Investigation of the influence of starter cultures on the acceleration ripening period finished meat products

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Abstract. The article presents the results investigation of the influence of starter cultures on the acceleration ripening period finished meat products and microstructure of the finished sausage. Established that the preliminary processing of raw materials by starter cultures (lactic acid and propionic acid microorganisms) in a ratio of 1:1 at a higher temperature, contributes to the intensification of the technological process. Microstructural studies show that the treatment of meat products with starter cultures leads to significant destructive changes, due to the synthesis of lipolytic and proteolytic enzymes, providing an effective softening effect. Introduction in experimental samples of plant raw materials, well balanced in carbohydrate and vitamin composition, has a positive effect on the balance of the finished product and increases their biological value. The obtained data present promising direction in the development of meat technology.

Key words. Meat, beef, lamb, sausages, microstructure, lactic acid bacteria, propionic acid bacteria, starter cultures, cereals, corn, lipase.

1. Introduction

Meat products are in high consumer demand. Reducing their cost is the most important condition for expanding the range and increasing the output of this type of product. Currently, one of the ways to solve this problem is the development and introduction of new technologies aimed at intensifying of complex biochemical transformations that occur in meat raw materials during its salting, ripening and precipitation in the production of sausages. One of the ways to solve such a problem is related to the biotechnological principle of meat raw material modification

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- directed regulation of the biotechnological, physical-chemical and microbiological processes, as a result of which the structure, color and flavor characteristics of the finished product are formed [1, 2].

The main factor for increasing the competitiveness of domestic meat products are highly efficient meat processing technologies.

Lamb and beef are one of the main types of raw materials in the production of food products for the population of Kazakhstan. For the rational use of lamb and beef currently provides for the establishment of the restructured meat products. The process of restructuring consists in combining individual pieces of meat into one monolithic piece, which, when cut into slices, will have a uniform shape and size. This process promotes rapid softening of the raw material during ripening, as well as giving the product a specific taste and aroma [3, 4]. It is proved that starting cultures change the structure of sausages, forming new substances that contribute to improving the quality of the product [5].

These microorganisms are able to inhibit undesirable microflora by synthesizing various antibacterial metabolites, such as organic acids, carbon dioxide, hydrogen peroxide, diacetyl and bacteriocins [6, 7]. The available data on the use of lactic acid and propionic acid bacteria in the meat processing industry indicate the possibility of their use in the technology of production of meat products in order to increase production by reducing the time of the ripening process with raw material salting, reducing production costs, and improving the quality of finished products and increasing their yield [5, 8]. As a result of these starter cultures, intensive accumulation of volatile fatty acids and amine nitrogen is observed, which contributes to the formation of a specific taste and aroma of the finished product. When enriching the meat product is widely used in the diet of new types of plant raw materials. Studies of Kazakh and foreign authors have shown the promise of using in the technology of combined meat products of grain processing products that provide high nutritional and biological value of the product, contribute to improving the balance of formulations, the stable and uniform distribution of ingredients, minimizing losses in the production process, which ultimately leads to creating a product of stable quality. To increase the amount of antioxidants in plant raw materials, the method of germination of grains. The use of germinated grains is considered to be very timely and timely. At the same time, enrichment of production with food fibers, minerals, vitamins.

The method of histological analysis is widely used in biology and medicine. However, in this case, the meat is subjected to research after thermal and other types of technological impact. Histological studies are used to determine the effect of starter cultures on the muscle and connective tissues of finished meat products. In connection with the above, a study of the influence of starter cultures on the acceleration of the maturation of meat and the study of the microstructure of ready sausages is urgent.

2. Materials and methods

The objects of research were ready meat products - restructured semi-smoked meat products. The formulation of the control and test samples is given in Table 1.

Composition of the control sample		Prototype composition		
The name of raw materials and spices, kg per 100 kg				
Lamb	51.0	Lamb	51.0	
Beef	49.0	Beef	49.0	
Sodium nitrite	0.0075	Sodium nitrite	0.0075	
Salt	3	Salt	3	
		Granulated sugar	0.12	
Granulated sugar	3	Bacterial starters	0.05	
		Corn germ	1	

Table 1. Formulation of control and prototype

2.1. Technological process

- 2.1.1. Preparation of a control sample. Comminuted meat raw materials on the gyroscope with a hole diameter of 25–35 mm grating. Further, the dry salt was made by table salt at the rate of $100\,\mathrm{kg}$ of raw material and $3.0\,\mathrm{kg}$ of salt. Stirring was carried out in a minced stirrer for 5–10 minutes. Then the raw material was left in the refrigerator for $20\,\mathrm{h}$ at $+40\,^{\circ}\mathrm{C}$. After 20 hours, the raw materials were fed into a stirrer, the necessary spices were added according to the formulation. Stir until cooked for $10\,\mathrm{minutes}$. Finished meat raw materials are injected into the shells.
- 2.1.2. Preparation of a prototype. Comminuted meat raw materials on the gyroscope with a hole diameter of 25–35 mm grating. In order to activate the growth of propionic acid bacteria Propionibacterium freudenreichii and lactic acid bacteria Leuconostoc lactis in the ratio 1:1 in meat, a preliminary exposure of the meat chopped into slices was provided at a temperature of $(20\pm2)^{\circ}$ for 4 hours at the selected dose of fermentation of 5 units of activity. After holding the crushed meat in a liquid bacterial culture, vegetable raw materials were added at a ratio of 1% and the above mentioned spices.

In the course of the experimental studies, the following indices were determined: the pH value by the potentiometric method in accordance with State Standard 3624-87. Quantitative counting of cells of starting cultures was made by the method of limiting dilutions on the medium according to Technical requirement 10-02-789-192-95 and histological examination according to State Standard 31479-2012.

Studies on the determination of pH and the number of starter cultures were carried out in the Accredited Test Laboratory of Food Safety of the Scientific Research Institute at the Almaty Technological University. Histological studies were conducted in the "Federal Scientific Center of Food Systems. V. M. Gorbatov " RAS, Moscow.

3. Results and discussion

One of the promising areas should be recognized the creation and use for the production of meat products of biologically active substances on the basis of the products of vital activity of microorganisms. When using microorganisms in the technology of meat products, their biochemical activity, resistance to sodium chloride, sodium nitrite and acid-forming capacity, which is important in regulating the pH of the medium during the ripening of meat.

Consistency of meat products, among other factors, depends on the action of muscle proteins (sarcoplasmic and myofibrillar). The more proteolysis develops in the meat product, the more tender it becomes. Bacterial cultures affect the consistency due to their proteolytic activity and through lowering the pH. When the pH of meat is lowered to values equal to the isoelectric point of sarcoplasmic proteins, the latter precipitate, releasing water, which contributes to the formation of a good consistency of the product. With inoculation with microorganisms, the decrease in pH occurs faster, which also leads to a faster development of the corresponding consistency. In the process of manufacturing a number of meat products, pH control is necessary for many reasons. For processes of solidification of sausage stuffing, a low pH value is very important. At low pH about 5.2–5.3, swelling of collagen, hydrolysis of intermolecular bonds and activation of cellular enzymes, in particular cathepsins, whose optimal pH value is 3.8–4.5. In addition, a rapid and continuous reduction in the pH of minced meat to 5.2–5.4 suppresses the development in it of pathogenic and toxicogenic bacteria [5].

In the study of starter cultures, lactic acid bacteria and propionic revived in whey at $T=30\,^{\circ}\mathrm{C}$ for 20–24 hours. According to the experiments conducted by culturing for 24 hours the number of bacteria was $10^6~\mathrm{CFU/g}$ at 5 unit of applying ferment activity.

Given that strains of Propionibacterium freudenreichii develop at a temperature of 30–35 °C and neutral acidity (pH about 6–7), and strains of Leuconostoc lactis at a temperature of 22–30 °C and acid medium (pH about 5–6), further studies were aimed at studying biochemical activity of starter cultures.

In order to activate the growth of starter cultures in meat, preliminarily holding the meat chopped into slices was provided at a higher temperature (20 ± 2) °C for 4 hours at the selected dose of starter culture application.

The activity of starter cultures was assessed by the reaction medium and the change of content of viable cells. In Table 2 and Fig. 1, the data presented indicate that when the meat is ground into slices, stable growth of viable cells of starter culture is observed which added 5 units of activity. In this case, the pH varies insignificantly.

Thus, the increase in temperature increases the biochemical activity of the introduced microorganisms and after 4 hours of fermentation the number of starting cultures is $107\,\mathrm{CFU/g}$.

As a result of carbohydrate metabolism of microorganisms are formed products that participate in the formation of aroma. The pyruvic, acetic acids, ethyl alcohol, acetoin and other substances formed along with lactic and propionic acid give the raw material and subsequently the meat product the taste and aroma.

Table 1. Quantitative account starter cultures of cells and pH change during preincubation meat

Investigated indicators	Preincubation time (h)	Number of bacteria (CFU/g)	рН
Starter cultures (Propionibacterium freudenreichii and Leuconostoc lactis in the ratio 1:1)	1	2×10^6	6.5
	2	5×10^6	6.4
	3	8×10^6	6.1
	4	1×10^7	5.8

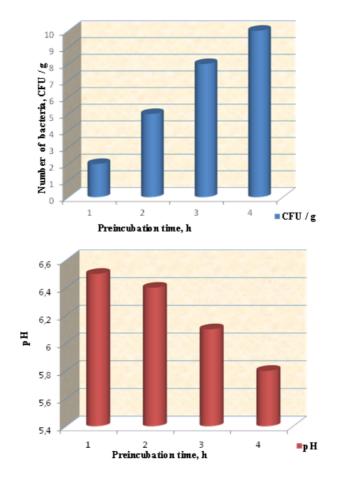


Fig. 1. Quantitative account starter cultures of cells (top) and pH change during preincubation meat (bottom)

An important role in the formation of aroma belongs to the products of the splitting of fats: free fatty acids and carbonyl compounds. It has been proved that

the bacteria of the genus Leuconostoc have the ability to produce lipases involved in this process. Scientifically proven that the enzymes can significantly accelerate processes, increase the output of finished products, improve its quality and are able to change the destructive functions of meat. However, the role of lipases in relation to the above characteristics has been little studied. Therefore, further studies were aimed at studying the effect of lipolytic and proteolytic enzymes of starter cultures on the microstructure of prepared sausages.

In a microstructural study, it was found that the mass of a control sample of a meat product is formed by large fragments of muscle, connective and adipose tissue $(0.7\text{--}1.4)\,\mu\text{m}$ (Fig. 2). In the studied samples of meat products, the muscle fibers are straight, swollen, closely adhered to each other, often fragmented. Between the coarse-grained structural elements of the contents, a fine-grained protein mass is distributed, formed as a result of mechanical action on the muscle tissue during the grinding of meat raw materials. In fine-grained protein mass, spice particles, fat droplets with a size $(12\text{--}100)\,\mu\text{m}$, uniformly distributed over the sample mass, are detected. The superficial coagulation layer adheres tightly to the shell. The muscle fibers in the bunches that have retained their integrity are closely adhered to each other, swollen, the boundaries between them are difficult to distinguish. The transverse striation is wide, retained in separate fibers, in the bulk of the muscle fibers. Structure is homogeneous, marked disintegration of myofibrils violation orderly arrangement with respect to each other. The fibers are homogeneous. Destructive changes are revealed in the form of separate microcracks.

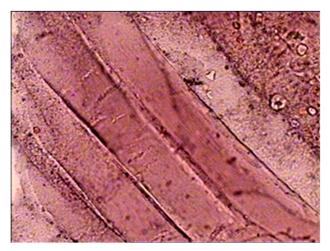


Fig. 2. Enlargement \times 340: microstructure of a control sample of a semi-smoked sausage

In experimental samples, between the coarse-grained structural components of the sample is a fine-grained proteinaceous mass, which includes in its composition fragments of cornmeal, spice particles, plant components. The layout of the sample is dense, the presence of large slits or cavities loosening the sample mass is not revealed, the structural components of the meat are closely related. The fine-grained protein

mass is permeated with microcapsules of round shape with a size of $250-350\,\mu m$ (Fig. 3). Fragments of muscle tissue that retained their microstructural features were characterized by swollen muscle fibers, the boundaries between them are poorly distinguishable, transverse striation is weakened or not found in individual sections of the sample.

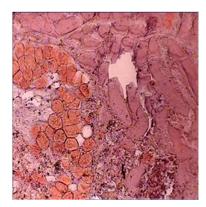




Fig. 3. Enlargement \times 240: microstructure of a prototype of a semi-smoked sausage

Destructive changes are of a multiple nature, the degree of destruction of fibers is higher compared to control samples.

The fiber cores are homogeneous or shady. The microflora is found in a fine-grained protein mass in the form of small microcolonies measuring (0.2–0.3) μm , and between the fibers, under the sarcolemma, in the areas of fiber destruction and connective tissue interlayers - is predominantly diffuse. The arrangement of the structural elements of the contents is dense, vacuoles with clearly delineated boundaries, sometimes merged with each other, in size 70–300 μm . Analyzing the obtained data, it should be noted that the directed use of starter cultures allows to accelerate destructive changes in the basic structural elements of meat, and, consequently, its secondary structure formation. Sausages produced using starter cultures are characterized by a greater degree of swelling and destruction of muscle fibers. Destructive changes cover a significant part of the fibers and are detected in the form of multiple decays of the myofibrillar substance to a fine-grained protein mass.

An intensive formation of fine grain protein mass promotes formation of a compact monolithic mass of meat pieces after the heat treatment forming dense space frame. Increasing the compactness of pieces of meat in relation to sausages, produced by traditional technology, is expressed in a decrease in its porosity, adecrease in the size and number of vacuoles.

It could be argued that treatment of meat products starter cultures leads to substantial destructive changes due to the synthesis of lipolytic and proteolytic enzymes providing effective softening effect on the basis of histological studies.

4. Conclusion

Based on the research carried out to study the influence of starter cultures on the acceleration of the maturation of meat and the study of the microstructure of finished sausages, the following technological solutions:

- use of starter cultures, namely pure cultures of lactic acid and propionic acid microorganisms in the ratio 1:1, with a selected dose of starter 5 units of activity, contributes to the intensification of physicochemical and biochemical processes in the salting, ripening of meat and the formation of optimal functional and technological properties in more short time;
- the feasibility of using plant raw materials, namely 1% of crushed corn germinated when creating restructured semi-smoked meat products;
- processing of sausage products with starter cultures leads to a change in the microstructure of meat products.

Thus, a high biochemical activity of bacteria was revealed, which contributes to the intensification of the technological process. Microstructural studies show that treatment of meat products with starter cultures destructively changes a significant portion of the fibers, which are detected as multiple decompositions of the myofibrillar substance to a fine-grained protein mass. These destructive changes provide an effective softening effect due to the synthesis of lipolytic and proteolytic enzymes.

References

- [1] Y. M. UZAKOV, O. V. CHERKASOV, V. V. PRYANISHNIKOV: Functional products from scientific theory to practical implementation. Almaty (2017), p. 408.
- [2] Y. M. Uzakov: Slaughter and production of meat products using the Halal technology. Almaty: "Evero" (2014), p. 268.
- [3] Y. S. Khamagaeva, Y. A. Khankhalaeva, L. Y. Zaigraev: Use of probiotic cultures for the production of sausages. Ulan-Ude: Izdatelstvo VSGTU (In Russian) (2006), p.,204.
- [4] F. Leroy, L. de Vuyst: Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science & Technology 15 (2004), No. 2, 67–78
- [5] I. S. KHVYLYA, V. A. PCHELKINA, S. S. BURLAKOVA: Standardized histological methods for assessing the quality of meat and meat products. All about meat (2011), No. 6, 32–36.
- [6] V. V. PRYANISHNIKOV: Properties and application of plant raw materials in the technology of meat products. Voronezh (2007), p. 50.
- [7] I. S. Khamagaeva, I. V. Khamaganova, N. V. Darbakova, N. A. Zambalova: Influence of the culture liquid of propionic acid bacteria on the quality of sausages. All about meat (2011), No. 5, p. 37–39.
- [8] All about meat: Trends in the use of biologically active substances of microbial origin in the production of meat products. All about meat (2000), No. 2, p. 16.