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Impact of metabiotic on the quality and safety of cooked sausages

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Abstract:

This article shows theoretical and practical fundamentals of sausage cooking by using a biologically active additive based on metabolites of probiotic microorganisms. Results of a comprehensive analysis of the culture liquid of a propionic acid bacteria concentrate containing viable and disintegrated cells are presented. It has been defined that the culture liquid of propionic acid bacteria concentrate is a good source of commercially valuable metabolites, the number of which depends on the species and strains. A metabiotic is characterized by intense catalase, peroxidase and superoxide dismutase activity. It has been revealed that the disintegration of propionic acid bacteria cells promotes an increase in the content of antioxidant enzymes and vitamin B_{12}. The method of capillary electrophoresis helped to reveal a certain high content of citric and succinic acids with intense antioxidant activity. The efficiency of using the new generation probiotic for improving the quality of cooked sausages with due safety has already been proved.

Keywords: cooked sausages, probiotic microorganisms, metabolites, safety, quality.

INTRODUCTION

In the Russian Federation, the formation of a healthy food system for the population is one of the state policy priorities [1, 2]. The main problem of nutrition is that the products of everyday demand contain a lot of dyes, preservatives, antioxidants and other unsafe food supplements against the increasing shortage of vital biologically active components [3–7].

In addition to meeting the physiological needs in food and energy, the consumption of food must fulfill preventive and curative functions [8, 9].

While developing new generation products, it is very useful to apply microorganisms that are able to acclimate in the human intestine and have a positive effect on our immune system, as well as to synthesize a number of biologically active substances [10].

Propionic acid bacteria have unique immunostimulating, antimutagenic and antioxidant properties [11–13]. Valuable metabolites are known to not only accumulate in biomass, but also to remain in the culture liquid of propionic acid bacteria, and therefore while producing safe food products their use is of much interest.

Metabiotics (a new generation of biotics) make it possible to create a controlled intestinal microbiocenosis, because cellular components, metabolites of probiotic microorganisms may have greater importance than the quantitative content of the microorganisms themselves [14].

Taking into account the current ecological, social and economic situation, the authors have shown that the development of innovative competitive meat products with functional properties is urgent.

Study Goal is the theoretical substantiation and development of the sausage cooking technology by using a biologically active additive based on metabolites of probiotic microorganisms.

MATERIALS AND METHODS

Theoretical, methodical, experimental study and practical developments were carried out in certain specialized laboratories of the Eastern Siberian State University of Technology and Management, the approved testing lab center of the Ulan-Ude branch of the Federal Center for Hygiene and Epidemiology of Railway Transport (Ulan-Ude), and the research laboratory of the Siberian Institute of Plant Physiology and Biochemistry (Irkutsk).

The culture liquid of the propionic acid bacteria concentrate of the strains *P.freudenreichii ssp. shermanii* AC-2503, *P.freudenreichii ssp. freudenreichii* AS-2500, *P.cyclohexanicum Kusano* AC-2260 (the culture liquid of these strains with disintegrated bacterial cells) was chosen as the study material. Certain propionic acid bacteria (provided by the All-Russian Research Institute of Genetics and the Institute of Biochemistry and Physiology of Microorganisms (Moscow)) were activated by the previously developed biotechnological method and cultured on a serum medium supplemented with growth components [15].

For this study, certain meat raw materials, semi-finished products, model samples and finished products (cooked sausages) were selected as objects of the study. The cooked sausage “Chainaya”, second chop (GOST R 52196-2011), was chosen as a control sample.

During the study tests, organoleptic, physical and chemical, microbiological and biochemical indices were analyzed. The main physical and chemical and sanitary-hygienic quality indicators of meat, sausage meat and cooked sausages were determined by standard methods [16, 17].

Quantitative counting of the propionic acid bacteria cells was carried out by using the method of limiting dilutions in the hydrolyzate lacteal medium and hydrolyzate lacteal and maize medium. The antimutagen activity was determined by the Ames test; a test strain of *Salmonella tuphimurium* TA-100 was used as a mutagenicity indicator.

The peroxidase activity was determined spectrophotometrically with the o-dianisidine reagent; the catalase — by colorimetric method; and the superoxide dismutase — according to the autooxidation of adrenaline.

The amino acid composition of the proteins was determined by the AAA-339 amino acid analyzer.

The method of capillary electrophoresis by applying the Kapel-105M device was used to determine the content of organic acids.

The concentration of exopolysaccharides was determined by the anthrone method; the content of vitamin B_{12} — by the spectrophotometric method.

First, the tests were performed with the model systems, and then with the finished products. The technology was verified experimentally and industrially in the meat workshop of Vazhenka Ltd (Ulan-Ude).

All tests were performed in 3-fold replication. The obtained data were processed by using the Excel statistical software package with the Mann-Whitney test. Statistically significant differences were discussed at $p < 0.05$.

RESULTS AND DISCUSSION

When creating innovative meat products by using the QFD methodology, the following directions of improving their quality were taken into account: improvement of structural and mechanical properties, juicing of elastic consistency; giving an

intense meat taste and aroma; increasing the shelf life of finished products; saving product cost; increasing the product usefulness by increasing the content of vitamins, essential amino acids, antimutagenic substances, as well as reducing products of peroxidation. To achieve these goals, it was offered to use a biologically active additive based on the products of the vital activity of propionic acid bacteria [18, 19].

Probiotic microorganisms produce a considerable amount of various physiologically active substances and various compounds [20–23].

When being cultivated in the logarithmic phase of growth, propionic acid bacteria are known to secrete valuable metabolites that are not only accumulated in biomass, but remain in the culture liquid. The culture liquid obtained after biomass separation was analyzed; the results are given in Table 1.

The study results show that the culture liquid is characterized by a sufficiently high content of viable cells, which is important when producing cooked sausages, the technology of which uses raw materials of the second class quality.

Over the recent years, due to the problem of comprehensive processing of microbial biomass for the production of a number of products of microbiological synthesis, the disintegration of microorganisms has been of great practical importance. Ultrasonic disintegration of the cell membrane was used to extract intracellular compounds. The results of studying the effect of disintegration on the content of valuable metabolites before and after their treatment are presented in Table 2.

Table 1 — Qualitative Characteristics of the Culture Liquid

Indicator	*P. freudenreichii* ssp. *Shermanii* AC-2503	*P. freudenreichii* ssp. *Freuden-reichii* AC-2500	*P.cyclohexanicum Kusano* AC-2260
Taste and smell	Acidic, whey without foreign flavors and odors		
Color	Light yellow		
Consistency	Homogeneous		
Number of propionic acid bacteria, CFU/cm^3	$9\cdot10^8$	$8\cdot10^8$	$7\cdot10^8$
pH	5.8	5.8	5.8
Weight of product (in which not allowed), cm^3			
Coliform bacteria	10.0		
S. aureus	10.0		
Pathogenic, incl. *Salmonella*	50.0		
Yeast, CFU/g	< 5		
Molds, CFU/g	< 5		

Table 2 — Metabolites in the Culture Liquid

Indicator	*P. freudenreichii* ssp. *Shermanii* AC-2503		*P. freudenreichii* ssp. *Freudenreichii* AC-2500		*P.cyclohexanicum Kusano* AC-2260	
	before	after	before	after	before	after
Catalase, monoclonal antibodies/ml	2,102.0	3,902.0	1,275.0	2,075.0	1,709.1	2,609.1
Superoxide dismutase, U/mg protein	0.94	1.24	0.92	1.02	1.01	1.55
Peroxidase, nmol/(min·mg protein)	1.003	2.313	1.102	3.273	0.705	1.805
Exopolysaccharides, μg/ml	0.514	0.908	0.302	0.502	0.411	0.846
Vitamin B_{12}, μg/ml	4.464	14.285	3.1	9.28	2.2	6.31
Antimutagenic activity, % inhibition	31.7	45.9	31.5	44.6	30.8	44.2

According to Table 2, a considerable increase in the content of valuable metabolites is observed in the culture liquid with disintegrated cells. This is due to the fact that during the disintegration of cells, intracellular metabolites are extracted, which are associated with subcellular particles and membranes, and are released into the medium after cell destruction. Taking into account that $1\cdot10^8$ viable cells are found in the culture liquid, their destruction promotes an additional accumulation of biologically active compounds.

Antimutagenic substances released into the culture liquid are of practical interest. The tests show that coincubation of the culture liquid with mutagen sodium azide before the introduction into a medium inoculated with *S. typhimurium* results in inhibition of mutagenesis. Moreover, the culture liquid is a source of exopolysaccharides and vitamin B_{12}. It is necessary to note that in the culture liquid of the strain *P.freudenreichii ssp. shermanii* AC-2503 the greatest amount of valuable metabolites is accumulated.

It is interesting that propionic acid bacteria, along with propionic and acetic, synthesize a high amount of succinic and citric acids. Table 3 shows the test results.

It is known that propionic acid bacteria can synthesize all amino acids by assimilating nitrogen. A wide spectrum of amino acids, as well as a high content of leucine, threonine, and glutamic acid were found in the culture liquid. As a result of analyzing the quantitative and qualitative amino acid composition of the culture liquid, 16 amino acids were identified with a total of 0.692 g/l, including 6 essential ones.

As a result, it was found that the culture liquid of the propionic acid bacteria concentrate contained a high amount of biologically active substances capable of having a positive effect on the quality of cooked sausages.

Table 3 — Content of Organic Acids in the Culture Liquid

Acid	Mass fraction of organic acid, mg/dm^3
Formic	54.5 ± 10.9
Citric	3065 ± 613.0
Succinic	9,415.0 ± 1,883.0

Based on the test results, a certain sausage cooking technology was developed. It provides the replacement of process water to the culture liquid of propionic acid bacteria (see Figure 1). A metabiotic is introduced at the stage of making minced meat at cuttings in the amount of 7% per 100 kg of raw materials. The optimal dose is chosen taking into account its impact on the functional and technological and organoleptic properties of the minced meat. To produce cooked sausages, beef, pork and bacon are used as the main raw materials.

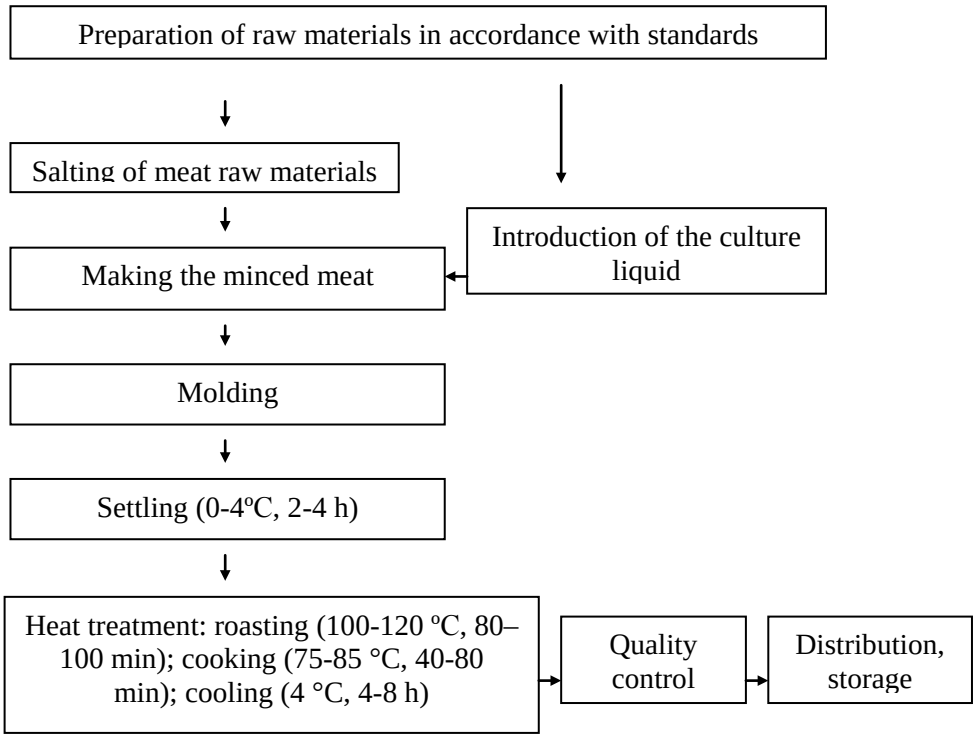

Figure 1 — Technological Scheme for the Production of Cooked Sausages

Table 4 — Qualitative Characteristics of the Cooked Sausages

Indicator	Control	Test
Appearance	Baloneys with clean and dry surface, free of damage, broth and fatty stains	
Type and color on the cut	Pink, minced meat is evenly mixed with slices of bacon, fine porosity	Pink, minced meat is evenly mixed with slices of bacon
Taste and smell	Inherent to this type of product with the aroma of spices, moderately salty, without foreign flavors and odors	Intense, specific taste, without foreign flavors and odors
Consistency	Elastic	Elastic, dense
Juiciness	Juicy	Very juicy
Mass fraction of edible salt, %	2.2 ± 0.15	2.2 ± 0.15
Moisture content, %	67.4 ± 1.1	70.1 ± 0.8
Mass fraction of fat, %	18.5 ± 1.5	18.4 ± 1.0
Mass fraction of protein, %	14.6 ± 1.5	14.5 ± 1.5
Mass fraction of sodium nitrite, %	0.004 ± 0.0002	0.002 ± 0.0001
The content of vitamin B_{12}, µg/100 g	0.9 ± 0.08	2.1 ± 0.1
Residual activity of acidic phosphatase, %	0.006 ± 0.0001	0.006 ± 0.0001
QMA & OAMO, CFU/g	$2.5 \cdot 10^3$	
Coliforms in 1 g	Not detected	
Sulfite-reducing clostridia in 0.01 g	Not detected	
S. aureus	Not detected	
Pathogenic, incl. *Salmonella* in 25 g	Not detected	

Immediately after the production of cooked sausages, the quality control was performed. Table 4 shows the qualitative characteristics of the control and test samples of cooked sausages.

Color is one of the most important quality indicators, according to which every consumer first of all gets an idea of the product's presentation. When assessing the sausages by the members of the Testing Committee, it was noted that the test samples were brighter than the control ones. This is probably due to the fact that propionic acid bacteria contain constitutive nitrate reductase and restore nitrites as final acceptors in the utilization of lactate. Thus, the impact of the culture liquid on the formation of nitrosopigments was studied. Table 5 shows the test results.

Table 5 — Nitrosopigments and Color Stability

Indicator	Control	Test
Sodium nitrite added, mg/100 g of minced meat	5.0	5.0
Nitrosopigments, % to total pigment	71.2	87.0
Color stability, %	66.5	88.0

The test results show that the test samples are characterized by an increased content of nitrosopigments and

color stability. This may be explained by the fact that propionic acid bacteria are able to form tetrapyrolic pigments, iron-containing protoporphyrin and sirohydrochlorine complexes constituting a prosthetic group of hemoproteins that include hemoglobin and myoglobin, which contributes to the formation of a large number of nitroso compounds that ensure stable coloration of the finished products.

The smell and taste of all samples meet the requirements, while the test samples have more intense flavor-aromatic characteristics.

Sausages produced with a culture liquid have denser and elastic consistency as compared to the control, which may be explained by the high content of exopolysaccharides in the metabiotic, which increases the proportion of bound moisture and ensures the juiciness of the finished product. It is important to note that there is some increase in the content of bound moisture in the test samples, which results in an increase in the juiciness of the finished product.

As for the physical and chemical characteristics, the test results show a certain decrease in residual nitrite in the test samples. The increased content of vitamin B_{12} in the test samples may be explained by the ability of propionic acid bacteria to synthesize this vitamin in considerable amounts.

At the next stage of our study, antimutagenic properties of the finished products were analyzed because the antimutagenesis test is important, first of all, with respect to bacteria used in the production of additives and probiotic products. The test results show that the sausages cooked by using a culture liquid have antimutagenic action against the mutagenesis induced by sodium azide. This is due to the fact that propionic acid bacteria synthesize significant amounts of antimutagenic substances. The antimutagenic activity amounts to 14.8% inhibition.

According to the test results, the culture liquid containing disintegrated cells of propionic acid bacteria may be characterized by some fairly balanced amino acid composition and high biological value due to such deficient amino acids as leucine, tyrosine, and isoleucine.

At the next stage, the amino acid composition of cooked sausages was studied. The test results are shown in Figure 2.

The analysis of the amino acid composition of the control and test samples of the cooked sausages showed the advantage of the latter. For instance, in respect to essential amino acids, the test samples exceed the control ones by 8.6%.

The amino acid score is an indicator characterizing the biological value of a protein (Table 6).

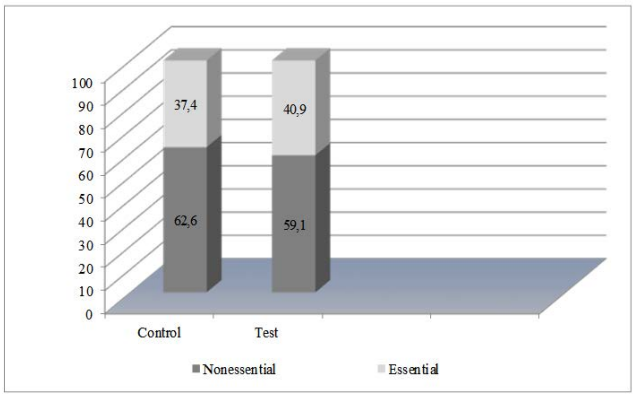

Figure 2 — Amino Acids in the Cooked Sausage Samples

Table 6 — Amino Acid Score in the Cooked Sausages

Amino acid	FAO/WHO standard	Amino acid content in the sample (g/100 g protein)/score	
		Control	Test
Tryptophan	1.00	1.32/132	1.38/138
Leucine	7.00	7.36/105.1	8.98/128.3
Isoleucine	4.00	4.05/101.3	4.50/112.5
Valine	5.00	5.20/104	5.42/108.4
Threonine	4.00	4.39/109.8	4.57/114.2
Lysine	5.50	5.37/97.6	5.76/104.7
Methionine + cystine	3.5	3.61/103	3.68/105.1
Phenylalanine + tyrosine	6.00	6.08/101.3	6.61/110.2
Total	36.00	37.38	40.9

According to the data presented in Table 6, both samples of the sausages exceed the standard proposed by FAO/WHO in relation to the composition of essential amino acids. The difference between the composition of essential amino acids in the control and test sausage samples may be explained by the use of the metabiotic containing amino acids in the amount of 0.286 g/l.

Despite the fact that the ratio of the three most important amino acids (tryptophan, methionine plus cystine, and lysine) in the control samples corresponds to the optimal formula, the amino acid score in the control samples lacks such amino acid as lysine. In the test samples, the amino acid score amounts to > 100% for all amino acids.

The high biological value of cooked sausages is proved by the protein quality index of the control (1.02) and test (1.15) samples where the index of essential amino acids is 1.05 and 1.16, respectively.

As a result of the conducted research, it was established that the cooked sausages with a more balanced amino acid composition were obtained when using the culture liquid of propionic acid bacteria.

The results of the microbiological test show that the sausages produced by using the culture liquid of the propionic acid bacteria concentrate meet the requirements of the current regulatory documents.

Thus, during the conducted tests it was determined that the developed sausages were superior to the control samples by organoleptic and physicochemical parameters.

In the next series of experiments, the impact of the metabiotic on the oxidative processes occurring when storing finished products was studied. The test results are shown in Figure 3.

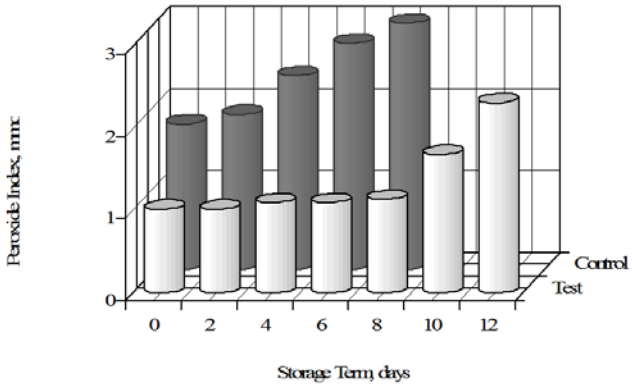

Figure 3 — Change in the Peroxide Index for Cooked Sausages when Storing them

The analysis of the presented data shows that the use of the culture liquid contributes to considerable inhibition of the

oxidation processes that take place in the finished product during the storage. The control sample reaches a value of 2.361 mmol O_2/kg by the 4th day of storage, while the test samples reach this value only on the 12th day. This is due to the synthesis of superoxide dismutase enzymes, peroxidase, and catalase characterized by high antioxidant properties. The dynamics of the change in the acid index also indicates the inhibition of oxidative processes. This is probably due to the fact that propionic acid bacteria synthesize a significant amount of succinic and citric acids that are active antioxidants.

Thus, as a result of the conducted study, it was determined that the use of the culture liquid in the production of cooked sausages not only improved their quality characteristics but also inhibited the oxidation processes in the finished product, which increased its shelf life. The developed sausage cooking technology fully meets the requirements of consumers, such as safety, health benefits and good taste.

CONCLUSION

According to the study results, it has been determined that a wide range of biologically active substances accumulating in the culture liquid of the propionic acid concentrate can increase the consumer properties of meat products. The greatest amount of valuable metabolites is accumulated in the culture liquid of the strain *P.freudenreichii ssp.shermanii* AC-2503. There is a high exopolysaccharide potential of 0.514 µg/ml and antimutagenic activity of 31.7% inhibition. The primary metabolite of propionic acid bacteria, vitamin B_{12} , in the amount of 4.464 µg/ml is found. The specific activity of antioxidant enzymes makes up catalase — 2,102 monoclonal antibodies/ml, peroxidase — 1.003 nmol/(min·mg protein), and superoxide dismutase — 0.94 U/mg protein. It has been found that the synthesis of formic acid that has vivid bactericidal properties is not that significant — 54.5 mg/dm^3. It is necessary to note that the culture liquid contains a high amount of citric acid (3,065.0 mg/dm^3) and succinic acid (9,415.0 mg/dm^3).

It is proved that the disintegration of cells by ultrasound promotes an increase in the content of valuable metabolites accumulating in the culture liquid of the propionic acid bacteria concentrate.

The technology of cooked sausages has been developed. It fully meets the requirements of consumers such as safety, health benefits and good taste. At the same time, this innovative technology should satisfy manufacturers according to practical and economic indicators, which is confirmed by the results of the pilot industrial verification of technology in the production conditions.

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