

## Study of the Influence of Lactic Acid Bacteria on Hydrolytic and Oxidation Processes in Stuffed Meat

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### Abstract

A significant problem in maintaining the quality of fermented meat products is the instability of their lipid fraction during storage. In meat products and semi-finished products, the fat content can reach 30 - 40%, and therefore, during long-term storage of such products, hydrolytic and oxidative processes in the lipid component are almost inevitable. The substances formed during the oxidation of lipids not only worsen the quality characteristics of meat products, but also can cause significant harm to human health.

The leading role in the creation of fermented meat products is played by starter cultures of lactic acid bacteria, however, at present, their effect on the hydrolytic and oxidative processes of meat lipids is not well understood.

In order to create safe meat products developed on the basis of lactic acid fermentation of raw materials, it seems advisable to select and include in the consortium of starter cultures of such cultures of lactic acid bacteria that would effectively suppress the processes of damage to meat lipids during storage.

The lipolytic activity of new strains of lactic acid bacteria exhibiting high functional and technological properties was studied. It was revealed that strains of *Lbm. casei*, *Lbm. acidophilum*, *Lmb. bavaricus* do not have lipolytic activity. High lipolytic activity, show strains of *Lmb. sakei* and *lmb. brevis* - 12 and 14.5 U / ml, respectively.

It was found that strains of lactic acid bacteria *Lbm. casei* and *lmb. bavaricus* exhibit high antioxidant properties, actively inhibit oxidative and hydrolytic processes in lipids of beef, pork and chicken.

### Keywords

*Lactic Acid Bacteria, Lipolytic Activity, Hydrolytic and Oxidative Processes.*

### Introduction

To date, in the scientific and technical literature there is a sufficient amount of information about the beneficial effect of foods containing cultures of microorganisms - probiotics on the human body, including the ability to reduce the risk of malignant neoplasms, weaken the manifestations of food allergies, strengthen immunity, and have a cholesterolemic effect, remove toxic substances from the body. It is well known that lactic acid bacteria are probiotic cultures that form the normal microflora of the human gastrointestinal tract.

At the present stage, a lot of attention is paid to lactic acid bacteria, as starting crops in the production of fermented meat products. Many scientists in their studies focus on cultures of lactic acid bacteria, the use of which leads to a decrease in pH and an improvement in organoleptic characteristics of meat products, as well as on methods for introducing starter cultures and fermentation modes of raw meat by cultures of the studied microorganisms [1-4].

When storing meat products under the influence of biological and physico-chemical factors in the adipose tissue, lipids decompose due to hydrolytic and oxidative changes in lipids.

The main role in the hydrolytic processes of fat breakdown is played by lipolytic enzymes of native fats and associated microorganisms. The basis of biochemical transformations of lipids is a sequence of free-radical chain reactions under the influence of physical and chemical factors (oxygen, air, light, heat, the presence of salts of heavy metals, etc.) [5-10].

During long-term storage of fermented products from meat raw materials, lipid oxidation products accumulate, which limits the shelf life of this group of food products and negatively affects their organoleptic characteristics, nutritional value and safety.

In addition to the oxidative changes in lipids that are unavoidable during storage, hydrolytic processes involving lipolytic enzymes can also lead to spoilage of fat-containing products.

The role of lipolytic enzymes of lactic acid bacteria in the maturation of fermented meat products is not fully understood. On the one hand, lipolysis is a prerequisite for the formation of a specific taste and aroma of meat products, on the other hand, it should not exceed a certain critical level, since hydrolytic processes involving lipolytic enzymes can lead to spoilage of these fat-containing products, the formation of defects such as rancid taste and smell, unclean and greasy aftertaste.

Fat lipolysis plays an important role in the formation of the aroma of fermented meat and dairy products, during which di- and monoglycerides, volatile fatty acids (acetic, butyric, caproic, valerianic, etc.) and their decomposition products (aldehydes, ketones, methyl ketones, ethers), alcohols). Specialists note that this process is carried out mainly by lactic acid bacteria that produce lipolytic enzymes involved in the conversion of fats [8,11-13].

The resulting volatile low molecular weight fatty acids can be oxidized to peroxides, which, under the action of the catalase activity of microorganisms, turn into carboxyl compounds, which contribute to the formation of taste and aroma of the product.

The rate of lipolytic processes can vary in a wide range depending on the type of raw material, the qualitative and quantitative composition of microflora, the conditions and duration of fermentation. Free fatty acids are the main products of lipid hydrolysis; it is by their content that it is customary to evaluate the degree of lipolysis of fats.

In connection with the foregoing, studies aimed at finding a scientifically based selection of starter cultures for fermented meat products that can not only provide ripening and obtain a product enriched with probiotic microorganisms, but also effectively suppress oxidative processes that occur in the fat fraction of meat products during storage, are relevant.

The aim of this work was to study the effect of starter cultures of lactic acid bacteria on the chemical stability of minced meat lipids during storage.

### Materials and Methods

The objects of study were cultures of lactic acid bacteria of the river. *Lactobacterium*: *Lbm. delbrueckii*, *Lbm. fermentum*, *Lbm. acidophilum*, *Lbm. plantarum*, *Lbm. bavaricus*, *Lmb. brevis*, *Lmb. paracasei*, *Lmb. sakei*, with high functional and technological properties [3,4,14].

Lactic acid bacteria cells were grown in MRS medium at a temperature of 37 °C for 24 hours; after incubation, the biomass was separated from the culture fluid by centrifugation (8000 rpm, 15 min.)

The activity of lipolytic enzymes of lactic acid bacteria was determined using the modified method of Ota and Yamada [15]. 0.9 ml of neutral olive oil was pipetted into a dry flask with a capacity of 20-30 ml with a ground stopper, 0.7 ml of phosphate buffer (pH = 7.0) was added and shaken vigorously. Then 1 ml of culture fluid was added, shaken for 2-3 minutes on a rocking chair. The hydrolysis was carried out for 2 hours at 37 °C, after which 10 ml of ethanol was added to terminate the reaction. The hydrolysis products were titrated with a 0.05 M NaOH solution in the presence of a 1% thymolphthalein solution. Control samples were prepared similarly, excluding the hydrolysis step. The specific activity of lipase was expressed in micromoles of oleic acid released in 1 hour by hydrolysis of a substrate (olive oil) of 1 ml of culture fluid.

During the experimental work, the highest quality beef, bold pork, and pectoral muscles of chickens were used as raw meat. For research, prototypes of model-stuffing systems were made from raw meat with a 25% content of raw fat. Meat was minced in a laboratory meat grinder with a grill diameter of 4 mm. Lactic starter cultures in the amount of 107 CFU / g were added to the resulting stuffing and mixed thoroughly. Control samples were prepared without lactic acid bacteria. Stuffing-model systems were stored chilled at a temperature of  $2 \pm 2$  °C.

The influence of starter cultures was determined by the oxidation of meat fats by acid number (GOST R 55480-2013), peroxide number (GOST R 54346-2011) and thiobarbituric lipid number (GOST R 55810-2013).

### Results and Discussion

The studied cultures of lactic acid bacteria exhibit a wide range of functional and technological properties necessary for the production of high-quality meat products, such as high acid accumulation activity, antagonistic activity in relation to sanitary-indicative microflora of food products, resistance to adverse environmental factors, etc. [3, 4, 14].

Culture	Shape and size		Optimum growth temperature, °C	Type of lactic acid fermentation
	Cells	Colonies		
<i>Lbm. casei</i>	sticks (1,5-2,0) µm	small, whitish domed,	30-32	homoenzymatic
<i>Lbm. delbrueckii</i>	sticks (0,5-0,8×3,5) µm	small, convex, whitish, shiny	35-37	homoenzymatic
<i>Lbm. fermentum</i>	sticks (0,5-0,9) µm	small, round, white	35-40	heterofermentative
<i>Lbm. acidophilum</i>	sticks (0,6-0,9 × 4,5) µm	small, round, white	37-45	homoenzymatic
<i>Lmb. plantarum</i>	sticks (0,9-1,2 × 6) µm	small, flat, whitish,	30-32	homoenzymatic
<i>Lmb. bavaricus</i>	sticks (0,8-1,0 × 5) µm	medium, round, grayish	32-37	homoenzymatic
<i>Lmb. brevis</i>	sticks (2-4 × 0,7-1) µm	small, convex, whitish, shiny	30-32	heterofermentative
<i>Lmb. paracasei</i>	sticks (1,5-2,0) µm	teardrop, whitish cream	30-32	homoenzymatic
<i>Lmb. sakei</i>	sticks (0,7-3) µm	round, convex, whitish translucent	35-37	homoenzymatic

**Table 1: Characterization of Lactic Acid Bacteria**

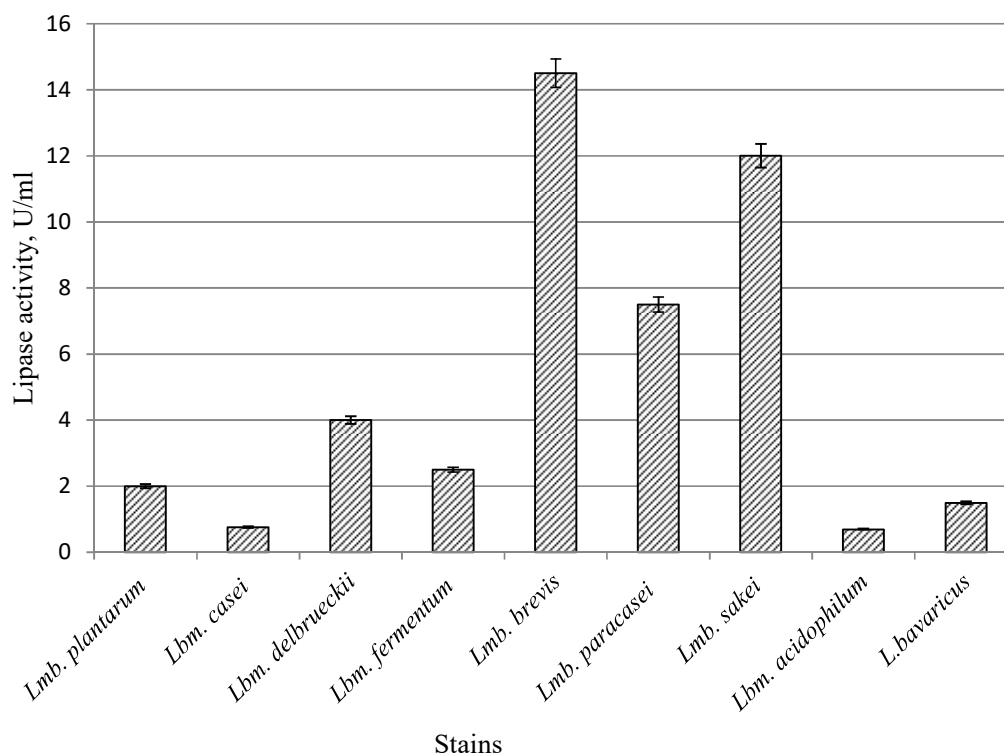
The leading role in the technology of fermented meat products is played by starter cultures of lactic acid bacteria, however, their influence on the hydrolytic and oxidative processes of the lipid fraction of meat is not well understood.

The inclusion of a consortium of microorganisms for the maturation of fermented meat products, in particular, dried and smoked sausages, strains of lactic acid bacteria producing lipases, is very promising from the point of view of creating a characteristic taste, color, aroma and consistency of this type of product. The prospect of this kind of research is also explained by the fact that lipolytic enzymes also take part in accelerating the ripening of sausages. However, in domestic practice, such studies are practically absent. Therefore, issues related to the practical application of starter cultures producing lipases in the technology of fermented meat products are of considerable interest.

In order to study the effect of starter cultures of lactic acid bacteria on the hydrolytic processes of lipid conversion, we studied the ability of lactic acid bacteria to produce lipolytic enzymes.

It should be noted that lipids are not sources of energy for lactic acid microflora, the presence of lipolytic activity is due solely to its biosynthetic needs.

The results of the study of lipolytic activity of the studied cultures of lactic acid bacteria are presented in Figure 1. The values of lipolytic activity varied among various types of studied lactobacilli, which, obviously, is associated with the individual characteristics of the studied cultures.

**Figure 1: Lipase Activity of Lactic Acid Bacteria**

It was revealed that strains of *Lbm. casei*, *Lbm. acidophilum*, *Lmb. bavaricus* do not have lipolytic activity. A small amount of lipolytic enzymes (4-5 U / ml) is synthesized by lactic acid bacteria *Lbm. delbrueckii* and *Lmb. curvatus*. High lipolytic activity, over 10 U / ml, is shown by strains of *Lmb. sakei* and *lmb. brevis* - 12 and 14.5 U / ml, respectively.

At the next stage of the study, the oxidative changes in the lipids of minced meat were studied when starting cultures of the studied lactic acid bacteria were introduced into them. At this stage, we used strains that do not exhibit lipolytic activity - *Lbm. casei*, *Lbm. acidophilum*, *Lmb. bavaricus*, in order to exclude the influence of hydrolytic processes occurring in lipids with the participation of lipases produced by lactic acid bacteria.

The results of studies of indicators of oxidative damage to lipids of model-minced systems are presented in the Table. 2.

Culture	Peroxide value, 10 <sup>-2</sup> g/100 g			Acid value, mg KOH/g			TBA numbers, mg MDA/kg		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Beef									
Control	5,2	12,3	14,5	0,3	1,6	2,6	0,12	0,26	0,42
Lbm. casei	5,0	9,9	11,1	0,2	0,4	1,4	0,12	0,14	0,28
Lbm. acidophilum	4,8	10,8	12,8	0,3	1,0	1,7	0,10	0,19	0,31
Lmb. bavaricus	5,1	11,6	13,2	0,3	0,8	1,8	0,12	0,16	0,24
Pork									
Control	5,6	12,9	15,3	0,7	1,3	2,8	0,14	0,39	0,56
Lbm. casei	5,5	10,0	11,7	0,6	1,0	1,7	0,15	0,22	0,31
Lbm. acidophilum	5,6	11,4	12,0	0,6	1,4	1,9	0,15	0,27	0,44
Lmb. bavaricus	5,6	10,5	12,6	0,8	1,2	2,2	0,13	0,25	0,39
Chicken									
Control	6,02	13,6	16,4	1,1	2,6	3,9	0,10	0,41	0,62
Lbm. casei	5,8	10,2	12,6	1,0	2,0	2,5	0,11	0,18	0,35
Lbm. acidophilum	5,9	11,5	13,1	1,1	1,9	2,4	0,10	0,33	0,49
Lmb. bavaricus	5,8	11,9	14,2	1,2	2,2	3,0	0,10	0,24	0,38

**Table 2: Effects of Lactic Acid Bacteria on Peroxide and Acid Value, TBA Numbers of Lipids**

The initial stage of lipid oxidation is associated with the formation of hydroperoxides, by a change in the amount of which one can judge the depth of the process.

An analysis of the obtained data on the content of peroxides in the experimental samples (Table 2) showed that the process of their formation proceeds more intensively in the first hours of storage, since after 24 hours their amount increases on average by 2 times, while with further storage the acid number does not increase intensively. After 48 hours of storage of model minced systems at a temperature of  $2 \pm 2$  °C, the number of peroxides increased on average 2.4 times from the initial values, while the introduction of cultures of lactic acid bacteria allows to slow down the formation of peroxides. Acid numbers of prototypes after 48 hours of minced meat storage are 15–21% lower, depending on the type of raw meat and the introduced culture of lactic acid bacteria. The lowest acid values of minced meat from beef, pork and chicken were obtained using the Lbm culture. casei.

The depth of hydrolytic processes occurring in model-minced systems was judged by the content of fatty acids, which is characterized by an acid number. The acid number is used to judge the hydrolytic decomposition of triglycerides with the formation of low molecular weight fatty acids, it is with these processes that experts associate a characteristic change in the taste of meat products.

The results of the study show that in the control model-forcemeat systems, hydrolytic processes proceed more intensively than in the experimental samples prepared with the introduction of cultures of lactic acid bacteria Lbm. casei, Lbm. acidophilum, Lmb. bavaricus, in this case, in the samples from chicken this process proceeded more intensively than from pork and beef. So, the acid numbers of control minced beef, pork and chicken after 48 hours were 2.6, 2.8 and 3.9 mg KOH / g, while with starter cultures of lactobacilli - 1.6, 1.9 and 2, 0 mg KOH / g, respectively. Thus, when cultures of lactic acid bacteria are introduced into model-forcemeat systems, the intensity of hydrolytic decomposition of meat lipids is significantly reduced (by 1.5 - 2.0 times, depending on the type of raw meat used).

The number of secondary products of fat oxidation reacting with 2-thiobarbituric acid, primarily malondialdehyde, is used to judge the rate of oxidation processes in food lipids.

It was found that the initial content of malondialdehyde in minced meat was 0.10 - 0.15 mg MDA / kg and did not significantly depend on the type of raw material used. However, after 24 hours of storage of minced meat in a cooled form, there was a significant decrease in the rate of formation of secondary products of meat lipid oxidation in experimental samples (using cultures of lactic acid bacteria) compared to control samples. The amount of malondialdehyde in the experimental samples after 48 hours of storage is on average 35% less compared to the control. It is shown that the use of lactobacilli Lbm. casei and lmb. bavaricus can significantly reduce oxidative processes in lipids of beef, pork and poultry.

### Summary

By the level of lipolytic activity, they are most effective for the production of fermented food products with long-term storage of lactic acid bacteria cultures: Lbm. casei, Lbm. acidophilum, Lmb. bavaricus. On the other hand, strains with high lipolytic activity, such as Lmb, may be of practical interest. sakei and lmb. brevis, for the production of fermented meat products with an accelerated ripening cycle and time-limited shelf life.

Studying the effect of lactic acid bacteria Lbm. casei, Lbm. acidophilum, Lmb. bavaricus on the chemical stability of beef, pork and chicken lipids showed that the studied cultures can significantly reduce the rate of hydrolytic and

oxidative processes that occur in the lipid fraction during storage, which indicates a high antioxidant activity of these lactobacilli. An interesting fact is that starting cultures of lactic acid bacteria can significantly reduce the amount of secondary lipid oxidation products in meat, which is especially important in view of the fact that these components pose a danger to human health. The most effective crop in this regard is *Lbm. casei* exhibiting high antioxidant properties.

### Conclusion

Lactic acid bacteria cultures *Lbm. casei* and *lmb. bavaricus* exhibit high antioxidant properties, effectively inhibit oxidative and hydrolytic processes that occur in meat lipids during storage.

The results presented in this article on the effect of lactic acid bacteria on the chemical stability of meat lipids (beef, pork, and chicken) will subsequently serve to develop a consortium of new starter cultures based on lactobacilli for fermented meat products, which also have antioxidant properties.

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