

# Food Science and Applied Biotechnology

e-ISSN: 2603-3380

Journal home page: [www.ijfsab.com](http://www.ijfsab.com)  
<https://doi.org/10.30721/fsab2018.v1.i2>



## Research Article

### Stimulation of vital activity of lactic acid bacteria in the preparation of a liquid acid-forming ferments in bakery products

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#### Abstract

Following The influence of the new flour nutrient substrates with the introduction of phyto raw materials on the viability and activity of lactic acid bacteria cultured in liquid acid-forming ferments, which are used for making bread from rye flour and mixture of rye and wheat flour, are established. It was established that the phyto raw materials leads to the stimulation of lactic acid bacteria. The highest stimulating ability is *Echinaceae purpurea herba*, and then *Salviae Folia*, *Artemisia absinthium herba* and *Cortex Quercus*. Practical applications: Phyto raw materials as a component of flour nutrient substrates improves microbiological characteristics of liquid acid-forming ferments, in particular increases the total number of lactic acid bacteria and increases their activity. New conditions contribute to the accumulation of flavoring and aromatic substances that produce these microorganisms. This improves biotechnological properties of liquid acid-forming ferments, and thus will lead to increasing of consumer properties of bread from rye flour and mixture of rye and wheat flour.

**Keywords:** lactic acid bacteria, flour nutrient substrates, phyto raw materials, liquid acid-forming ferments, bread from rye flour and mixture of rye and wheat flour.

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#### Article history:

Received 12 December 2017

Reviewed 5 June 2018

Accepted 14 September 2018

Available on-line 10 October 2018

<https://doi.org/10.30721/fsab2018.v1.i2.20>

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## Introduction

The preservation of quality indicators and consumer properties of bread from rye flour and mixture of rye and wheat flour at constant level is important for both producers and consumers. This requirement must be performed in the various production modes of operation of bakeries. A high level of quality can be maintained only when using liquid acid-forming ferments by traditional technology in the process of making bread.

A large quantity of liquid acid-forming ferments used in the Republic of Belarus. They differ in the cultivated microorganisms and the number of process steps. Four production stages are used for the preparation of national kinds of bread from rye flour and mixture of rye and wheat flour. Lactic acid bacteria *Lactobacillus delbrueckii* (strain 76), *Lactobacillus plantarum* (strain I-35) and yeast cells race «Ivanovskaja» are introduced at these stages. These microorganisms are cultured on specially prepared flour nutritional substrates with maintaining certain process parameters in the continuous mode. A constant amount of microorganisms and their activity are preserved in such conditions. Biotechnological properties of liquid acid-forming ferments are stable. This development of lactic acid bacteria and yeast cells contributes to the accumulation of large quantities of substances that form the complex flavor of bread from rye flour and mixture of rye and wheat flour (Auermann 2009; Royter 1972). Production modes of making bread change daily currently. It reduces the activity of the cultivated microorganisms, and sometimes leads to their death. Therefore, it is necessary to look for new directions in the preparation of liquid acid-forming ferments which retain the cultured microorganisms. A new direction is the use of flour nutrient substrates that contain natural biologically active substances. These substances stimulate the intracellular metabolism of microorganisms, increase the permeability of their cells for nutrients. Biotechnological properties of liquid acid-forming ferments are stable in such conditions. Phyto raw materials is a source of natural biologically active substances. *Cortex Quercus*, *Echinaceae purpurea herba*, *Salviae Folia* and *Artemisia absinthium herba* is used widely in the Republic of Belarus.

In previous studies, were established methods of preparation and introduction of phyto raw materials, the concentration of phyto raw materials in the composition of flour nutrient substrates and were obtained prototypes of new flour nutrient substrates that contain these non-traditional raw materials (Samuylenko et al. 2016; Samuylenko 2017).

## Materials and Methods

The work was funded by the Belarusian Republican Foundation for fundamental research. The research was conducted in the laboratories of the Department of technology of bread products of the Mogilev State University of Food Technologies. Experiments were repeated 3–5 times. The results were processed by statistical methods with the probability of 0.95. Error experience of 5.0 %. The article presents the arithmetic means of the values obtained.

### Materials

**Raw materials.** Flour nutrient substrates without introduction and with introduction of various types of phyto raw materials (*Cortex Quercus*, *Echinaceae purpurea herba*, *Salviae Folia* and *Artemisia absinthium herba*) with different concentrations were used for this research. Liquid acid-forming ferments, which are used for making bread from rye flour and mixture of rye and wheat flour in the Republic of Belarus, were used as a source of cultivated lactic acid bacteria.

**Equipment and auxiliary materials.** Porcelain cups, glass swizzle sticks, flasks with a volume of 1000 cm<sup>3</sup>, pipettes with a volume of 1 cm<sup>3</sup> or 2 cm<sup>3</sup>, glass slides, microscope, glass beakers, graduated cylinders, test tubes, thermostat.

**Chemicals.** Tap water, alcohol with the introduction of formalin (75.0 % alcohol in the amount of 98.0 % and formalin in the amount of 1.9 %), dye – methylene blue.

## Methods

The total amount of lactic acid bacteria and their activity in the cultivation of new flour nutrient substrates were installed in the presented research. Method Burgvits was used to estimate the total amount of lactic acid bacteria (Starovoitova et al. 2002; Karnyshova and Sevastey 2008). The method is based on the production of fixed and colored preparations from liquid acid-forming ferments and on the counting of microorganisms in these preparations using a microscope. Method Jurgenson and Romanov (1958) was used to assess the activity of lactic acid bacteria. The method is based on the speed of color change of a dye (blue color to colorless in color) (Afanasyeva 2003).

**Preparation of samples.** Flour nutrient substrates (100.0 g) without any phyto raw materials and with the introduction of phyto raw materials with different concentrations are mixed with water with a temperature of 95°C – 97°C. Moisture of flour suspension should be 72.0 % – 78.0 %. The flour suspension temperature should range from 63°C to 67°C. Flour suspension is left at this temperature for 60 min and then cooled on a temperature of (50±5)°C for 60 min. Flour suspension is mixed with the liquid acid-forming ferments, which was previously prepared according to traditional technology. The ratio between the components is 1:1. New liquid acid-forming ferment is placed in a thermostat at a temperature of (50±5)°C for 480 min. Every 60 min analyzed the total amount of lactic acid bacteria and their activity.

Preparation of samples for determining the total amount of lactic acid bacteria.

New liquid acid-forming ferments (10.0 g) is mixed with tap water (500 cm<sup>3</sup>) in a porcelain cup after the required period of time. The suspension is transferred into a flask with a volume of 1000 cm<sup>3</sup>. The flask is closed, the suspension is mixed for 1 min. One drop of the suspension is transferred to a glass slide using a pipette and distributed in a square of 4 cm<sup>2</sup> evenly. The suspension on the glass slide needs to air dry. Further, the alcohol with formalin is applied on a glass slide and dried again. At the end of applied methylene blue, after 10-15

minutes the excess dye is washed with water carefully and dried again.

Preparation of samples for determining the activity of lactic acid bacteria.

New liquid acid-forming ferments (10.0 g) is mixed with tap water (20 cm<sup>3</sup>) with a temperature of 40 °C in a porcelain cup after the required period of time.

The experiment.

Determination of total amount lactic acid bacteria

Prepared samples are analyzed at the microscope using the immersion lens 90x and the eyepiece 15x. Each sample can be seen 50 fields of view, which counts the amount of cells of lactic acid bacteria. The counting results are summed up. The amount of lactic acid bacteria in 1.0 g of liquid acid-forming ferment (N) is determined by the formula (1):

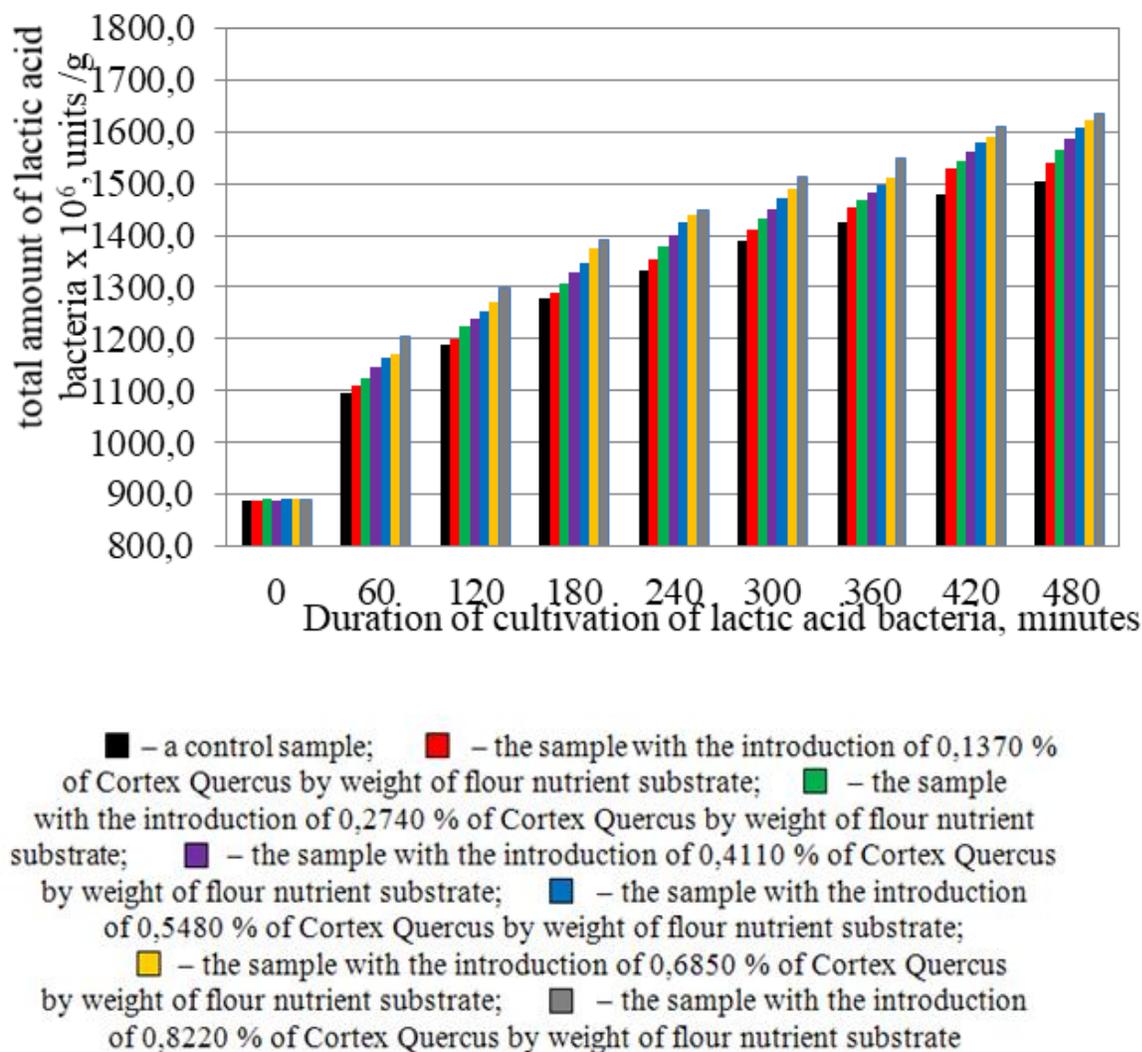
$$N = (n \times P \times Q \times 1.0) / p \times q \times g, \quad (1)$$

where  $n$  – the arithmetic mean of the number of cells of microorganisms in a single field of view;  $P$  – the square of the preparation from liquid acid-forming ferment (400 mm<sup>2</sup>);  $Q$  – the amount of water required for diluting liquid acid-forming ferment (500 cm<sup>3</sup>);  $p$  – the square of the field of view of the microscope, mm<sup>2</sup>;  $q$  – the volume of one drop of suspension, cm<sup>3</sup>;  $g$  – the amount of liquid acid-forming ferment, which is taken for research (10.0 g).

**Determination of the activity of lactic acid bacteria.** The prepared sample (of 10.0 cm<sup>3</sup>) is transferred into 2 tubes using the graduated cylinder. In a test tube is added with methylene blue in the amount of 1.0 cm<sup>3</sup>. The second tube serves as a control sample. The tubes are placed in thermostat at a temperature of 40°C. Sets the time of the transition of blue coloring dye to colorless. The result is expressed in minutes.

## Results and Discussion

Fig. 1 presents the results of research total amount of lactic acid bacteria *Lactobacillus delbrueckii* (strain 76), cultivated in liquid acid-forming ferments using a new flour nutrient substrates.



**Figure 1.** The change in the amount of lactic acid bacteria *Lactobacillus delbrueckii* (strain 76) using the new flour nutrient substrates (with the introduction of *Cortex Quercus*)

Fig.1 shows that the use of phyto raw materials, particularly *Cortex Quercus*, in flour nutrient substrates leads to the stimulation of vital activity of lactic acid bacteria. The change in the total amount of lactic acid bacteria depends on the concentration of *Cortex Quercus* in flour nutrient substrates and the duration of cultivation of microorganisms. The total amount of lactic acid bacteria increases by

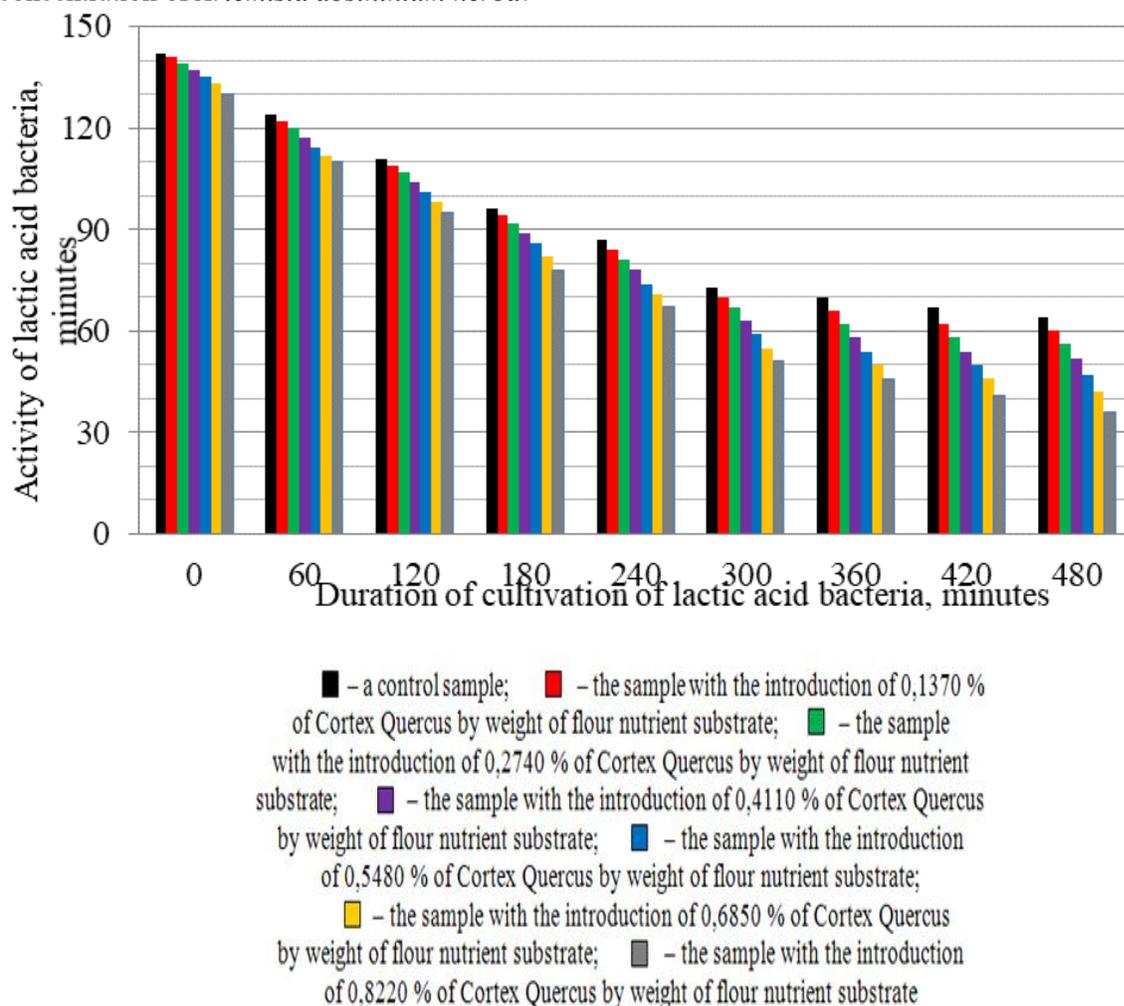
(110.7–131.3) x 10<sup>6</sup> units.g<sup>-1</sup> at a constant duration of cultivation and the introduction of the maximum concentrations of *Cortex Quercus*. The total amount of lactic acid bacteria increased by 617.8 x 10<sup>6</sup> units.g<sup>-1</sup> for control sample and (653.7–748.3) x 10<sup>6</sup> units.g<sup>-1</sup> for prototypes in a joint increase in the duration of cultivation of microorganisms and concentration of *Cortex Quercus*.

The same research were performed by culturing the lactic acid bacteria *Lactobacillus delbrueckii* (strain 76) using the new flour nutrient substrates with the introduction of *Echinaceae purpurea herba*, *Salviae*

*Folia* and *Artemisia absinthium herba* were set identical to the dynamics of the development of lactic acid bacteria. The total amount of lactic acid bacteria increases:

by  $(158.6-193.5) \times 10^6$  units.g<sup>-1</sup> at a constant duration of cultivation and the introduction of the maximum concentration of *Echinaceae purpurea herba*; by  $(139.7-162.9) \times 10^6$  units.g<sup>-1</sup> at a constant duration of cultivation and the introduction of the maximum concentration of *Salviae Folia*; by  $(127.9-153.0) \times 10^6$  units.g<sup>-1</sup> at a constant duration of cultivation and the introduction of the maximum concentration of *Artemisia absinthium herba*.

The increase in duration of cultivation of microorganisms and concentration of phyto raw materials increases the amount of lactic acid bacteria: by  $(664.3-807.5) \times 10^6$  units.g<sup>-1</sup> when using flour nutrient substrates with the introduction of *Echinaceae purpurea herba*; by  $(658.2-777.9) \times 10^6$  units.g<sup>-1</sup> when using flour nutrient substrates with the introduction of *Salviae Folia*; by  $(639.9-767.0) \times 10^6$  units.g<sup>-1</sup> when using flour nutrient substrates with the introduction of *Artemisia absinthium herba*.



**Figure 2.** The change in the activity of lactic acid bacteria *Lactobacillus delbrueckii* (strain 76) using the new flour nutrient substrates (with the introduction of *Cortex Quercu*

Analyzing the obtained results we can say that the highest stimulating ability for lactic acid bacteria *Lactobacillus delbrueckii* (strain 76) has *Echinaceae purpurea herba*, then *Salviae Folia*, *Artemisia absinthium herba* and *Cortex Quercus*.

Fig. 2 presents the results of research activity of lactic acid bacteria *Lactobacillus delbrueckii* (strain 76), cultivated in liquid acid-forming ferments using a new flour nutrient substrates. Fig. 2 shows that the use of new nutrient substrates with the introduction of phyto raw materials, particularly *Cortex Quercus*, reduces (improves) the activity of lactic acid bacteria *Lactobacillus delbrueckii* (strain 76). The change in the activity of lactic acid bacteria is also dependent on the concentration of phyto raw materials of flour nutrient substrates and the duration of cultivation of microorganisms. Activity of lactic acid bacteria is reduced by 12-28 min for the experimental samples at a fixed time of cultivation and at entry maximum concentrations of *Cortex Quercus*. Activity of microorganisms is reduced by 78 min for control sample and 81-94 min for prototypes in a joint increase in the duration of cultivation of microorganisms to 480 min and the concentration of *Cortex Quercus*.

*Echinaceae purpurea herba*, *Salviae Folia* and *Artemisia absinthium herba*. Activity of lactic acid bacteria reduces: by 12-44 min at a fixed duration of cultivation and the introduction of the maximum concentration of *Echinaceae purpurea herba*; by 12-35 min at a fixed duration of cultivation and the introduction of the maximum concentration of *Salviae Folia*; by 11-32 min at a fixed duration of cultivation and the introduction of the maximum concentration of *Artemisia absinthium herba*. A joint increase in the duration of cultivation of microorganisms to 480 min and the concentration of phyto raw materials reduces the activity of lactic acid bacteria: by 85-110 min when using flour nutrient substrates with the introduction of *Echinaceae purpurea herba*; by 83-101 min when using flour nutrient substrates with the introduction of *Salviae Folia*; by 81-99 min when using flour nutrient substrates with the introduction of *Artemisia absinthium herba*.

The obtained results confirm the previous conclusions. The highest stimulating ability for lactic acid bacteria *Lactobacillus delbrueckii* (strain 76) has *Echinaceae purpurea herba*, then *Salviae Folia*, *Artemisia absinthium herba* and *Cortex Quercus*.

## Conclusions

Research show that the use of new flour nutrient substrates with the introduction of phyto raw materials stimulates lactic acid bacteria *Lactobacillus delbrueckii* (strain 76), which are cultivated in a liquid acid-forming ferments used in the production of bread from rye flour and mixture of rye and wheat flour. The highest stimulating ability for lactic acid bacteria have flour nutrient substrates containing *Echinaceae purpurea herba*, then *Salviae Folia*, *Artemisia absinthium herba* and *Cortex Quercus*. The use of the new flour nutrient substrates in the technology of liquid acid-forming ferment will improve their biotechnological properties and, accordingly, the consumer properties of bread based on them.

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