2015). The results from the table. 1 show that the development of microorganisms from starter cultures is accompanied by the destruction of *E. coli* bacteria, and this effect is more emphasized with the use of the combined starter culture. All these beneficial effects from the use of starter cultures with bifido bacteria also affect the organoleptic characteristics of the final product. The results of the organoleptic evaluation are shown in Table 2.

Table 2. Sensor mark of the boiled-smoked sausage produced with starter cultures, n=6 ($\bar{x} \pm SD$).

Indicator	<i>B. longum</i> (B_2): <i>L. plantarum</i> (L_2) 2:1	<i>L. plantarum</i> (L_2)	Control (without starter culture)
Outside appearance	8.32 \pm 0.04	8.29 \pm 0.05	7.21 \pm 0.04
Color of cross-section	8.54 \pm 0.05	8.31 \pm 0.05	7.20 \pm 0.03
Aroma	8.46 \pm 0.05	8.43 \pm 0.04	6.42 \pm 0.05
Taste	8.11 \pm 0.04	8.15 \pm 0.05	6.55 \pm 0.02
Consistency	8.19 \pm 0.08	7.82 \pm 0.07	6.40 \pm 0.04
General evaluation	8.25 \pm 0.04	8.10 \pm 0.03	6.75 \pm 0.03

\bar{x} – mean, Sd – standard deviation; n- number of pieces

The obtained results for changes in the sensory properties of the control and experimental tests of the tested product show that the used starter cultures have a major influence on the formation of those qualitative indicators that increase them. The displayed data show that, in terms of indicators, the colour of cross-section, aroma, taste, consistency and general evaluation, there is a statistically significant difference between the samples from the control and experimental tests.

The surface of all the studied sausages is dry, clean and smoked. The type of cut area is determined immediately after the cut-off. The data in the Table.2, show that in regard to the sensory feature of the intersection colour "in the experimental sausages, statistically different higher grades were determined (dully 8.54 \pm 0.05 и 8.31 \pm 0.05) compared to the control test (7.20 \pm 0.03). In this connection, it has to be pointed out that in our conducted studies the pH values in the samples with incorporated yeast showed decrease (Indjelieva et al., 2015). Furthermore, pH values also depend 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 was 5.72 (Indjelieva et al., 2015). According to a number of authors, this circumstance plays an extremely important role in the specific formation and stabilization of the colour of meat products (Leroy et al., 2006; Pidcock et al., 2008, Ruiz Moyanos et al., 2008).

The observed changes in the flavour values of the studied sausages show significantly higher grades in experimental researches produced with starter cultures. The results of the sensory analysis do not contradict the ones which are obtained by determining the aromatic

compounds with auxiliary gas chromatography, (Indjelieva et al., 2015). These results confirm the fact that the addition of starter cultures creates the necessary conditions for the proper activity of the flavouring processes as a result of which the specific taste of the finished products is formed.

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 product 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 (Indjelieva et al., 2015).

It was found that the examined sausages produced by starter cultures obtained a higher mark for taste (Table 2). In addition, the taste of the experimental tests is evaluated with significantly higher values compared to those of the control test. The more commonly used bacterial strains have the ability to manage proteolytic processes during digestion and ripening and contribute to the pleasant and characteristic taste of sausage.

A similar trend is also observed in the sensory trait of consistency, where significant differences have been identified, as there are significant differences in the grades between the control and experimental trials. The higher levels of the organoleptic evaluation of the consistency of the experimental samples are associated with the occurrence of destructive processes in the pulp mass and mainly partial hydrolysis of the meat whites under the influence of the introduced starter cultures.

The complex sensor mark shows similar tendency that the tests produced with a starter culture obtained significantly higher marks compared to the control test.

The analyses of the obtained results is a basis to conclude that the applied starter cultures provide the necessary conditions of the proper activity of the bio-chemical processes, and as a result a sensory characteristic of the sausage is formed which promotes the final

product with a high sensor mark.

The experiment results from the research of the changes of some chemical indicators included in the normative documents as a dry material of Sodium Chloride and nitrites content are shown in Table 3.

Table 3. Chemical indicators of the finished product cooked-smoked and dried sausage "Burgas", n = 6 ($\bar{x} \pm Sd$).

Indicator	<i>B. longum</i> (<i>B.</i> ₂): <i>L. plantarum</i> (<i>L.</i> ₂) 2:1	<i>L. plantarum</i> (<i>L.</i> ₂)	Control (without starter culture)
pH	5.73±0.02	5.79±0.03	5.90±0.01
Dry matter, % of general mass	65.98±0.05	65.12±0.04	62.75±0.03
Proteins, % of dry matter	26.94±0.05	26.28±0.05	25.26±0.03
Fats, % of dry matter	61.83±0.04	62.78±0.06	64.82±0.05
Sodium chloride, % of general mass	2.80±0.01	2.86±0.03	2.95±0.04
Nitrite, mg/100g product	3.12±0.01	3.21±0.01	4.08±0.01

\bar{x} – mean, *Sd* – standard deviation; n- number of pieces

From the results it can be seen that the used starter cultures do not have an impact on the indicators of the proteins and fat in the final product from both the control and the experiment tests.

The results show that the contents of nitrites in the sausages produced with starter cultures is significantly lower compared to

the control test 3.12±0.01 and 3.21±0.01 and 4.08±0.01 (Tab. 3). Considering the results from the previous studies (Indzelieva et al., 2015) it can be concluded that the starter cultures which contain bifid bacteria speed the achievement of the standard values of the physical and chemical indicators.

CONCLUDING REMARKS

1. The development of the micro-flora of the starter cultures prevents the growth of *E.coli* at the very early stage of the production of the boiled sausage and increases the sanitary and hygienic characteristics of the final product.
2. The obtained results of the sensor analyses and the control and examination tests of the

- researched product show that the applied starter cultures have a significant influence over the formation of the qualitative indicators and they even increase them.
3. The starter cultures which contain bifid bacteria speed the achievement of the standard values of the physical and chemical indicators.

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ВЛИЈАНИЕ НА СТАРТЕР КУЛТУРА ОД МЛЕЧНО-КИСЕЛИ БАКТЕРИИ И БИФИДОБАКТЕРИИ ВРЗ САНИТАРНО-ХИГИЕНСКИТЕ, СЕНЗОРНИТЕ И ФИЗИЧКО- ХЕМИСКИТЕ ПОКАЗАТЕЛИ НА ВАРЕНОДИМЕНИ ТРАЈНИ КОЛБАСИ

Дијана Индјелиева¹, Катја Велкова Јоргова², Дарко Андроников³, Ацо Кузелов⁴

¹Универзитет Проф. др. Асен Златанов, Колеџ по Туризам Проф. Јакимов Бургас Република Бугарија,

²Технолошки Факултет, Универзитет за прехранбени технологии Булевар Марица 23 3200 Пловдив
Бугарија,

³Техничко технолошки факултет, Универзитет „Гоце Делчев“, 2000 Штип, Република Македонија,

⁴Земјоделски факултет, Универзитет „Гоце Делчев“, 2000 Штип, Република Македонија,

dindjelieva@abv.bg; katiavjorgova@gmail.com; darko.andronikov@ugd.edu.mk; aco.kuzelov@ugd.edu.mk

Резиме

Целта на ова испитување беше да се проучи влијанието на млечнокиселите бактерии и бифидобактериите врз растот на санитарно-индикаторните бактерии од *E. coli* во процесот на цедење на варенодимен траен колбас. Беше испитувано и влијанието на овие стартер култури врз физичко-хемиските и сензорни показатели на готовиот производ. Во експериментот беа користени два типа стартер култури: монокултура *B. longum* (B_2) и комбинирана култура во сооднос *B. longum* (B_2) : *L. plantarum* (L_2) - 2 : 1. За испитување на нивното влијание врз протекувањето на технолошкиот процес и квалитативните карактеристики на производот беа произведени 10 kg модел на варенодимен траен колбас бургас. Добиените резултати ни даваат основа да констатираме дека стартер културите го потиснуваат развитокот на *E. coli* уште во почетокот од производството на варенодимен колбас и ги зголемуваат санитарно-хигиенските показатели на готовиот производ. Исто така, придонесуваат за формирањето и подобрувањето на сензорните карактеристики на колбасот и го забрзуваат достигнувањето на стандардните вредности на физичко-хемиските показатели.

Клучни зборови: месни производи, стартер култури, млечно-кисели бактерии, бифидобактерии