Biotechnological potential of new strains of lactic acid bacteria

S V Kitaevskaya¹, V Y Ponomarev², A F Hasanova² and N K Romanova¹

¹Department of food production technology, Kazan National Research Technological University, 68 Karl Marx str., Kazan, Republic of Tatarstan, 420015, Russia
²Department of technology of meat and dairy products, Kazan National Research Technological University, 68 Karl Marx str., Kazan, Republic of Tatarstan, 420015, Russia

E-mail: kitaevskayas@mail.ru

Abstract. The paper presents data on the ferment effect of acid bacteria on model protein substrates in order to determine their proteolytic potential for biotransformation of fresh meat. The proteolytic activity of new starter cultures of lactic acid bacteria was evaluated. Strains of lactic acid bacteria Lbm. casei and Lbm. curvatus, exhibiting high proteolytic activity, can be recommended for inclusion in the composition of starter cultures.

1. Introduction
According to the traditional technology for the production of raw smoked and dry-cured products, lactic microorganisms play an increasing role in the formation of the qualitative characteristics of the finished product. Their paramount importance was established at the beginning of the twentieth century and further studies only confirmed and expanded information about the biochemical processes that occur in fresh meat in fermentation [1].

The investigation of the main microflora of minced and uncooked smoked dry-cured sausages sausage mincemeat showed that it is mainly represented by lactic acid bacteria and further investigation were aimed at searching and subtracting psychrophilic lactic acid microorganisms. The representatives of lactobacilli such as Lactobacillus plantarum, Lactobacillus brevis and Lactobacillus fermenti were among the first microorganisms that were widely used in the industrial production of meat products. Later, atypical lactic acid bacteria Lactobacillus sake (Lactobacillus sakei) and Lactobacillus curvatus were evolved. Their concurrent use with typical lactobacilli, significantly accelerated the aging of raw smoked and uncooked sausages, and also improved the quality of the fermented meat products [2].

These microorganisms have enzyme systems producing catalase and nitrite reductase. It helps more efficient breakdown of sodium nitrite to nitric oxide, which in turn has a positive effect on the color formation of the finished product. Also, these strains showed significant antagonistic activity against putrefactive microflora and pathogens [3].

Lactobacillus bacilli Lactobacillus acidophilus, Lactobacillus bulgaricus, used in most starter cultures, are of the greatest industrial importance. Their distinctive feature is resistance to acids and salts, a wide temperature ranges within which they are able to develop [2]. A peculiarity of lactic acid rods is also the presence of the concrete enzymatic proteolytic system. As a result, they are able to transfer up to 30% of casein to a soluble state [4]. The presence of proteolytic activity is the most
important factor determining the quality of the finished product. Free amino acids generated in the process of enzymatic hydrolysis participate in the formation of the taste of sausages, and also affect the biological value. The spectrum of accumulated amino acids depends on the species structure of the starting cultures and it is determined by the fact that a certain set of amino acids is necessary for the life of lactic acid microorganisms. Regardless of the species, lactic acid bacteria accumulate such amino acids as valine, arginine, leucine, glutamic acid and proline. Some strains accumulate aspartic acid, leucine, lysine, etc. Therefore, by varying the species structure of the starter culture, it is possible to ensure the accumulation of a certain set of amino acids in the finished product, which affects the taste and aroma of the finished product [5].

The presence of micrococci with lipolytic activity in the starter culture promotes the accumulation of low molecular weight fatty acids that oxidize to peroxides and subsequently, under the action of catalases, which turn into carboxyl compounds. They also affect the taste formation of the product [6].

Thus, the technological effect of microorganisms included into the starter cultures is due to the formation of biologically active components that contribute to improving the sanitary-microbiological and organoleptic characteristics of the finished product and intensifying the processes of aging and drying [7].

Despite a significant amount of work devoted to the proteolytic activity of lactic acid bacteria, the criteria for selecting strains for this indicator for the consortium of starter cultures have not yet been determined. Currently, only for the production technology of cheeses with a high temperature drying [7].

The aim of this work was to estimate the biotechnological potential of promising strains of lactic acid bacteria according to the level of their proteolytic activity to establish the possibility of their use for the production of raw smoked and uncooked sausages with prolonged period of storing.

2. Materials and methods

The objects of the study are new starter cultures containing valuable, from industrial point, strains of lactic acid bacteria of the p. Lactobacterium: Lbm. fermentum, Lbm. plantarum, Lmb. casei, Lmb. curvatus, having a complex of high biotechnological properties [9, 10].

The characteristics of the starter cultures are presented in Table 1. All the selected starter cultures have an optimum growth temperature in the range of 30-32 °C, of 15-40 °C. They perform mainly homofermentative lactic acid fermentation with the exception of Lactobacterium fermentum strains.

Starter cultures of lactic acid bacteria were introduced as 1 ml of overnight culture and kept for 30 minutes. To obtain an overnight culture, cells of microorganisms were grown in MRS medium at the temperature of 37 °C for 12 hours.

Samples of model-and-minced systems of premium beef were also developed for the research. Meat was minced in a laboratory meat grinder with a grill diameter of 4 mm. Lactic starter cultures in the amount of 10^7 were added to the resulting stuffing and mixed thoroughly.

| Stains             | Shape and size of colonies | Growth temperature°C | Type of fermentation                       |
|--------------------|---------------------------|----------------------|--------------------------------------------|
| Lactobacterium plantarum | rods (4.5 × 0.6-1) μm | Average, dome-shaped, whitish | 30-32 | 15-40 | Homo-fermentative lactic acid bacteria |
| Lactobacterium curvatus | rods (1-1.2 × 4.5) μm | Small, rounded, white | 30-32 | 15-40 | Homo-fermentative lactic acid bacteria |

Table 1. Characteristics of starter cultures.
The investigation of the starter cultures of lactic acid bacteria was carried out by the absorption spectra registration in the wavelength range of 220-280 nm according to the method of Warburg and Christian [11] and also according to the method [12].

3. Results and discussion
The studies for the effect evaluation of the microorganism enzyme systems on model substrates were conducted to identify the proteolytic potential of the considered microorganism strains.

Enzymatic hydrolysis products were registered by sedimentation of the substrate after fermentation with ATC starter cultures. It allowed the precipitation of high molecular weight proteins and peptides. It should be noted that ATC precipitates only proteins and does not precipitate the products of their hydrolysis, which is important for the protein and non-protein nitrogen determination [12].

All the starter cultures under consideration have an intense proteolytic effect on the model substrate containing 1% solution of serum albumin. However, the effect of proteolytic influence is not the same for each type of leaven. The greatest effect of reducing the protein background registered in the wavelength range of 230-300 nm was observed for starter cultures Lmb. casei and Lmb. fermentum. For these samples, an intense accumulation of hydrolysis products in the wavelength range of 220-280 nm was registered; it is typical for such amino acids as tryptophan, tyrosine; phenylalanine [11]. For strains of Lbm. plantarum and Lmb. curvatus this effect was less advanced.

The investigation of the lactic acid starter cultures effect on the protein content in experimental samples of model minced systems showed that during the fermentation process an intensive decrease in the protein content was observed relative to the control values, with the most advanced changes of Lmb. casei and Lmb. curvatus characteristic. For these cultures of lactic acid bacteria, a degree of hydrolysis was 65% and 72%, respectively (figure 1).

![Figure 1. Hydrolysis degree of the model minced systems.](image-url)
4. Conclusion
The results of studies on the starter cultures’ effect of lactic acid bacteria on the protein content in model protein systems indicate a high proteolytic ability of Lbm. casei and Lbm. curvatus cultures. These strains of microorganisms effectively affect not only model protein substrates, but also protein complexes of fresh meat.

The obtained results concerning the study of the lactic acid bacteria proteolytic activity in the future will serve to develop a consortium of new starter cultures based on lactobacilli for fermented meat products with functional purposes.

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