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EFFECT OF BACTERIAL PREPARATION ON QUALITY PARAMETERS OF COOKED SAUSAGES WITH REDUCED SODIUM NITRITE CONTENT

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***Subject.** Technology of cooked sausages with reduced sodium nitrite content with the addition of the bacterial preparation Iprovit-Sc$_n$ based on starter cultures of Staphylococcus carnosus. **Purpose.** To investigate the effect of the bacterial preparation Iprovit-Sc$_n$ based on starter cultures of Staphylococcus carnosus on the quality indicators of cooked sausages with reduced sodium nitrite content. **Methods.** Samples of cooked sausages with reduced sodium nitrite content made with the addition of the bacterial preparation Iprovit-Sc$_n$ were the object of the study. The content of water, protein, fat (by weight) and pH were determined using known laboratory methods; water activity (a_w) – using an AquaLab 3 TE device; water-binding capacity – by pressing the test sample; the amino acid composition of proteins was studied after hydrolysis of product samples; the content of volatile fatty acids was determined by distillation from an acidified aqueous extract with live steam, followed by titration of the distillate; The content of heme pigments was assessed by the total amount of pigments, the content of nitrosomyoglobin and the color fastness; the content of sodium nitrite was determined by the reaction with N-1-naphthylethylenediamine dihydrochloride in an acidic medium to form diasocompounds, the color intensity of which was measured photometrically. **Results.** The quality parameters of cooked sausages with reduced sodium nitrite content produced with the addition of the bacterial preparation Iprovit-Sc$_n$ based on starter cultures of Staphylococcus carnosus with increased nitrite-reducing activity were studied compared to sausages produced using traditional technology. It was found that the use of the bacterial preparation in the test sample contributed to an increase in the total content of free amino acids by 6.8%, which improves the taste and aroma of cooked sausages. The greatest increase was observed in relation to valine (+7.8%), phenylalanine + tyrosine (+9.8%) and isoleucine (+10.8%), which play a key role in the formation of an intense meat flavor. Lysine (+8.1%) also showed a significant increase. Therefore, adding the bacterial preparation Iprovit-Sc$_n$ based on Staphylococcus starter cultures in the production of cooked sausages allows to reduce the mass fraction of added sodium nitrite from 5.0 mg/100 g to 2.5 mg/100 g, improves physical and chemical parameters, and forms a more intense and effective taste. **Scope of results.** A technology for the production of cooked sausages with a reduced content of sodium nitrite has been developed. The developed technology has been tested under industrial conditions.*

***Key words:** pH value, quality indicators, sodium nitrite, cooked sausages, bacterial preparation*

ВПЛИВ БАКТЕРІАЛЬНОГО ПРЕПАРАТУ НА ПОКАЗНИКИ ЯКОСТІ ВАРЕНИХ КОВБАС ЗІ ЗНИЖЕНИМ ВМІСТОМ НІТРИТУ НАТРІЮ

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***Предмет.** Технологія варених ковбас зі зниженим вмістом нітриту натрію з додаванням бактеріального препарату Іпровіт-Sc$_n$ на основі стартових культур Staphylococcus carnosus.*

Мета. *Дослідити вплив бактеріального препарату Інровіт-Sc_n на основі стартових культур Staphylococcus carnosus на показники якості варених ковбас зі зниженим вмістом нітриту натрію.* ***Методи.*** *Об'єктом досліджень слугували зразки варених ковбас зі зниженим вмістом нітриту натрію, виготовлених з додаванням бактеріального препарату Інровіт-Sc_n. Масову частку вологи, білка, жиру та значення pH визначали за допомогою відомих лабораторних методів; активність води (a_w) – за допомогою приладу AquaLab 3 TE; вологозв'язуючу здатність – методом пресування досліджуваної проби; амінокислотний склад білків досліджували після гідролізу зразків продукту; вміст летких жирних кислот визначали їх відгонкою з підкисленої водної витяжки гострою парою з наступним титруванням дистиляту; вміст гемових пігментів оцінювали за загальною кількістю пігментів, вмістом нітрозоміоглобіну та стійкістю забарвлення; вміст нітриту натрію визначали за реакцією з N-1-нафтилетилендіаміном дигідрохлориду в кислому середовищі з утворенням діазосполучень, інтенсивність забарвлення яких вимірювали фотометрично.* ***Результати.*** *Досліджено показники якості варених ковбас зі зниженим вмістом нітриту натрію виготовлених з додаванням бактеріального препарату Інровіт-Sc_n на основі стартових культур Staphylococcus carnosus з підвищеною нітритредукувальною активністю порівняно з ковбасами виготовлених за традиційною технологією. Встановлено, що використання бактеріального препарату у дослідному зразку сприяло збільшенню загального вмісту вільних амінокислот на 6,8%, що покращує смак та аромат варених ковбас. Найбільше зростання спостерігалося щодо валіну (+7,8%), фенілаланіну + тирозину (+9,8%) та ізолейцину (+10,8%), які відіграють ключову роль у формуванні інтенсивного м'ясного смаку. Лізин (+8,1%) також показав значне підвищення. Отже, додавання бактеріального препарату Інровіт-Sc_n на основі стартових культур Staphylococcus під час виготовлення варених ковбас дозволяє знизити масову частку доданого нітриту натрію з 5,0 мг/100 г до 2,5 мг/100 г, сприяє покращенню фізико-хімічних показників, формуванню інтенсивнішого кольору, смаку та аромату готового продукту.* ***Сфера застосування результатів.*** *Розроблено технологію на виробництво варених ковбас зі зниженим вмістом нітриту натрію. Розроблена технологія випробувана у промислових умовах.*

Ключові слова: *величина pH, показники якості, нітрит натрію, варені ковбаси, бактеріальний препарат*

Introduction. One of the most important tasks of the food industry, and the meat industry in particular, is to maintain the quality and safety of manufactured products, since meat and meat products are a valuable source of proteins, fats, vitamins, and minerals. Today, consumer demand is increasingly focused on obtaining food products of excellent quality, made from natural ingredients, safe, with attractive nutritional and sensory properties, and with a long shelf life. Animal protein is of great importance in the human diet due to its high biological and nutritional value [1]. Meat provides essential amino acids, as well as minerals and vitamins with high bioavailability. This product contains from 16.5 to 20% protein and is an excellent source of thiamine, riboflavin, niacin and other B vitamins. In addition, meat is a good source of essential fatty acids such as linoleic, linolenic and oleic [2-3]. The concept of "quality" combines a wide range of parameters characterizing a product, including the absence of substances harmful to human health that may enter the product from outside or form during technological processing [4].

Sodium nitrite ($NaNO_2$) is one of the sources of technological components in the meat processing industry. It is used to stabilize color, prevent lipid oxidation [5] and prevent the development of pathogenic microflora (in particular *Clostridium botulinum*) [6], as well as to form a characteristic taste and aroma [7].

However, sodium nitrite is a very toxic substance. Numerous studies indicate negative risks to human health (causes bowel cancer, chronic obstructive pulmonary disease) associated with high consumption of nitrites. The main threat is the formation of nitrosocompounds, among which the most dangerous are nitrosamines – compounds with a carcinogenic effect [8]. They can form in products during heat treatment or in the human gastrointestinal tract [9].

According to the recommendations of the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee, the permissible daily intake of nitrites should not exceed 0.06 mg / kg of body weight [10]. For example, if an adult weighing 60 kg consumes 60 g of sausage, his nitrate intake will be below his dosage (0.188 mg/kg body weight / day < 3.7 mg/kg body weight / day), but nitrite intake will be above the daily tolerable level (0.139 body weight /

kg / day). Therefore, in countries where the dosages for nitrites and nitrates exceed 0.06 mg/kg and 3.7 mg /kg body weight / day, respectively, more stringent regulatory restrictions are required. In this regard, the food industry is actively looking for alternative technologies that can reduce the use of sodium nitrite in meat products while maintaining their color stability, safety and sensorial properties.

According to the literature, the main strategies for the use of nitrites in meat products are based on their reduction or elimination and the replacement of synthetic nitrites with "natural" ones by adding nitrate-rich plant extracts.

A study was conducted on the gradual reduction of nitrite addition levels to minimize the likelihood of nitrosamine formation. Green leafy vegetables are a good source of nitrates, so they can be used as an alternative to synthetic nitrite [11]. For example, parsley (*Petroselinum crispum* Mill.) and spinach (*Spinacia oleracea* L.) contain high levels of nitrates (average content from 1000 mg/kg to 2500 mg/kg) [12].

Researchers also aimed to replace the effect of nitrites on color by optimizing the formation of natural myoglobin derivatives or using red ingredients for coloring nitrite-free meat products (e.g. beetroot and celery extracts).

Horsch et al. [13] noted that the addition of celery (in the form of a simple concentrate or a concentrate with added citric acid) did not affect the redness of the product; the redness value was similar to that of the samples with the addition of sodium nitrite. However, they recorded changes in the parameters of lightness and yellowness with an increase in the amount of celery concentrate – the pork ham was more yellow and darker than the control sample. In another study, the authors compared the effect of young radish and industrial vegetable powder (250 g / kg) on various parameters of cooked pork sausage. According to their results, the powders created darker and less red products than sausage with the addition of sodium nitrite.

It has been shown that the use of celery powder alone or together with synthetic nitrite in the production of heat-treated cloth does not reduce the risk of nitrosamines. The content of N-nitrosopiperidine increases with an increase in the ratio of celery powder. Furthermore, this increase becomes higher as the cooking time increases [14].

Sucu and Turp [15] observed an increase in the redness parameter values of sujuk when beetroot extract (*Beta vulgaris*) was added. They obtained the highest values in a sample containing 0.35% beetroot extract. A similar study was carried out by Perko T. The aim of her study was to produce hot dog sausages with a modified meat emulsion composition and to compare their sensory properties and microbiological stability. For this purpose, three meat emulsion samples were prepared. The control sample (sample 1) was prepared according to a traditional recipe from beef and organic ingredients. Beetroot powder was added to the second emulsion (sample 2). In the third emulsion (sample 3), half of the iodized sea salt was replaced by salt with nitrite. The products were analyzed for total microorganism counts on days 1, 7, 14, and 21 of storage. The sensory quality of the hot dogs produced was also assessed. [16].

One of the methods recommended for reducing the concentration of nitrites in products was the use of protein preparations produced on the basis of the blood of slaughtered animals. This method has been widely used [17].

Ukrainian scientists have considered the possibility of reducing the content of sodium nitrite by increasing the amount of color-forming pigment in cooked sausages using the blood of slaughtered animals. The effect of added blood of slaughtered animals in combination with sodium nitrite on the microbiological stability of cooked sausages and their shelf life has been studied [18].

To minimize the amount of nitrosamines formed, it was proposed to reduce the content of sodium nitrite by introducing the antioxidant ascorbic acid (vitamin C) into the product, for which purpose the standard for its addition in the USA was introduced at 0.055% (550 mg/kg). A similar effect is achieved by introducing α -tocopherol (vitamin E) into food products [19].

The most promising solution for reducing sodium nitrite content is a biotechnological approach that uses bacterial preparations with different functions, consisting of several different strains and types of microorganisms, the combined action of which has a more effective synergistic, preventive and metabolic effect compared to the use of any one. Bacterial preparations may include lactobacilli responsible for reducing pH, color formation, formation of aromatic components, staphylococci and micrococci, molds, yeast [20, 21].

All these microorganisms are capable of reducing nitrites when creating a favorable acidity of the environment [22]. For this purpose, lactic acid bacteria or their mixtures with bacteria of other taxonomic groups are additionally introduced into the starter cultures. Among the options used for fermentation of meat systems, a mixture of microorganisms of the strains *Staphylococcus carnosus, Lactobacillus sakei* and *Staphylococcus xylosus* is most often used [23].

Proper use of Staphylococcus strains in the meat processing process can be useful in reducing the amount of sodium nitrite added to thermally processed meat products by reducing nitrates formed from oxidized nitrite [24]. They also have proteolytic and lipolytic activity responsible for the formation of low molecular weight compounds including peptides, amino acids, aldehydes, amines and free fatty acids that contribute to the flavor development of fermented meat products. In addition, they have antioxidant enzymes including catalase and superoxide dismutase (SOD), which reduce the formation of undesirable compounds such as superoxide radicals (O^{2-}) and hydrogen peroxide, precursors of oxidative rancidity in food.

Cooked sausages are one of the most widely consumed categories of meat products. Along with the stable demand in the market, an important factor for including cooked sausages in the production program of meat processing enterprises is their technological acceptability, i.e. the possibility of using a combination of composition and the use of new ingredients without deteriorating the quality and food safety of the product [25]. When producing cooked sausages, it is important to ensure:

- uniform pink color, which is traditionally formed due to the interaction of sodium nitrite with the muscle pigment myoglobin [26];
- microbiological safety, especially with respect to anaerobic bacteria that can multiply under heat treatment and storage conditions;
- flavor and aroma characteristics, largely dependent on fermentation processes, the formation of nitrosopigments and reactions with proteins and lipids [27].

If the level of sodium nitrite in cooked sausages decreases, the product may have a gray or pale tint, sodium nitrite will not inhibit the development of many pathogenic bacteria, and the amount of volatile fatty compounds that affect the taste and aroma of the finished product will decrease [28].

With the active study of the effect of bacterial preparations on the quality indicators of meat products, their use in cooked sausages with a reduced content of sodium nitrite remains insufficiently studied, and accordingly, requires more detailed study.

Thus, our study is an assessment of the effectiveness of using the bacterial preparation Iprovit-Sc_n based on *Staphylococcus carnosus* in cooked sausages with a reduced content of sodium nitrite and determining its effect on the quality characteristics of the finished product. This study is aimed at developing a safe and high-quality alternative to traditional methods of processing cooked sausages, which meets modern requirements for food safety and healthy eating.

The purpose of the work is to study the effect of the bacterial preparation Iprovit-Sc_n based on starter cultures *of Staphylococcus carnosus* on the quality indicators of cooked sausages with a reduced content of sodium nitrite.

Materials and methods. The objects of the research were samples of cooked sausages manufactured in accordance with DSTU 4436:2005 [29] using traditional technology with a mass fraction of $NaNO_2$ of 5 mg / 100 g (Control), as well as samples with a reduced content of sodium nitrite with a mass fraction of $NaNO_2$ of 2.5 mg / 100 g, made from premium beef and semi-fat pork mince at the stage of salting the bacterial preparation Iprovit-Sc_n (Experiment). The water content was determined by drying the sample to constant mass at a temperature of 105ºC; the content of protein was determined by the content of total nitrogen according to Kjeldahl with subsequent distillation of ammonia; the content of fat – by the Soxhlet method; pH – potentiometrically; water activity (a_w) – using an AquaLab 3 TE device with a measurement accuracy of up to ±0.003; moisture-binding capacity – by pressing the test sample; the amino acid composition of proteins was studied after hydrolysis of the product samples; the content of volatile fatty acids was determined by their distillation from an acidified aqueous extract with live steam, followed by titration of the distillate; The content of heme pigments was assessed by the total amount of pigments, the content of nitrosomyoglobin and the color fastness; the content of sodium nitrite was determined by the reaction with N-1-naphthylethylenediamine dihydrochloride in an acidic medium with the formation of diasocompounds [30], the color intensity of which was measured photometrically on a UNICO 2100 spectrophotometer.

Research results. Physical and chemical parameters are critical for determining the quality and safety of meat products, as they affect the sensorial characteristics (taste, aroma, texture) and the preservation of the useful properties of the product during its shelf life. In the case of cooked sausages, such parameters as moisture content, fat, protein, moisture-binding capacity, active acidity and water activity (aw) are important.

The results of the study of the physical and chemical composition of the control and experimental samples of cooked sausages are presented in Table 1.

Table 1

Physical and chemical parameters of cooked sausages

Parameter	Control	Experiment
Water content,%	63.8	64.2
Protein content,%	14.8	14.6
Fat content,%	17.5	17.2
Ash,%	2.4	2.3
Water binding capacity,%	58.3	60.1

Analyzing the results (Table 1), it was determined that the experimental sample had a slightly higher moisture content (+0.4%), which may be due to the influence of the *Staphylococcus carnosus* starter cultures on the water holding properties of proteins. The chemical composition of cooked sausages is characterized by a balanced protein-fat composition and meets the requirements of this group of products.

Water holding capacity determines the ability of sausage to retain water, which directly affects its texture, juiciness and resistance to losses during heat treatment. A higher level of water holding capacity (60.1% versus 58.3%) confirms the positive effect of the starter culture on the structural characteristics of the product.

The pH value is an indicator of the activity of physical and chemical processes occurring in meat during its technological processing. The use of starter cultures allows for a targeted influence on the course of the noted processes, causing, under certain conditions, an increase in the quality indicators of finished products.

The results of studies of changes in active acidity (pH) and water activity (a_w) at different stages of the production of cooked sausages: before salting, after salting and in the finished product are shown in Figure 1. From Fig. 1 it is evident that in the experimental samples the pH value throughout the production process is somewhat lower compared to the control sample, which is explained by the result of the vital activity of staphylococci.

Similarly to the change in pH, the water activity index changed (Fig. 1). Analyzing the results of water activity in both samples of cooked sausages, a decrease in a_w was noted, however, in the experimental sample this occurred more intensively compared to the control and in the finished product it reached 0.938, which shows a balanced water state of the product.

It is known that the use of bacterial preparations in salting meat products allows creating optimal conditions for the process of color formation with maximum recovery of nitrites, which is necessary for the production of products with a reduced content of sodium nitrite.

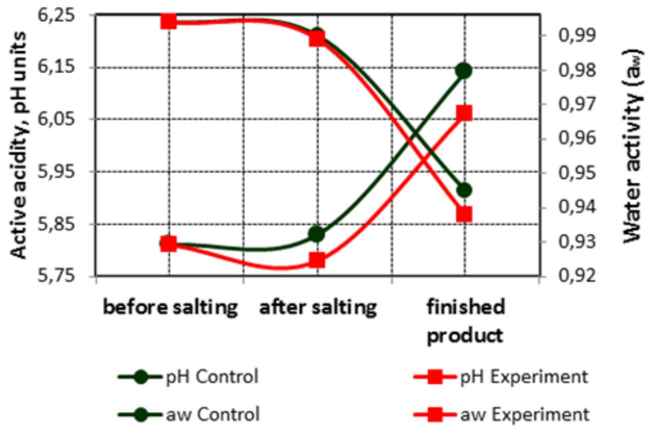

Fig. 1. Effect of starter cultures on changes in pH and aw of cooked sausages during production

Ensuring a stable color can be achieved by creating appropriate conditions in minced meat with the help of beneficial microflora, using starter cultures with denitrifying activity.

Among the most important biochemical processes in the manufacture of meat products is the formation of nitrosopigments. These are compounds that are formed under the influence of sodium nitrite and give the product a characteristic red color. The course of the color formation reaction can be tracked by changes in the total amount of pigments, nitrosopigments and residual sodium nitrite content (Table 2)

Table 2

Color characteristics of cooked sausages

Parameter	Sample	
	Control	Experiment
Total amount of pigments (optical density)	0.166	0.172
Content of nitroso pigments,%	81.3	82.5
Color fastness (optical density)	0.92	0.89
Residual sodium nitrite,%	2.65	0.70

Amid a weakly acidic reaction, under the influence of bacterial nitrite reductase, sodium nitrite is reduced to nitric oxide, which then reacts with meat pigments to form stable nitro-somyoglobin. As can be seen from Table 2, the content of nitrosopigments mg/100 g is almost at the same level as their content in the control, which did not affect their color fastness.

It should be noted that reducing the corresponding amount of sodium nitrite, which was added to the minced meat of cooked sausages, made it possible to significantly reduce its residual level. Thus, in the experimental sample, the residual content of sodium nitrite in the finished product was lower than the control by 74%.

One of the key aspects of the quality of cooked sausages is their flavor and aroma properties, which are formed due to volatile fatty acids and free amino acids (Table 3). The study analyzed the effect of the bacterial preparation Iprovit-Sc_n on the formation of these indicators in cooked sausages.

Table 3

Free amino acid content in cooked sausages

Amino acid	Sample	
	Control	Experiment
Leucine	8.0	8.2
Methionine + cystine	4.1	4.4
Valin	5.1	5.5
Lysine	6.2	6.7
Threonine	4.3	4.3
Phenylalanine+ tyrosine	6.1	6.7
Tryptophan	1.1	1.1
Isoleucine	4.6	5.1
Total	39.5	42.2

The content of volatile fatty acids (VFA) in cooked sausages of the control and experimental samples differed significantly from each other.

The control sample demonstrated a lower level of accumulation of volatile fatty acids and amounted to 9.43 mg / 100 g. This indicates a lower intensity of biochemical processes affecting the formation of taste and aroma.

The experimental sample was characterized by a higher VFA content and amounted to 11.95 mg / 100 g, which may be a consequence of the use of a bacterial preparation, which probably contributes to a more active breakdown of lipids and amino acids, which leads to the formation of a more intense taste and aroma of sausage and has a positive effect.Analyzing the results of the studies (Table 3), it was determined that the use of the bacterial preparation in the experimental sample contributed to an increase in the total content of free amino acids by 6.8%, which improves the taste and aroma of cooked sausages.

The greatest increase was observed in valine (+7.8%), phenylalanine + tyrosine (+9.8%) and isoleucine (+10.8%), which play a key role in the formation of an intense meat taste. Lysine (+8.1%) also showed a significant increase, which also contributes to the richness of the taste.

Based on the results of the studies, a technology was developed for the production of cooked sausages with a reduced content of sodium nitrite. The developed technology was tested under industrial conditions.

Conclusion. Therefore, the above results allow us to state that the addition of the bacterial preparation Iprovit-Sc_n based on *Staphylococcus* starter cultures in the production of cooked sausages allows reducing the content of added sodium nitrite from 5.0 mg/100 g to 2.5 mg/100 g. The obtained results indicate the possibility of optimizing the technological process and improving the sensorial, physical and chemical characteristics of the finished product. The use of the bacterial preparation in the production of cooked sausages helps to intensify biochemical processes, which improves the quality characteristics of the sausage and increases its consumer properties.

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