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THE STUDY OF ANTIOXIDANT POTENTIAL OF COMMERCIALLY VALUABLE STARTER CULTURES OF LACTIC ACID BACTERIA

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Abstract

One of the main causes of pathological changes in the human body, leading to the development of many diseases such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, and premature aging is an excessive accumulation of oxygen free radicals in the biological fluids. To prevent oxidative stress in recent years a lot of attention is paid to antioxidant therapy, i.e., providing the necessary amount of antioxidants, primarily natural, in the diet.

The paper studies the antioxidant potential of new biotech valued cultures of lactic acid bacteria, that are recommended for the production of fermented functional foods. The study of antioxidant activity of lactic acid bacteria was carried out at the level of cell extract and at the level of extracellular metabolic products. It was found that all investigated new strains of lactic acid bacteria (*Lbm. Delbrueckii, Lmb. casei, Lmb. curvatus*) had a high antioxidant potential. The strains of lactic acid bacteria *Lbm. casei and Lbm. curvatus* with the highest antioxidant properties can be recommended for being included into the starter cultures for the production of fermented functional food stuff of different meat.

Keywords: lactic acid bacteria, antioxidant activity, lipid oxidation.

Introduction

During the processing and storage, the food lipids can be subjected to free-radical oxidation, which results in the reduction of their quality and bioavailability. The basis of biochemical transformations of lipids is a sequence of free-radical chain reactions under the influence of physical and chemical factors (atmospheric oxygen, light, heat, heavy metals in the environment, etc.).

The peroxide and hydroperoxide formed at the initial stage of oxidizing do not significantly affect the organoleptic properties of the products but can be toxic and are conductive to the destruction of liposoluble vitamins and polyunsaturated fatty acids; the secondary products of oxidation (aldehydes and ketones) tincture the product with corresponding specific foreign flavors [1]. Moreover, the intake of foodstuff such as food with oxidized lipids can cause the body's pathological changes, so the search for substances with a high antioxidant activity protecting the lipids from initiation of peroxidation in them is important not only to lengthen the period of storage but also to maintain the food bioavailability.

Numerous epidemiological and clinical studies have confirmed the important role of antioxidants in protecting against premature aging, preventing cancer and cardiovascular conditions.

From our point of view, promising is the inclusion of the starters for food production, which are based on the process of fermentation of raw products with lactic acid bacteria cultures that exhibit high antioxidant activity [2-4] and can effectively inhibit the oxidation processes in the lipid fraction of food products during storage.

The aim of this study is to evaluate the antioxidant capacity of commercially valuable cultures of lactic acid bacteria.

Materials and Methods

The objects of study were cell extracts and culture fluids of commercially valuable lactic acid bacteria cultures *p. Lactobacterium: Lbm. delbrueckii, Lmb. casei, Lmb. curvatus* with a complex of high biotechnological properties being stored in the museum of the Department of CCI KSTU [5].

To obtain culture fluid (CF), the microbial cells were grown in MRS medium at 37° C until the stationary growth phase, at the end of incubation the biomass was separated by centrifugation (8000 rev/min, 15 min.), the supernatant fluid was sterilized by permeating it through the membrane filter (“Synpor”) with pore diameter of 0,45 microns. To obtain the cell extract (CE) of lactic acid bacteria, the cells were sonicated.

The antioxidant activity of cells and metabolites of lactic acid bacteria was evaluated by the reducing power [6]; antiradical activity (according to the DPPH method) [7]; oxidation products entering into reaction with 2-thiobarbituric acid, [8]; oxidation products of β-carotene with hydrogen peroxide [9].

The influence of starter cultures on the intensity of the oxidation process of fats of raw meat product was evaluated by acid (GOST R 55480-2013), peroxide (GOST R 54346-2011) and thiobarbituric numbers of lipids (GOST R 55810-2013). The samples of model-stuff systems were prepared from minced raw meat (chicken, pork, beef) in a laboratory meat grinder with a grating diameter of 4 mm. The content of raw fat in the samples was 25%. The

obtained minced meat was added in an amount of lactic acid starters of 10^7 KOH/g and mixed thoroughly. To intensify the processes of oxidation, model-stuff systems were stored at room temperature.

Results and Discussion

At the present time to determine the antioxidant activity of substances the methods based on the determination of the effect of antioxidants on the oxidation state of the different lipid substrates are widely used, at that, the reaction velocity or completeness is measured. As a rule, antioxidant activity is studied in relation to the free chemical macrobiotic radicals.

In our experiments using spectrophotometric methods for determining the antiradical activity of the microorganisms of the products reacting with 2-thiobarbituric acid, as well as the inhibition of β-carotene destruction (Table 1).

Table 1 – Antioxidant Activity of Lactic acid Bacteria.

Stains	Objects	Reducing power, %	DPPH radical scavenging activity,%	Inhibition of lipid peroxidation, %	MDA concentration, μmol/ml	Inhibition of β-carotene destruction ,%
Lmb. casei	KE	57,3±0,32	84,7±0,72	62,3±0,26	0,49±0,054	82,1±0,73
	CF	52,1±0,84	78,2±0,61	75,1±0,49	0,31±0,04	94,5±0,81
Lmb. curvatus	KE	60,1±0,35	44,6±0,12	69,8±0,43	0,41±0,036	87,3±0,44
	CF	53,9±0,76	61,2±0,31	64,9±0,17	0,45±0,071	80,2±0,55
Lbm. delbrueckii	KE	45,7±0,12	54,8±0,26	50,2±0,23	0,6±0,042	91,2±0,69
	CF	40,8±0,17	72,1±0,37	71,1±0,33	0,36±0,081	86,8±0,35

The findings of investigation have shown that both the cell extracts of lactic acid bacilli and their metabolites exhibit antioxidant activity. *Lbm. casei and Lmb. curvatus* lactic acid bacilli have the greatest reducing power. *Lbm casei* cell extract (84%) and *Lbm. casei* (78%) and *Lbm. delbrueckii* (72 %) culture fluids have maximum antiradical activity.

The total antioxidant activity determined from the oxidation products, reacting with 2-thiobarbituric acid of the culture fluids lies in the range of 65-75%, of the cell extracts - 50-70%. The maximum antioxidant activity according to this method is exhibited by *L. casei* and *L. delbrueckii* culture fluids.

In studying the antioxidant activity of the cultures of lactic acid bacteria in β-carotene - H_2O_2-models, it has been shown that all studied objects have a high antioxidant potential (in the range of 80-95%).

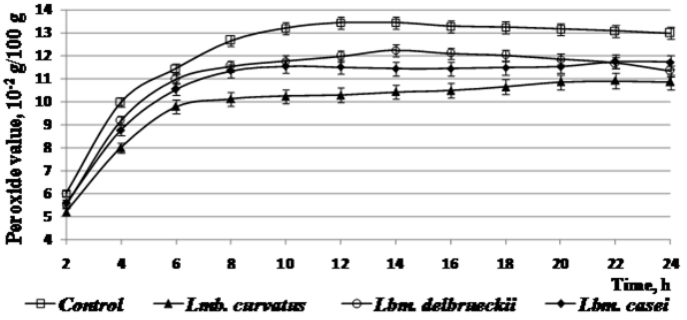
Thus, the studied cultures of lactic acid bacteria exhibit different antioxidant properties, which is determined by the individual metabolic characteristics of the test cultures.

The initial phase of lipid oxidation is connected with the formation of hydroperoxide, by the dynamics of the ongoing quantitative changes of which one can judge about the intensification of this process. The analysis of the data obtained on the content of peroxides in the test samples (Fig. 1) has shown that the process of their formation prevails

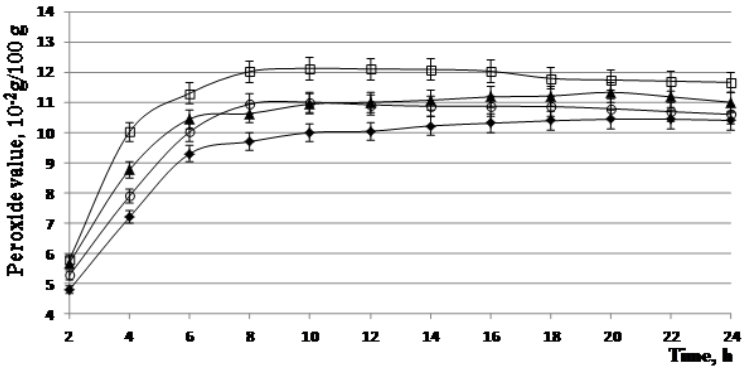
over the destruction up to 14 hours of storage of model-stuff systems at the room temperature. After 14 hours, there was a decrease of the peroxides, i.e. the process of peroxides disintegration is more intense than their formation.

The intensification of hydrolytic processes occurring in model-stuff systems was evaluated by the content of fatty acids which are characterized by acid value. The acid number indicates the hydrolytic decomposition of triglycerides and is characterized by taste change caused by the formation of low molecular fatty acids.

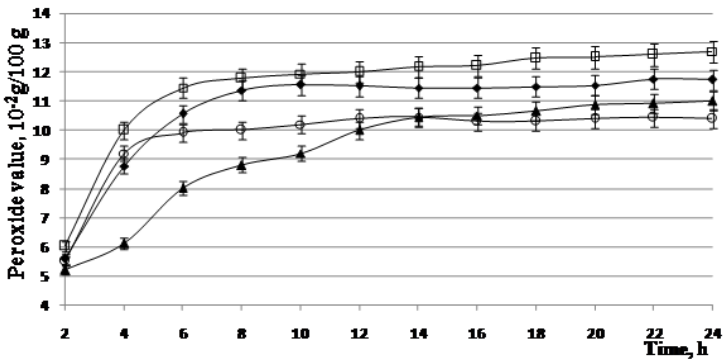
The analysis of the control model-stuff systems showed that the hydrolytic processes in them took place more intensively than in test samples (with starter cultures of lactic acid bacteria being applied). It should be noted that in the red meat samples process was more intensive than in the test pieces of white meat (Fig. 1, 2).



a) chicken

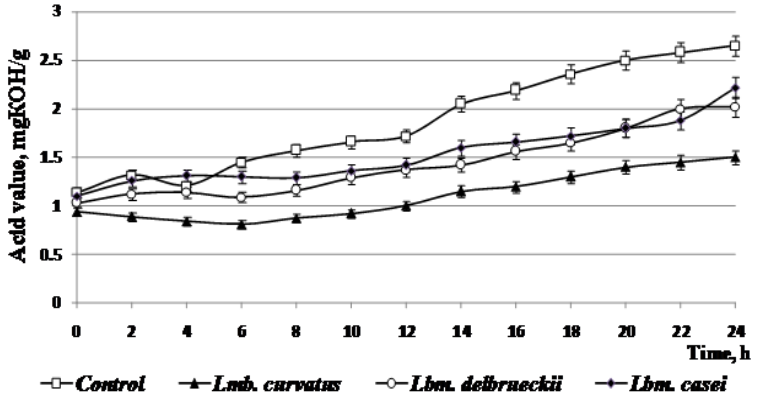


b) beef

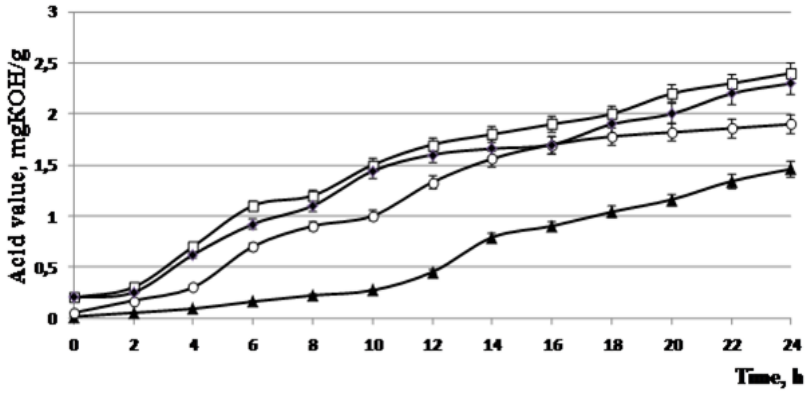


c) pork

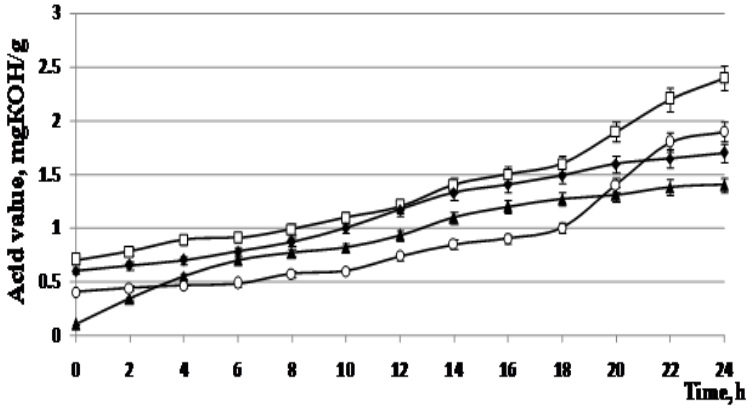
Figure 1- Peroxide value of lipids of meat minced



a) chicken



b) beef



c) pork

Figure 2 - Acid value of lipids of meat minced.

The intensity of oxidizing processes in the lipid fraction of minced meat during the period of storage was judged by the accumulation of secondary products of fat oxidation and especially malondialdehyde reacting with 2-thiobarbituric acid.

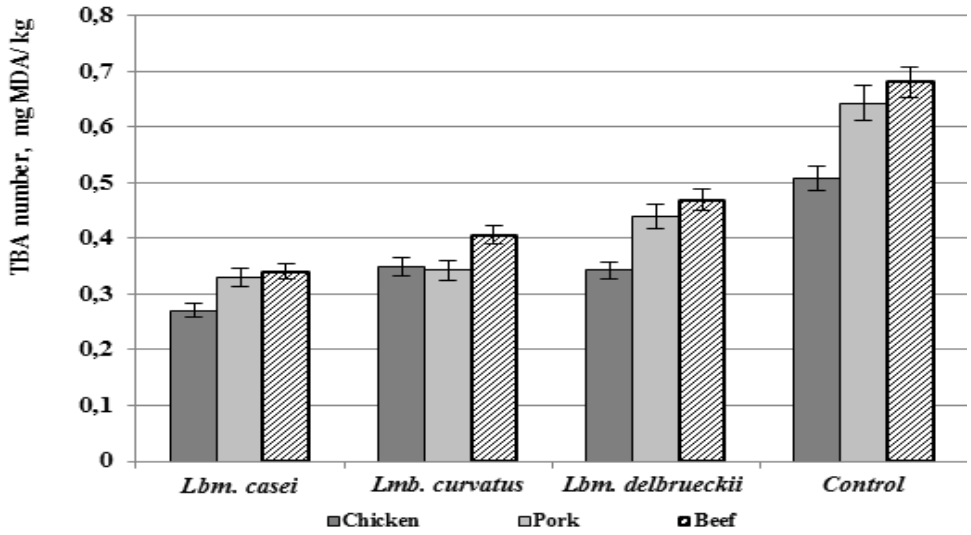


Figure 3 - TBA (thiobarbituric acid) numbers of lipids of meat minced.

The results have shown that the starter cultures of lactic acid bacteria can significantly reduce the rate of accumulation of secondary lipid oxidation products (Figure 3). The best results were obtained when applying *Lbm casei*. in model-making minced system. By the end of stuffing storage (24 hours) in the test-pieces of white and red meat, the content of malondialdehyde was by 2-2,5 times lower than in the control samples.

Summary

The study of antioxidant properties of the new strains of cultures of lactic acid bacteria has shown that they have antioxidant potential. An interesting fact is that the total antioxidant activity of culture fluids of lactic acid bacteria was higher than in cell extracts. It can be assumed that the antioxidant properties of these strains are connected with low-molecular compounds such as proteins, amino acids, etc., which are produced by the cells of lactic acid bacteria into the culture fluid. The results of studies on the influence of starter cultures of lactic acid bacteria on the process of oxidation of lipids of chicken, pork and beef mince demonstrate the potential of lactic acid bacteria to exhibit antioxidant action against oxidation of animal fats when preserving. Thus, the introduction of starter cultures of lactic acid bacteria with high antioxidant potential, can slow the process of lipid oxidation, which can serve as a basis for the development of new starters for fermented foods, including of long-term storage.

Conclusion: Cultures of lactic acid bacteria *Lbm. casei* and *Lbm. curvatus* exhibit high antioxidant properties, which proves the feasibility of their use as a potential antioxidant strains in biotechnology of development of functional foods.

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