International Journal of Recent Technology and Engineering (IJRTE)
ISSN: 2277-3878, Volume-8 Issue-4, November 2019

Properties and Compatibility of Microflora for Creating Starter Cultures in Sausage Production Technology

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Abstract*: In recent years, the success of scientific research in the field of biotechnology has led to the development of new technologies to accelerate the production of smoked sausages, improve their organoleptic properties and significantly increase the guarantee of production of high-quality products. One way to intensify the process of raw smoked sausages is to use starter crops. The paper presents the results of a study of individual strains and a consortium of microflora used in the production of raw smoked sausages. The results and justification of changes in the functional and technological properties of raw meat after making starter crops are presented.

***Keywords* : starter cultures, microflora, functional and technological properties, meat raw materials.**

I. INTRODUCTION

One of the most complex and labor-intensive technological processes is the technology of production of raw smoked sausages. During their production, special requirements are made to the qualifications of personnel, the quality of raw materials and the sanitary condition of equipment and premises [1].

An analysis of foreign and domestic sources allows us to conclude that the technology for the production of raw smoked sausages made it possible to use modern technologies that accelerate ripening. As a rule, starter cultures are used to accelerate ripening [2, 3].

Studies conducted by L.S. Kudryashov and S.V. Kuznetsova, showed that fermentation in raw smoked sausages during the ripening period is accelerated if you add the strain Lactobacillus plantarum NRRL - B-5461, as a source of formation of "soft" lactic acid. To improve its action, they recommend using a mixture with cultures of Pediococcus cerevisiae, Streptococcus lactis, Leuconostoc citrovorum, Streptococcus diacetilactis [4].

In a number of countries, various bacterial preparations Bactoferment 61, Duploferment H, Pokelferment 77 are used for the production of raw smoked sausages, they include dentitrifying micrococci and microorganisms that produce lactic acid and improve the formation and color stabilization, reduce the nitrite content, improve the quality and shorten the manufacturing process sausages [5, 6].

Studies Nesterenko A. A., Koshchaev A. G., Kenijz N.V. allowed us to develop an accelerated technology for the production of raw smoked sausages using probiotic microorganisms as starter cultures for functional foods [8].

VNIIMP scientists have developed a technology for the production of semi-dry raw smoked sausages, which involves the use of the bacterial preparation PB-MP. The application of this technology allows to accelerate the production of raw smoked sausages by 17-19 days. At the same time, the yield of finished products is 68-69%, the reduction in energy costs is 20-24%, and high quality of finished products is ensured.

Studies on the creation of starter cultures for the production of smoked sausages have shown the need for additional nutrient media. This role is performed by dextrose. The introduction of sugar contributes to the best preservation of the finished product. The ongoing enzymatic processes hinder the active growth of undesirable microflora. Along with this, protein substances and fats are practically not used during fermentation, since starter cultures use easily broken down sugars as a nutrient medium [9, 10].

It is known that when selecting cultures for starter cultures, it is important that microorganisms are in a strong symbiotic relationship.

An important indicator of the quality of the starter culture is the suitability for the production of a given product, which should be verified by research. When compiling starter cultures, it is necessary to take into account the specific properties of the product being produced, temperature conditions of production, and the relationship between microorganisms.

Manuscript published on November 30, 2019.
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The most important eligibility criteria for combining individual strains into multi-strain starter cultures is the compatibility of species and strains. For their growth, the characteristics of the metabolism of different microorganisms are of great importance, since the mutual influence, applicability and stability of the starter culture in production depend on this. If possible, mutual stimulation of starter microorganisms and antagonistic action should occur, i.e. suppression of the development of extraneous microflora [8, 9].

The aim of the research is to study individual microflora strains when creating starter cultures for the production of raw smoked sausages.

II. MATERIAL AND METHODS

For the creation of the consortium, microorganism cultures common in the market and used for the treatment and prevention of the gastrointestinal tract microorganisms were selected: lactobacillus plantarum, lactobacillus casei, staphylococcus carnosus, bifidumbacterium siccum, bifidumbacterium bifidum.

The ground beef was prepared from premium beef 30%, semi-fat pork 45% and spike fat 25%. Raw meat was ground on a spinning top with a hole diameter of 3 mm, was mixed.

The studies presented in the work were carried out in accordance with the recommendations and methods of their implementation [7].

Determination of pH was carried out on a potentiometer pH-340 according to GOST R 51478-99 [10]. The pH of the hydrolyzate solutions was determined by the potentiometric method on a universal pH-150M ionometer.

Mass changes were determined by weighing on a balance and in a ratio in% to the mass of the feedstock.

III. RESULTS AND DISCUSSION

By the need for nutrients, lactic acid bacteria are among the most complex microorganisms. As a carbon source, they can use mono- and disaccharides, organic acids. They do not develop on ordinary nutrient media, but grow on media supplemented with amino acids, hydrolysates of meat proteins, lactalbumin, casein, and various types of flour. Most strains of lactic acid bacteria require amino acids: arginine, leucine, isoleucine, histidine, valine; vitamins: riboflavin (B_2), thiamine (B_1), pantothenic (B_3), nicotinic (PP), folic (Sun) acid, pyridoxine (B6) and others. Some peptides, purines, pyrimidines, fatty acids also stimulate the growth of some bacteria.

The need for microorganisms in vitamins is given in table 1 [8].

Table – I: Need for microorganisms in vitamins

Name	Lactobacillus		Bifidobacterium		Staphylococcus carnosus
	plantarum	casei	siccum	bifidum	
Riboflavin	±	+	±	-	±
Pyridoxal	-	+	-	+	+
Folic acid	-	+	+	-	+
B_{12}	-	+	+	+	-
Thymidine	-	+	-	-	+
B_6	-	-	-	+	-

In connection with the needs of microorganisms in carbon sources, a model experiment was conducted to study the effect of various mono- and disaccharides on the dynamics of the development of lactic acid microorganisms. The following sugars were selected for the experiment: arabinose, raffinose, glucose, lactose, maltose, sucrose. Sugars were introduced in an amount of 5% (by analogy with the lactose content in milk 4.7 - 5.2%), fermentation 5% and milk 5%, fermentation was carried out for 12 hours. The results were determined by the change in titratable acidity table 2.

Table – II: Various signs of the growth of microorganisms

Name	Lactobacillus		Bifidobacterium		Staphylococcus carnosus
	plantarum	casei	siccum	bifidum	
Growth at 4 °C	+	+	+	+	+
Growth at 15 °C	+	+	+	+	+
Growth at 45 °C	±	±	±	±	±
Fermentation:					
arabinose	+	-	-	-	±
glucose	+	+	-	-	±
lactose	+	+	+	+	+
maltose	+	+	+	±	∓
raffinose	+	-	+	-	+
sucrose	+	+	+	±	±

Note: (+) - titratable acidity of 90 °T and above, () - titratable acidity from 70 to 90 °T, () - titratable acidity from 50 to 70 °T, (-) - titratable acidity of less than 50 °T.

From the above data, we see that the introduction of sugars leads to an increase in the acidity of the product, but not in all types of microorganisms. When making arabinose, glucose, raffinose, low acidity for bifidobacteria, in the case of maltose, low acidity for bifidobacteria and staphylococci. In the case of lactose, acidity increases in all selected types of microorganisms to 90 °T or more, which corresponds to the acidity of traditional fermented milk products.

To determine the concentration of table salt, the cell preparedness, table salt of various concentrations (from 0 to 12% by weight of the medium) was introduced into the nutrient medium. Inoculation of cultures was carried out with MRS culture medium under sterile conditions. Then they were cultured in an autoclave at 30 ° C for 48 hours and the cells were counted.

The effect of sodium chloride concentration on cell survival of selected microorganisms is shown in Figure 1.

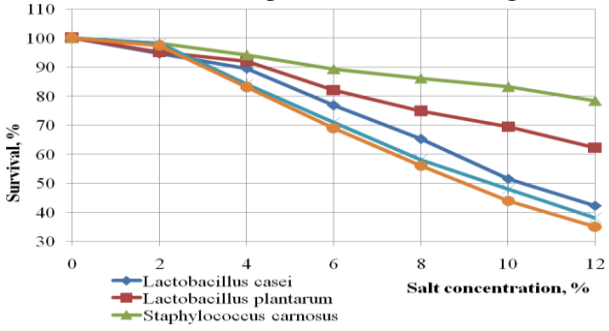

Fig. 1. Cell survival of microorganisms depending on salt concentration

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The data obtained indicate a greater tolerance to table salt of staphylococcus carnosus culture, in contrast to the rest of the studied cultures.

In the technology of production of smoked sausages, the pH of the minced meat is of great importance, by which the fermentation rate and acid accumulation are judged. A quick decrease in the pH of minced meat also contributes to the inhibition of the development of pathogenic microflora and improves the quality of the finished product.

To determine the rate of decrease in pH, microorganisms were seeded on nutrient media under sterile conditions in a box; the inoculum dose was 1 g / cm3, after which it was cultured in an autoclave for 12 h at a temperature of 30 °C. Then the pH of the medium was determined.

The dynamics of pH changes during the cultivation of microorganisms is presented in Figure 2.

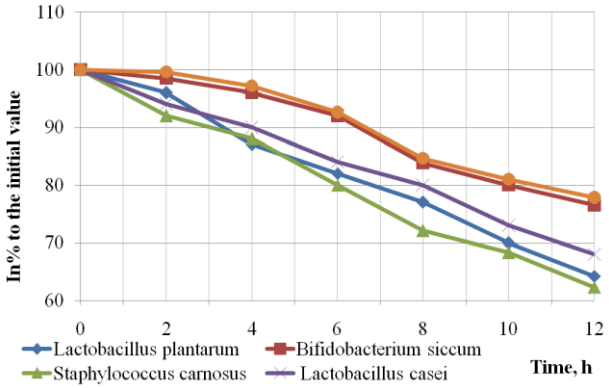

Fig. 2. Change in pH of the medium during the cultivation of microorganisms

Based on the above characteristics, we selected three strains for a consortium of microorganisms from five strains: Lactobacillus plantarum, Bifidobacterium siccum, Staphylococcus carnosus

Lactobacillus plantarum was chosen because of its high salt tolerance and less vitamins needed for growth compared to lactobacillus casei, bifidobacterium siccum for its high salt tolerance and proteolytic activity compared to bifidobacterium bifidum.

In our further work, we analyzed the biochemical activity of the selected cultures on nutrient media.

For these purposes, we took a model stuffing, consisting of second-class veined beef.

During cultivation on model minced meat, the following indicators were determined, indicating the growth of microorganisms, a change in the pH of the medium (Fig. 3), the dynamics of the accumulation of lactic acid (Fig. 4), and the dynamics of hydrolysis of the proteins of the nutrient medium (Fig. 5) during 24 hours of cultivation.

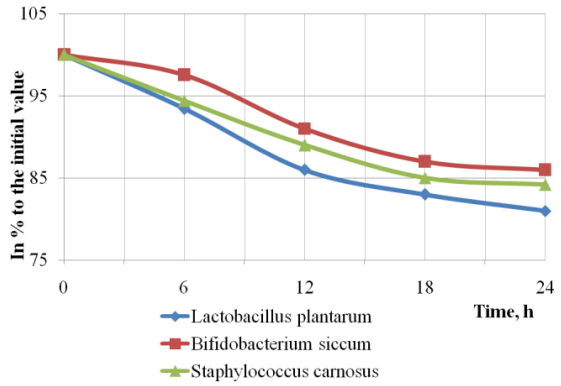

Fig. 3. Changing the pH of the medium with the growth of bacteria on a model stuffing

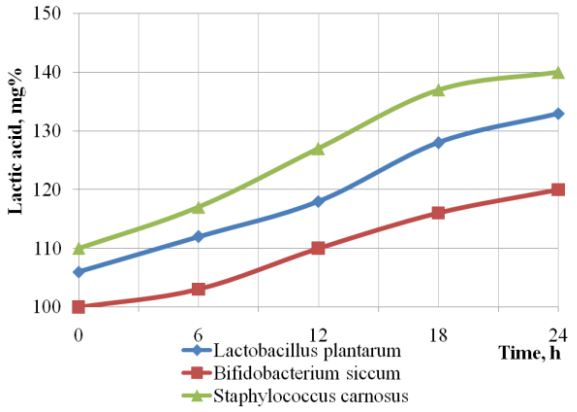

Fig. 4.Dynamics of the accumulation of lactic acid in the process of growth of microorganisms on model stuffing

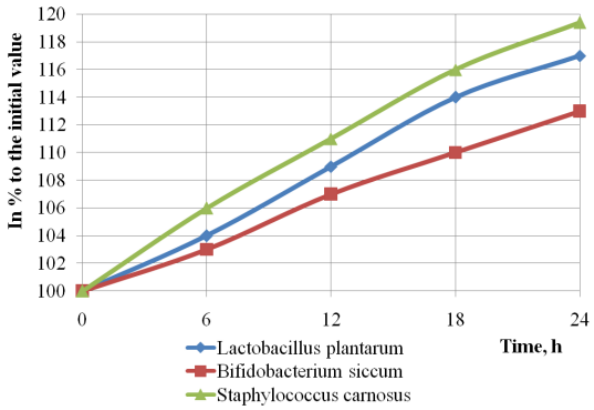

Fig. 5. Dynamics of protein hydrolysis of minced meat by cultivation of microorganisms

During the cultivation of Lactobacillus plantarum, the pH of the minced meat decreased by 19% compared to the initial value by 24 hours of cultivation, the amount of accumulated lactic acid was 27 mg%, the degree of protein hydrolysis was 17% of the initial value. During cultivation of Bifidobacterium siccum, the pH decreased by 14%, the amount of lactic acid was 20 mg%, the degree of hydrolysis of proteins was 13% of the initial value. For Staphylococcus carnosus, respectively, these data were pH decreased by 15.8%, the amount of lactic acid 30 mg%, the degree of hydrolysis of proteins 19% to the initial, respectively.

Analyzing the data obtained, it can be said that the selected strains of microorganisms grow on model ground beef, as evidenced by the accumulation of lactic acid and a decrease in the pH of the environment, there is also a breakdown of the proteins of the connective tissue of collagen, there is an accumulation of free amino acids and polypeptides, as evidenced by changes in the dynamics of protein hydrolysis.

To determine the degree of action of cultures in various ratios, we carried out cultivation of selected bacteria on a model stuffing in the following combinations - Lactobacillus plantarum + Bifidobacterium siccum, Staphylococcus carnosus + Lactobacillus plantarum, Staphylococcus carnosus + Bifidobacterium siccum. During cultivation, the same indicators were determined as in the cultivation of each species separately.

The research results are presented in figures 6-8.

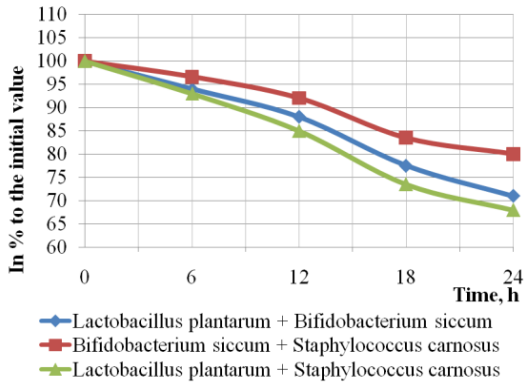

Fig. 6. The pH of the medium during the cultivation of bacteria in various combinations for model stuffing

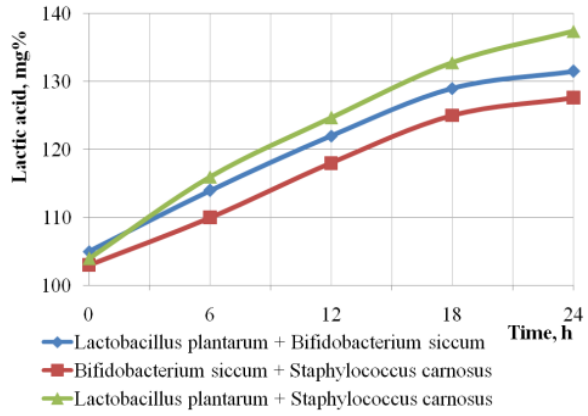

Fig. 7. Dynamics of lactic acid accumulation during bacterial cultivation in various combinations for model stuffing

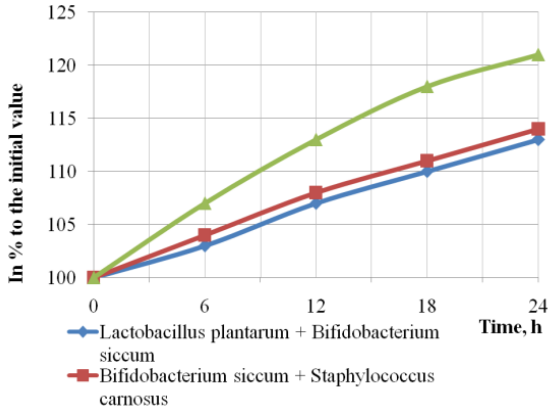

Fig. 8. Dynamics of protein hydrolysis of model minced meat during cultivation of bacteria in various combinations

Interpreting the results, it can be said that the selected strains of microorganisms grow well on the ground beef, not only individually, but in various combinations, as evidenced by the accumulation of lactic acid and a decrease in pH, which indicates a positive cohabitation of the selected strains of lactic acid and bifidobacteria.

The same can be said about the correctness of the selected cultures, since there is a breakdown of the proteins of the connective tissue of collagen, there is an accumulation of free amino acids and polypeptides, as evidenced by the dynamics of protein hydrolysis.

The growth of microorganisms was monitored by the dynamics of titratable acidity of the model minced meat during 24 hours. During the study, the results were shown in Figure 9.

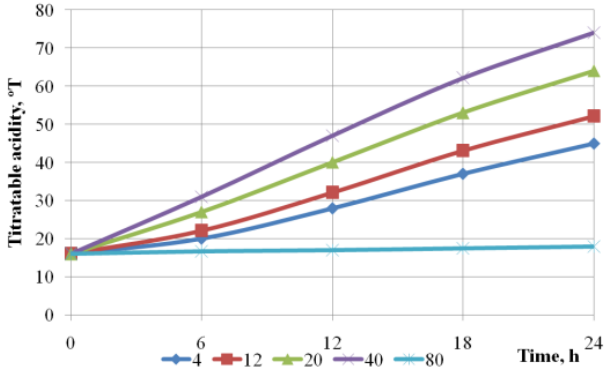

Fig. 9. Dynamics of acid formation during biomodification of raw materials at different ambient temperatures

An important factor in the application of the created consortium of microorganisms is the resistance of these types of bacteria to the action of various temperature parameters used in the production of meat products. To conduct the study, a consortium of microorganisms was activated for 12 hours in skim milk under sterile conditions at an optimum microorganism growth temperature of 30 °C in a thermostat. After activation, a consortium was added to the minced meat and studies were performed. The following temperature conditions were selected: + 4 °C, + 12 °C, + 20 °C, + 40 °C, + 80 °C.

The temperature parameters were not chosen randomly + 4 °C temperature of salting and ripening of meat, + 12 °C drying temperature, + 20 °C smoking temperature, + 40 °C hot smoking temperature, 80 °C cooking temperature that was chosen to assess the possibility of using starter cultures in the production technology of smoked and cooked smoked sausages.

According to the results obtained, it can be said that at all variants of the selected temperatures, except for the temperature of 80 °C, the titratable acidity of minced meat increases, which indicates the development of a consortium of microorganisms. At 80 °C, practically no acid formation was observed, which indicates the death of microorganisms.

IV. CONCLUSION

In the course of the work, the cultural and biochemical properties of microorganisms were studied: lactobacillus plantarum, lactobacillus casei, staphylococcus carnosus, bifidumbacterium siccum, bifidumbacterium bifidum, as well as their synergism on various nutrient media, including model minced meat. The laws of growth and changes in the biochemical properties of strains are established. The selection of strains for creating starter cultures for raw smoked sausages from low-value meat raw materials is justified.

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