EFFECTS OF PROPIONIC-ACID BACTERIA AND BIFIDOBACTERIA ON THE QUALITY OF RAW SMOKED SAUSAGES

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Abstract: Propionic-acid bacteria and bifidobacteria are widely used in food industry. Therefore, it is important to determine their biochemical activity and their aroma producing potential. In this study, the effect of the use of propionibacteria and bifidobacteria as starter cultures on the sensory characteristics of raw smoked sausages has been studied. The sausages were prepared with starter culture consisted of *Propionibacterium shermanii KM-186* and *Bifidobacterium longum B379M* and compared with the sausages with a commercial starter culture Bitec LS-25. The results have shown, that the sausages containing starter culture of propionic-acid and bifidobacteria, obtained better sensory scores, better quality characteristics compared to the sausages made with commercial starter culture. The analysis of volatile compounds has shown, that the addition of starter culture, containing propionic-acid bacteria and bifidobacteria leads to formation of additional compounds, including lactones, phenols, and terpenes. During sensory evaluation, experts noticed the presence of mild creamy note in experimental sample. It can be the result of lactones formation by propionic-acid bacteria and bifidobacteria. In addition, it was shown that the addition of propionic-acid bacteria and bifidobacteria reduces nitrite amount in sausages in several times (11 times approximately compared to commercial starter culture) due to nitroreductase activity of these bacterial strains.

Keywords: Raw smoked sausages, propionic-acid bacteria, bifidobacteria, starter cultures, volatile compounds, flavor, sausages

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INTRODUCTION

The characteristic taste and aroma of fermented sausages originates from the breakdown of carbohydrates, lipids, and proteins through the action of microbial and endogenous meat enzymes [1]. The aroma is a combination of many different non-volatile and volatile compounds. Some originate from added spices, others are metabolic or chemical products derived from carbohydrates, lipids and proteins during the fermentation and drying periods. Microbial growth in the sausage mince, together with the activity of enzymes from the meat and fat, are undoubtedly responsible for many of those components. However, autoxidative reactions initiated by metallo-compounds or other factors may be of great importance as well [2, 3].

Many volatile compounds have been identified in fermented products [4]. The volatile compounds produced during maturation of dry meat products may be of non-enzymatic origin, as during self-oxidation of lipids, or may result from a catalysis. This catalysis is due to endogenous enzymes in the case of non-inoculated products such as dry-cured ham, but depends also on exogenous enzymes in the case of fermented meat products [2].

It is unknown, which processes play the major role in the desirable aroma development of fermented sausages. One of the most studied of processes is the breakdown of triglycerides into free fatty acids, di- and monoglycerides during ripening and the increase of different carbonyl oxidation products like aldehydes and ketones [1].

Lipid oxidation is a reason of formation of the generation of nonbranched aliphatic compounds such as alkanes, alkenes, methyl ketones, aldehydes, alcohols and several furanic cycles. Compounds of low molecular weight form during the fermentation, for example, diacetyl, acetoin, butanediol, acetaldehyde, ethanol, acetic acid etc. [1].

Carbonyl compounds probably have an impact on flavor as they generally have very low sensory threshold values in the ppm to ppb range. Since fatty acids are precursors of carbonyls, lipolytic activity of the microorganisms present in the mince is believed to be of great importance and has been much investigated. However, it is doubtful whether large amounts of free fatty acids are necessary in obtaining the amount of oxidation products demanded for the characteristic sausage flavor, especially when carbonyls may arise from the intact triglycerides as well as from the free fatty acids.

Starter cultures are used in the meat industry to start and propagate the fermentation process, extend the shelf life of the product, improve its hygienic quality

and increase the acceptability of the final product [5]. There are many technologies of meat products, made with the use of starter cultures [6, 7, 8, 9].

Propionic-acid bacteria (PAB), the most abundant flora during ripening of Swiss-type cheeses, are directly involved in the characteristic eye formation and in the production of propionic and acetic acids. Also they play a key role in the formation of cheese flavor [10, 11, 12]. However, they are not widely used in meat industry.

Propionic-acid bacteria grow at low oxygen concentrations (anaerobic to aerotolerant) [13] and their growth is inhibited by high salt-in-moisture (S/M) levels, to an extent dependant on strain type [14, 15, 16]. PAB grow optimally between pH 6 and 7 with a pH maximum for growth at 8.5 and minimum at 4.6 [15, 17]. Cheese pH controls the rate of growth and metabolism of PAB [18] and a higher inocula is required for cheeses with a lower cheese pH [13]. The optimal growth temperature for PAB is 30°C, but growth also occurs between 7 and 45°C as reviewed by Langsrud et al. [15]. Park et al. [7] reported that 16 out of 33 strains of PAB tested grew at 7.2°C and 31 out of 33 grew at 12.8°C. No growth of P. freudenreichii was reported at 2.8°C.

Also dairy PAB have only a few nutritional requirements. Many PAB strains grow in the absence of organic nitrogen sources, in a basal medium containing a carbon and energy source, ammonium, minerals, and 2–4 vitamins (at least pantothenate and biotin are required) [11, 19, 20].

One of the important properties of PAB for use in meat industry is their proteolytic activity. A proteolytic system was found in all the species of dairy Propionibacterium [21]. Searles et al. [22] reported that propionic-acid bacteria, in a medium with acid casein, were able to increase the trichloroacetic-acid (TCA)-soluble nitrogen. Later, El Soda et al. [23] found a "general caseinolytic activity" in three strains of *Propionibacterium freudenreichii* and in one strain of *P. acidipropionici*. Intracellular peptidases have been studied in more details [15, 24, 25] found peptidases able to release proline into the medium. Perez Chaia et al. [26] showed that leucine aminopeptidase and proline iminopeptidase have the good activities in *P. freudenreichii.* This proline iminopeptidase was purified and characterized [27]. El Soda et al. [23] also found prolyl dipeptidyl aminopeptidase activity [21].

Propionic-acid bacteria produce some volatile compounds, which are important for cheese flavor. Thierry et al. [19, 20, 28, 29] studied the formation of these compounds by PAB in cheese. Compounds produced by PAB, which are responsible for flavor of cheese, are acetic, propionic and butanoic acids and 2-methyl and 3-methylbutanal. *P. freudenreichii* for example, produces flavor compounds of varied origins (fermentation, lipolysis, amino acid catabolism). The work of Thierry et al. [28] showed, that various biochemical changes were observed in Raclette cheeses with the addition of *Propionibacterium freudenreichii* compared to the control cheese: the fermentation of lactate to propionate and acetate, a marked enhancement of lipolysis, some modifications of the amino acid profile, the conversion of branched chain amino acid to a variety of aroma compounds, and the increase in other volatile compounds such as esters and ketones.

Bifidobacteria are mainly added to food products, because of their probiotic properties [30, 31, 32, 33]. Bifidobacteria, naturally present in the dominant colonic microbiota, represent up to 25% of the cultivable faecal bacteria in adults and 80% in infants. As probiotic agents, bifidobacteria have been studied for their efficacy in the prevention and treatment of a broad spectrum of animal and/or human gastrointestinal disorders, such as colonic transit disorders, intestinal infections, and colonic adenomas and cancer [32]. Mainly bifidobacteria are used in dairy industry as a component of fermented drinks, but also they are used as a starter culture (in combination with other bacteria) during the production of fermented sausages [31].

According to [34] the use of combined concentrate of propionic-acid bacteria and bifidobacteria has positive effect on sensory profile of dry smoked sausages. New compounds are formed as a result of bacterial action. The content of free fatty acids in the sample with these bacteria is by 20–30% higher than in traditionally produces sausages. It can be result of higher lipase activity of propionic acid bacteria and bifidobacteria. The main difference between the samples was in high amount of lactones in the experimental sample. A weak pleasant creamy tone in the aroma and flavor of the experimental sausage, obtained using propionic acid bacteria and bifidobacteria, probably, the result of presence of lactones in experimental sample.

Considering the above, the effect of propionic-acid bacteria on flavor development of fermented sausages is interesting issue to study. Thus, this work was conducted to investigate the potential benefit from the use of Propionibacteria and Bifidobacteria as starter cultures during the production of raw smoked sausages and their influence on the quality characteristics and chemical composition of sausages. The investigation of the influence of starter culture addition and flavor compounds as well as sensory evaluation, are very interesting objects to study. To determine this effect, sausages with starter culture of propionic-acid and bifidobacteria were compared with sausages with commercial starter culture.

OBJECTS AND METHODS OF STUDY

Manufacture and sampling. The sausages, used in this study, have been produced at the local factory (Republic of Buryatia, Russia). Ingredients of sausages are listed in Table 1. Mean values of results of three experiments were taken.

Raw smoked sausages were produced by using beef, lean pork, pork fat, NaCl, $NaNO_2$, sugar, brandy and spices. On the first step meat was minced, mixed with salt, spices and brandy. On the second step the 5 %-solution of sodium nitrite, brandy, lean pork, pork fat and spices were added. After mincing of meat and mixing, the mixture was divided into two batches: batch A was inoculated with $1cm^3$ of starter culture

containing propionic-acid bacteria and bifidobacteria (LLC Small Commercial Enterprise "Bifivit", Ulan-Ude, Russia) per 100 kg of raw meat, while the commercial starter culture Bitec LS-25 (Frutarom, Germany) was added to batch B was added with a concentration of 0.025%.

Table 1. Composition of raw smoked sausages with starter culture

Materials and ingredients	Percentage share
Basic ingredients	
Beef	35%
Lean pork	35%
Pork fat	30%
Additional ingredients and spices (in kg to 100 kg of basic ingredients)	
NaCl	3.5 kg
$NaNO_2$	0.01 kg (in 5%-solution)
Sugar	0.2 kg
Brandy	0.25 kg
Starter culture	1 cm^3
Black pepper	0.15 kg
Allspice	0.05 kg
Cardamom or Nutmeg	0.05 kg

Experimental starter culture contained strains *Propionibacterium shermanii KM-186* and *Bifidobacterium longum B379M*. Cell counts of each strain were 10 log CFU/cm^3. The starter culture was in the liquid form.

Commercial starter culture Bitec LS-25 contains strains *Staphylococcus carnosus* and *Lactobacillus sakei*. Total cell counts is 1.5 * 10 log CFU/g. The consistency of the starter culture was fine-grained, freeze-dried powder. The producer claims, that the starter culture creates a rapid acidification process and a typical fermentation aroma, accelerates color formation, improves color stability, fat stability and consistency. The strain of lactobacillus contained is highly suited to compete with the spontaneous flora.

The meat mixture was immediately stuffed in natural casings to obtain sausages with an initial weight of approx. 1400-1500 g, a length of approx. 60 cm.

The process of thermal treatment was carried out in termoagregate with automatic control for (3–4) days. On the first day sausages were kept at temperature 24°C, relative humidity 92.3% and air velocity (0.2–0.5) m/s. The temperature was then lowered to (22–20)°C and kept for one day. On the third day sausages were smoked for 4-6 hours, the relative humidity was reduced to 88.3%. Then, on the fourth day smoke intensity was increased, and process was carried out at (20 ± 2)°C, relative humidity (83.3%) and air velocity (0.05–0.1) m/s. The total duration of treatment with smoke was (8–12) hours.

The process of drying was carried out in the same termoagregate at (18 ± 2)°C and relative humidity 82.3% for 1 day. Further drying was carried out at temperature (13 ± 1)°C, relative humidity (77.3%) and air velocity (0.05–0.10) m/s for (17–20) days.

Microbiological analysis. Sausage samples (10 g) were homogenized with 90 ml distilled water. Decimal dilutions were prepared. Microbiological analyses were performed every day during 10 days of ripening.

The numbers of propionic acid bacteria were determined on GMK-1 medium (Biocompas-S, Russia), consisting of corn-milk mixture (30 g), peptone (30 g), lactose (18 g), ascorbic acid (1 g), sodium citrate (12 g), magnesium sulphate (0.24 g), potassium phosphate (monobasic) (4 g), sodium phosphate (dibasic) (2 g), agar (6 g), distilled water (2000 ml). Incubation was performed at 30°C for 5 days.

Bifidobacteria counts were determined using Blaurock medium (Blaurock, 1937) with neomycin (0.2 ml of solution of neomycin was added to 20 ml of medium). For getting of neomycin solution one bottle of neomycin (500000 ME) was dissolved in 50 ml of distilled water. Colonies were counted after incubation at 37°C after 3 days.

Chemical analysis. Analyses of the moisture content, pH, and $NaNO_2$ contents were performed in triplicate. The moisture content of the dry-fermented sausages was determined by dehydration at 103°C to constant weight by the ISO recommended methods (ISO, 1997). The pH was measured using a pH-150M pH meter (Gomel plant of measuring devices, Belarus).

Nitrite amounts were analyzed using spectrophotometric method on KFK-3 (Zagorsk optical and mechanical plant, Russia).

Sensory evaluation. The sensory evaluation was performed by nine trained experts. The experts were asked to evaluate sensory characteristics of the sausages. The sausage samples were sliced (the thickness was approximately 2 mm). Control and experimental batches were numbered and provided to each expert with degustation card. The sensory evaluation was conducted in individual rooms. Six sensory characteristics were evaluated by judges. General appearance, color, aroma, texture, taste, and juiciness were the attributes, evaluated during sensory analysis. The hedonic scale from 1 (extremely poor/ unacceptable) to 9 (extremely acceptable) was used in the study. The mean values of all experts' marks for each characteristic were calculated.

Gas chromatography. To extract volatile substances sausage samples (100 g) were minced, placed into 2-l flask. Content of volatile compounds was determined by gas chromatography (GC) using gas chromatograph Hewlett-Packard 5730 (Hewlett-Packard, USA) equipped with a flame ionization detector and capillary column SPB-1 (50 m * 0.32 mm, 0.25 μm) (Supelco, USA). Chromatographic conditions were as follows: isotherm at 60°C for 5 minutes, then the temperature programming to 250°C. Helium gas velocity through the column was 1.5 ml/ min. The identification of peaks in the sample chromatograms was performed using a system for the processing of chromatographic data Ecokhrom (Russia). The concentration of individual components expressed as μg/ 100 g of product.

Statistical analysis. The data obtained for pH, moisture content, nitrite amounts, volatile compounds,

sensory characteristics was assessed statistically to determine significant differences between products for these parameters. Analyses of each parameter were carried out in triplicate at various sampling times. Mean, standard deviation and standard error were calculated for all quantified variables. An analysis of variance (ANOVA) was carried out. Significance level $P < 0.05$ was used for all data. All the statistical analyses were performed using programs Statistica 6.0 and Microsoft Excel 2010.

RESULTS AND DISCUSSION

Microbiological analysis

The results for analysis of counts of propionic-acid bacteria and bifidobacteria are shown in Fig. 1. In both samples, we can see increasing amount of bacteria during the production process.

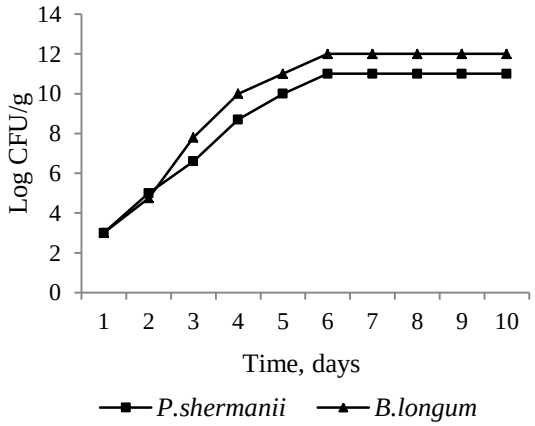

Fig. 1. Changes in growth of propionic-acid and bifidobacteria in raw-smoked sausages.

The initial quantity of probiotic microorganisms was approximately 5 log CFU/g. The quantity of viable cells of bifidobacteria and propionic-acid bacteria has gradually increased starting from second day of manufacturing. After 3 and 4 days the quantities of bifidobacteria were 9 log CFU/g and 10 log CFU/g, the quantities of propionic-acid bacteria were 10–11 log CFU/g. Sausages contained approximately 11–12 log CFU/g bifidobacteria and the same amount propionic-acid after 5 days of manufacturing. This amount of bacteria was stable during the smoking and drying.

The results of research have shown that PAB are resistant to high concentrations of NaCl and low temperatures during salting. The results of the study have shown that propionic acid bacteria and bifidobacteria can survive and grow in meat due to low nutrition requirements of bacteria and their ability to survive at low positive temperatures. This fact makes possible to use these bacteria for production of fermented sausages.

Results of chemical analysis

Results for analysis of pH are shown in Fig. 2. The initial pH of control sample was 6, the pH of sample with starter culture was 5.95, pH values of both samples drastically decreased after 5 days of fermentation. The decrease in sample with experimental starter culture was bigger, than in control sample. The pH values in both samples have slowly increased after (had increased) 10 days of fermentation due to proteolysis of proteins and formation of products of their decomposition. The pH value is close to isoelectric point after 20 days of fermentation.

The moisture content is one of the main characteristics of meat. For raw sausages it is very important. The development of structure of product and overall microflora also depend on the moisture content. In further studies the impact of combined concentrates to moisture content changes in the raw sausage during its production was studied.

The Fig. 3 shows, that water content after 5–10 days in experimental sample is lower, than in control sample. It is related to intensive decreasing of pH value during this period. The loss of water in experimental sample in 15–20 days of manufacture was 24–34%, on the other hand, the loss in control sample 20–30%. The difference in water loss was approximately 4%. After 20 days of manufacture the moisture content in control samples was 27.3%, the same value in control samples was just after 25 days of manufacture.

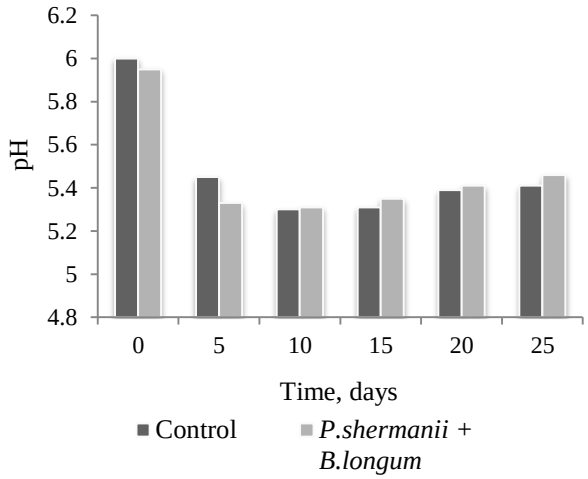

Fig. 2. The changes in pH of raw-smoked sausages added with propionic-acid bacteria and bifidobacteria.

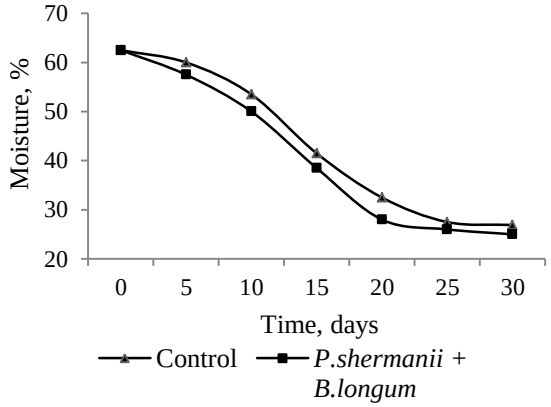

Fig. 3. Effect of the addition of starter cultures of propionic acid bacteria and bifidobacteria on the changes in moisture content.

The main chemical characteristics of control and experimental samples are shown in Table 2. The results show that moisture, protein and ash content were approximately the same in both samples. The difference in $NaNO_2$ content was found between samples. $NaNO_2$ content in experimental sample was 0.00036, and in control sample was 0.0040.

One of the main factors, which form quality of raw-smoked sausages, is pH. When pH values are close to 5.2–5.3, there is swelling of collagen hydrolysis-linking and activation of cellular enzymes, especially cathepsins. At the same value the development of pathogenic and toxicogenic bacteria suppresses. In this regard, the pH value was the main criterion for establishing the dose contributed bacterial concentrates in sausages. Therefore, the different amounts of bacterial concentrate were inoculated in the model samples of minced raw sausages.

It is well known that meat with pH value, which is close to isoelectric point, has the lowest water holding capacity. The lower water holding capacity can accelerate the drying process. In experimental sample pH values were closer to the isoelectric point than in control sample, probably, it is the reason of lower moisture content in this sausage. pH values close to isoelectric point can be a reason of better consistency of the product. The results of the study have shown that the bacterial concentrate of propionic-acid and bifidobacteria can reduce the time of manufacturing process that can have good practical application.

Nitrite has a number of functions in the curing of meat: it is involved in the development of color and odor; it has a preserving and antioxidant effect. Nitrite added in the formulation is reduced to nitric oxide, which interacts with meat pigments (myoglobin) to form nitrosomyoglobin and gives the characteristic cured red meat color. Some authors reported that addition of some propionic-acid bacteria strains can reduce nitrite content in products. According to [35] propionic-acid bacteria have different probiotic properties including reducing nitrates and nitrites with formation of nitrogen monoxide. Nitrate reduction is currently considered to be a key characteristic for the determinative grouping of strains of the Propionibacterium. In work [36] it was proven that strains of *P. acidipropionici*, *P. acnes* and *P. freudenreichii subsp. freudenreichii* are able to reduce nitrate on the yeast extract lactate and yeast extract media. It was found that nitrate reduction was strongly influenced by environmental factors such as oxygen, nitrate concentration, pH, media composition, incubation period and the presence of glucose [36]. As it was reported some strains of genes Propionibacterium can reduce nitrate to nitrite and further to nitrous oxide (N_2O) due to action of enzymes such as nitrate reductase.

Bifidobacteria can also eliminate nitrite by non-enzymic mechanism with generation of nitric oxide [37, 38].

The results of the present research correspond to results of previous research works. The content of $NaNO_2$ in experimental sample (0.00036%) was lower than in control sample (0.0040%) in several times (11 times approximately). It means more complete breakdown of $NaNO_2$ and its transformation to nitrosopigments by bacteria included in starter culture.

We can conclude that the use of combined bacterial concentrate of propionic-acid and bifidobacteria as a starter culture reduces nitrite content and processing time of meat products.

Sensory evaluation

Sensory analysis is one of the most valuable factors in quality evaluation and assessment. It is especially important for evaluation of new products. The sensory profiles of raw smoked sausages are shown in Fig. 4.

Table 2. Characteristics of raw smokes sausages

Sample	Content, %			
	Water	$NaNO_2$	Proteins	Fats
Control	27.0 ± 1.2	0.0040 ± 0.0001	23.3 ± 0.6	43.4 ± 1.4
P.shermanii + B.longum	26.5 ± 1.3	0.00036 ± 0.0001	24.2 ± 0.4	43.9 ± 1.2

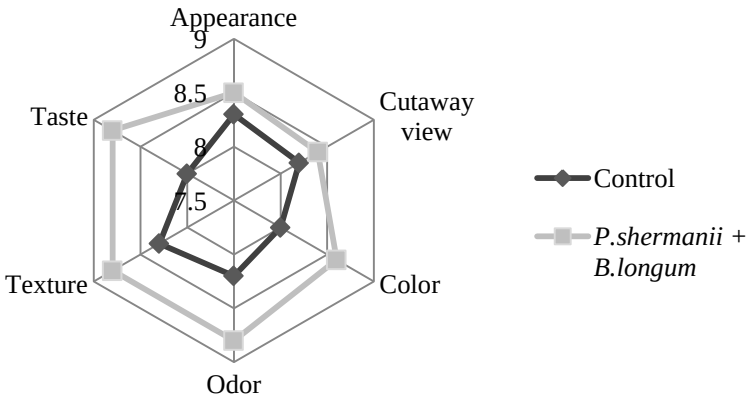

Fig. 4. Sensory profile of raw-smoked sausages.

There were better scores in experimental sample compared to control. Experimental samples had more intensive and pleasant aroma, and mild flavor with a sour milk tone. Consistency of experimental samples was more homogeneous compared to the control. The color characteristics were also different. Sample with propionic-acid and bifidobacteria was dark-red, and it had more uniform color.

The results of sensory analysis have shown that the sample with propionic- acid and bifidobacteria got higher scores in all sensory characteristics. It can be a result of action of starter culture on texture and flavor of products. Thus, the results show positive impact of combined concentrates to organoleptic properties of raw sausages.

Identification of volatile compounds

In the study approximately 140 substances were detected using gas chromatography (GC) and 85 of them were identified. The results from the GC analysis are shown in Table 3. It was found that the main volatile compounds of the sausages are saturated and unsaturated aliphatic aldehydes, alcohols, ketones, lactones, and fatty acids.

All identified compounds were grouped in nine classes including aldehydes, ketones, phenols, esters, terpenes, carbohydrates, alcohols, carboxylic acids, lactones. The content of volatile compounds in raw-cured sausages in percent is shown in Table 4. The most dominant classes of volatiles were Carboxylic acids, Phenols and Aldehydes.

Table 3. *Beginning*. Volatile compounds in fermented sausages

№ of peak	Retention Index	Component name	Concentration, mg/kg	
			Control	*P.shermanii* + *B.longum*
1	545	2-Methylpropanal	0.91	1.29
2	561	2-Methylpropanol	0.68	0.73
3	570	2-Butanone	0.71	0.91
4	579	Diacetyl	0.20	0.20
5	588	2-Butanol	0.43	0.52
6	597	Butanal	0.23	0.23
7	603	Ethyl acetate	0.53	0.57
8	607	2-Methylfuran	0.2	0.05
9	615	2-Butenal	0.21	0.23
10	628	3-Methylbutanal	0.09	0.15
11	639	2-Methylbutanal	1.23	1.47
12	687	Pentanal	0.24	0.27
13	718	2-Pentenal	0.68	0.76
14	760	2-Methylpentanal	0.07	0.12
15	765	Butyric acid	0.12	0.19
16	775	Hexanal	0.11	0.21
17	811	5-Methyl-2-hexanone	0.12	0.12
18	831	Trans-2-hexenal	0.20	0.36
19	866	2-11 anone	0.15	0.17
20	878	Heptanal	0.07	0.08
21	913	5-Methyl-2-heptanone	0.11	0.11
22	926	α -Thujene	–	0.09
23	932	α -Pinene	0.17	0.2
24	933	2-Heptenal	0.15	1.43
25	961	1-Octen-3-ol	1.02	0.71
26	968	Sabinene	0.15	0.60
27	974	β -Pinene	0.08	1.78
28	983	β -Myrcene	0.10	0.66

Table 3. *Continued.* Volatile compounds in fermented sausages

№ of peak	Retention Index	Component name	Concentration, mg/kg	
			Control	*P.shermanii* + *B.longum*
29	999	α -Phellandrene	0.04	0.65
30	1008	3-Carene	0.12	4.69
31	1011	α -Terpinene	0.29	0.58
32	1014	p-Cymene	–	0.70
33	1025	Limonene	0.25	5.81
34	1034	Ocimene	0.55	0.63
35	1052	γ -Terpinene	-	0.92
36	1054	m-Cresol	0.82	0.60
37	1064	Guaiacol	2.4	2.99
38	1084	Nonanal	0.11	0.22
39	1087	Linalool	0.16	0.40
40	1091	Phenylethyl alcohol	–	12.37
41	1127	1,3-Dimethoxybenzene	0.23	1.07
42	1128	2,6-Nonadienal	0.19	–
43	1145	2-Nonenal	0.18	0.15
44	1148	6-Nonenol	0.09	0.13
45	1157	Terpinen-4-ol	0.42	0.31
46	1161	Octanoic acid	0.97	1.19
47	1171	Methylguaiacol	3.07	4.09
48	1178	2,4-Nonadienal	0.14	–
49	1180	Methylchavicol	0.09	0.42
50	1184	Decanal	0.13	0.05
51	1200	Dodecane (internal standard)	5.00	5.00
52	1222	γ -Octalactone	0.26	2.64
53	1257	4-Ethylguaiacol	0.92	0.92
54	1289	Undecanal	0.33	0.56
55	1293	2,4-Decadienal	0.05	0.25
56	1334	Eugenol	0.35	0.52
57	1341	Neryl acetate	0.12	0.50
58	1355	Decanoic acid	4.28	5.05
59	1378	Dodecanal	0.25	0.26
60	1387	β -Elemene	0.04	0.48
61	1400	Tetradecane	0.02	0.15
62	1426	Caryophyllene	0.38	0.79
63	1432	γ -Decalactone	–	7.77
64	1465	δ -Decalactone	0.05	0.46
65	1500	Pentadecane	0.10	0.19
66	1505	β -Selinene	–	0.26
67	1545	Dodecanoic acid	1.24	1.59
68	1559	Germacren D	0.18	0.25

Table 3. *Ending.* Volatile compounds in fermented sausages

№ of peak	Retention Index	Component name	Concentration, mg/kg	
			Control	*P.shermanii* + *B.longum*
69	1588	Tetradecanal	–	0.22
70	1600	Hexadecane	0.12	0.16
71	1661	Bisabolol	0.11	0.14
72	1669	Farnesol	0.15	0.2
73	1682	Pentadecanal	0.46	0.47
74	1700	Heptadecane	0.30	0.42
75	1746	Tetradecanoic acid	4.79	6.05
76	1764	Phthalate	0.16	0.23
77	1777	Hexadecanal	0.09	0.12
78	1800	Octadecane	1.06	6.87
79	1900	Nonadecane	0.16	0.27
80	1924	Hexadecenoic acid	2.06	2.58
81	1948	Hexadecanoic acid	9.03	14.05
82	2000	Eicosane (Hydrocarbon - C20)	1.14	1.62
83	2118	Octadecenoic acid	3.64	2.44
84	2146	Octadecanoic acid	6.79	3.98
85	2175	Octadecadienoic acid	0.50	0.60

Table 4. Percentage of volatile compounds in raw-cured sausages

Samples	Class of compounds, mg/kg								
	Aldehyds	Ketons	Phenols	Esters	Terpenes	Carbohydrates	Alcohols	Carboxylic acids	Lactones
Control	6.06	1.18	6.66	0.53	2.05	5.91	1.49	21.83	0.37
P.shermanii + *B.longum*	9.06	1.40	13.45	0.57	7.25	9.68	14.14	20.43	10.87

Volatile compounds can be separated according to their possible origin. Spices could be responsible for the generation of terpenes and hydrocarbons. Phenols could be the components of smoke fume.

The study was shown that there is significant difference in contents of volatile compounds between sausage samples. Their qualitative compositions are different. Some compounds were found only in experimental samples. For example, α-Thujene, p-Cymene, γ-Terpinene, Phenylethyl alcohol, γ-Decalactone, Tetradecanal, β-Selinene. These results could be related to different activities of starter cultures used in the study.

Carbonyl compounds (aldehydes, ketones etc.) play the main role in formation of aroma profile of meat products. The content of aldehydes in sausage with propionic-acid and bifidobacteria was 30% higher than with control sample. The content of ketones was 17% higher in experimental sample.

In addition, the content of phenols was two times higher in experimental sample than in control. It was stated the high contents of α-Phellandrene, 3-Carene, Methylguaiacol in this sample.

The contents of esters and carboxylic acids were nearly the same in both samples.

It is important to note the difference in terpene content between the sausage samples. The content of terpenes in experimental sample was 3.5-fold higher than in control sample (For example, the content of α-Pinene was 22-fold higher, the content of β-Myrcene

was 6-fold higher, the content of γ-Terpinene was 2-fold higher). α-Pinene has pines odour, β-Myrcene has pleasant smell, γ-Terpinene has lemon odour.

The main difference between the batches was in the content of lactones. It was tenfold higher in experimental sample than in control sample. The contents of δ-Decalactone and γ-Octalactone (both of them have sweet, coconut notes) were 10-fold higher in experimental sample. γ-Decalactone wasn't found in the control sample, but was found in sample with experimental starter culture in concentration 7.77 mg/kg. The odour of γ-Decalactone can be characterized as sweet, fruity-peach and creamy.

It is well known fact that lactones are referred to uronic acids. For example, during fermentation of bifidobacteria D-glucose oxidizes with forming of D-glucuronic acid. The presence of lactones in experimental sample was the reason of mild creamy tone in sausage.

CONCLUSIONS

The study demonstrated that sausages produced with *Propionibacterium shermanii KM-186* and *Bifidobacterium longum B379M* as a starter culture contained several aroma-compounds that were not found in control samples. Also higher contents of terpenes, lactones, alcohols and phenols were found in sausages made with starter culture of propionic-acid bacteria and bifidobacteria compared to control samples. Results of sensory evaluation have shown that the experimental batch had better scores than the control batch. It indicates, that certain aroma compounds plays significant role in the formation of the aroma of raw-smoked sausages.

Thus, the finding of this study was to show that use of *Propionibacterium shermanii KM-186* and *Bifidobacterium longum B379M* as starter cultures has positive influence on sensory characteristics of raw-smoked sausages.

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