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Research Article

Effect of the composition of flour nutrient substrates on the activity of microorganisms from the bacterial concentrates

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Abstract
The need to develop the technology of liquid acid-forming enzymes using bacterial concentrates for the production of national types of bread in discrete mode was shown. The choice of bacterial concentrates was based on the optimal pH (4.5 - 5.5) and temperature (30 - 40°C) of flour nutrient substrates, which are necessary to ensure the directed cultivation of microorganisms in the technology of liquid acid-forming enzymes. The choice of bacterial concentrates was based on the maximum acidity that microorganisms could provide after two days of cultivation and which should be in the range of 100 to 180°T. The use of Belarusian bacterial concentrates on the basis of the comparative characteristics was proposed. Flour nutrient substrates for the cultivation of microorganisms from bacterial substrates have been proposed. They contained traditional components (rye flour, rye malt) and components for stimulation of activity of microorganisms from bacterial concentrates (dairy whey in liquid form, *Echinaceae purpurea* herba). The choice of the ratio between the components was based on the peculiarities of the technology of production of national types of bread and the possibility of providing microorganisms from bacterial concentrates with the necessary nutrients. Microorganisms of bacterial concentrate "IM-pro 1" (a consortium of dried lactic acid bacteriums and bifidobacteriums) using the proposed flour nutrient substrates during cultivation had the best activity for a shorter period of cultivation. This made it possible to propose the use of this bacterial concentrate as a source of microorganisms in liquid acid-forming enzymes for national types of bread and to ensure the preparation in one stage.

Keywords: national types of bread, liquid acid-forming enzymes, bacterial concentrates, microorganisms, flour nutrient substrates, activity

Abbreviations: "IM-pro 1" (bacterial concentrate containing a consortium of dried lactic acid bacteriums *Lactobacillus plantarum* and bifidobacteriums *Bifidobacterium adolescents*), "TV-M" (bacterial concentrate containing dried lactic acid bacteriums *Lactococcus lactis*), "LBC" (liquid bacterial concentrate containing lactic acid bacteriums *Lactobacillus* spp. 1), FNS (flour nutrient substrate).

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Introduction

National types of bread from rye flour and a mixture of rye and wheat flour have high consumer properties. This determines their demand in our country, which is confirmed by a constant increase in the share of applications of trade organizations for this range. National types of bread are also in demand abroad. The annual increase in their exports to the Russian Federation, Ukraine, Azerbaijan, Israel, the European Union, the United States of America and other countries testifies to this. This trend provides foreign exchange earnings to the country's economy.

High consumer properties of national types of bread completely depend on biotechnological properties of liquid acid-forming enzymes in which specific microorganisms are cultivated purposefully (Auermann 2009). The viability of these microorganisms, their qualitative and quantitative composition, activity, qualitative and quantitative composition of flour nutrient substrates at each stage of the production cycle affect the biotechnological properties of liquid acid-forming enzymes (Afanasyeva 2003).

At the round-the-clock operation of baking enterprises liquid acid-forming enzymes are prepared traditionally in a continuous mode in the production cycle in several stages. This preparation is associated with the use of stable technological parameters. Forced instability of technological parameters occurs, some stages of the production cycle of preparation of liquid acid-forming enzymes are not implemented in the current discrete mode of operation of bakery enterprises. This leads to a decrease in the viability of microorganisms, their death, instability of biotechnological properties of liquid acid-forming enzymes and, as a consequence, to a deterioration in the consumer properties of national types of bread. Full implementation of the traditional multistage production cycle of preparation of liquid acid-forming enzymes for national types of bread in a discrete mode of operation of baking enterprises is not possible (Kuznetsova 2003).

The question of developing a new technology of liquid acid-forming enzymes for national types of bread using bacterial concentrates in a discrete mode of operation of baking enterprises has become relevant at the present stage of development of the baking industry.

Selection of bacterial concentrates of domestic production for the preparation of liquid acid-forming enzymes and the study of the effect of different compositions of flour nutrient substrates on the activity of microorganisms from the proposed bacterial concentrates were the purpose of this study.

Materials and Methods

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.

Raw materials. Flour nutrient substrates of different composition were used in research. Bacterial concentrates "IM-pro 1", "TV-M" and "LBC" were used as a source of microorganisms. Liquid acid-forming enzymes using bacterial concentrates for national types of bread were used.

Equipment and auxiliary materials. Porcelain cups, conical flasks with a volume of 200 - 250 cm^3, glass beakers, graduated cylinders, test tubes, thermostat and potentiometer.

Chemicals. Distilled and tap water, sodium hydroxide solution with a concentration of 0.1 N, dye - methylene blue and phenolphthalein.

Methods. The acidity of bacterial concentrates was determined by titration and potentiometric method (Starovoitova et al. 2002; Karnyshova and Sevastey 2008; Afanasyeva 2003). Method of 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 colourless in color) (Afanasyeva 2003).

Preparation of samples. Flour nutrient substrates (100 g) of different composition are mixed with dairy whey in liquid form with a temperature of 95 - 97°C.

Moisture of flour suspension should be 72 - 78%. The flour suspension temperature should range from 63 to 67°C. Flour suspension is left at this temperature for 60 min and then cooled to a temperature of 30 - 35°C for 60 min. In the same period of time the microorganisms of bacterial concentrates activate the following way. The bacterial concentrate in the required amount (0.5 g) is introduced into the tube, then dairy whey in liquid form is added there with a temperature of 40°C in an amount of 10 cm^3 and kept in a thermostat at a temperature of 40°C for 10 min. The prepared bacterial concentrate is introduced into the flour suspension and maintained at a temperature of 30 - 35°C for 24 h. Every 3 h the activity of microorganisms from bacterial concentrates that are cultivated in flour nutrient substrates of different composition is evaluated. The maximum acidity of the liquid acid-forming enzymes was established after 48 h of cultivation of microorganisms from bacterial concentrates.

Preparation of samples for determining the activity of microorganisms from bacterial concentrates. Liquid acid-forming enzymes (10 g) are mixed with tap water (20 cm^3) with a temperature of 40°C in a porcelain cup after the required period of time.

Determination of the activity of microorganisms from bacterial concentrates. The prepared sample (of 10 cm^3) is transferred into 2 tubes using the graduated cylinder. In a test tube is added with methylene blue in the amount of 1 cm^3. 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.

Determination of titratable acidity of liquid acid-forming enzymes using bacterial concentrates. Liquid acid-forming enzymes (5 g) are poured into a conical flask, distilled water (50 cm^3) is added. The resulting suspension is stirred, a few drops of phenolphthalein are added and titrated with sodium hydroxide solution to a pink color. Titratable acidity *K*, in degrees, liquid acid-forming enzymes is calculated by the formula (1):

$$K = V \times 2, \quad (1)$$

Where *V* - the amount of sodium hydroxide solution consumed for titration, cm^3; *2* – conversion factor.

Determination of active acidity (pH) of liquid acid-forming semi-finished products. The active acidity (pH) of liquid acid-forming enzymes is determined by immersing the potentiometer electrodes in the test object and fixing the results of research on the electronic panel.

Results and Discussion

Mainly lactic acid bacteriums cause biotechnological properties of liquid acid-forming enzymes. Bacterial concentrates should be used for the preparation of liquid acid-forming enzymes in one stage in a discrete mode of production of national types of bread. It should be noted that such an experience of using domestic bacterial concentrates for the preparation of liquid acid-forming enzymes in discrete mode in the dairy industry exists. In addition, the types of lactic acid bacteriums, the stages of their development in liquid acid-forming enzymes are similar for the dairy industry and for the baking industry.

The following domestic bacterial concentrates containing lactic acid bacteriums have been proposed. Bacterial concentrate "IM-pro 1" is a consortium of dried lactic acid bacteriums *Lactobacillus plantarum* and bifidobacteriums *Bifidobacterium adolescents*. "IM-pro 1" has acid resistance, shows antimicrobial activity to contaminant microorganisms, promotes the accumulation of organic acids, vitamins, amino acids, enzymes, flavoring compounds in liquid acid-forming enzymes. Bacterial concentrate "IM-pro 1" was produced by the Institute of Microbiology of the National Academy of Sciences of Belarus. The optimum temperature, active acidity (pH) of the cultivation of microorganisms from bacterial concentrate have been established. The optimum temperature was 35 ± 2°C, the optimal active acidity (pH) - 5.0 ± 0.5. Titratable acidity of liquid acid-forming enzymes reached 120 - 180°T after 48 h of cultivation of microorganisms from bacterial concentrate at such technological parameters.

Bacterial concentrate "TV-M" contains dried lactic acid bacteriums *Lactococcus lactis*. It is mainly used to produce fermented milk products, produced by the Institute of meat and dairy industry of the National Academy of Sciences of Belarus. The optimum temperature of cultivation of microorganisms from bacterial concentrate was set in the range of 32 - 35°C, the optimal active acidity pH - 5.0 ± 0.5. Titratable acidity of liquid acid-forming enzymes reached 120°T. Bacterial concentrate "LBC" is a liquid containing lactic acid bacteriums *Lactobacillus* spp. 1. It is used mainly in the dairy industry, produced by the Institute of Microbiology of the National Academy of Sciences of Belarus. The optimum temperature of cultivation of microorganisms from bacterial concentrate was 30 - 40°C, active acidity (pH) was 4.8 - 5.5. Titratable acidity of liquid acid-forming enzymes reached 120 - 180°T.

Microorganisms from all presented bacterial concentrates develop at temperatures and active acidity (pH), typical for the conditions of cultivation of microorganisms in liquid acid-forming enzymes, which are used in bakery production. The gradual formation of acid-containing substances for 48 h in liquid acid-forming enzymes allows you to adjust the duration of their preparation. High titratable acidity of liquid acid-forming enzymes allows to preserve them.

The selection of raw materials for flour nutrient substrates used for the cultivation of microorganisms from bacterial concentrates was the next stage of research. The main nutrients (monosaccharides and disaccharides) should be part of the flour nutrient substrates to ensure the effective development of microorganisms from bacterial concentrates. In bakery production, traditional raw materials (traditional rye flour and unfermented rye malt) mainly provide microorganisms with monosaccharides and disaccharides. These nutrients are formed by hydrolysis of starch of traditional rye flour under the influence of enzymes of unfermented rye malt at certain technological parameters. This process is long and difficult to implement in a discrete mode of production of national types of bread. The use of extruded rye flour instead of traditional rye flour will provide the flour nutrient substrate with the necessary monosaccharides and disaccharides.

The use of fermented rye malt instead of un fermented rye malt will also provide the flour nutrient substrate with the necessary nutrients and additional substances that affect the taste and aroma of national types of bread. In addition, the use of these components does not require additional preparation, which significantly reduces the duration of the process and can be used in a discrete mode of production of national types of bread. The use of lactose is necessary for the activation of microorganisms from bacterial concentrates. Dairy whey is used as a source of lactose in the composition of flour nutrient substrates in bakery production. It was proposed to additionally use *Echinaceae purpurea* herba in the composition of flour nutrient substrates as a source of monosaccharides, disaccharides, amino acids, minerals, vitamins and other biologically active substances.

The choice of the ratio between raw materials components was based on their technological properties and known ranges of variation (Kuznetsova 2003). The qualitative and quantitative composition of the flour nutrient substrates used in the studies is presented in Table 1.

The study of the effect of the composition of flour nutrient substrates on the activity of microorganisms from bacterial concentrates was the next stage of research.

The activity of microorganisms from bacterial concentrates is shown in Fig. 1- 3.

Analysis of results presented in Figures 1- 3 showed that microorganisms from all bacterial concentrates reacted to the composition of flour nutrient substrates, as the activity of microorganisms changed. The activity of microorganisms was 140 - 142 min at the beginning of cultivation. The use of extruded rye flour, fermented rye malt, dairy whey in liquid form in the composition of flour nutrient substrates allowed to reduce, that is to improve the activity of microorganisms from all bacterial concentrates in comparison with the traditional sample. This trend was observed at each controlled stage of microbial cultivation. The additional application of *Echinaceae purpurea* herba to the samples of flour nutrient substrates contributed to the improvement of microbial activity to a greater extent than without the introduction of this raw material.

Table 1. Qualitative and quantitative composition of flour nutrient substrates

Name of raw materials	The ratio of raw materials, % by weight of flour in a unified formulation for national types of bread				
	FNS (0) traditional sample	FNS (1)	FNS (2)	FNS (3)	FNS (4)
Traditional rye flour	17.5	16.0	16.0	10.0	10.0
Extruded rye flour	–	3.0	3.0	6.0	6.0
Unenzymeed rye malt	4.5	–	–	–	–
Enzymeed rye malt	–	3.0	3.0	6.0	6.0
Echinaceae purpurea herba	–	–	0.05	–	0.05
Water to provide the required humidity of liquid acid-forming enzyme	according to calculations	–	–	–	–
Dairy whey in liquid form to provide the required humidity of liquid acid-forming enzyme	–	according to calculations			
Humidity liquid acid-forming enzyme, %		78.0			

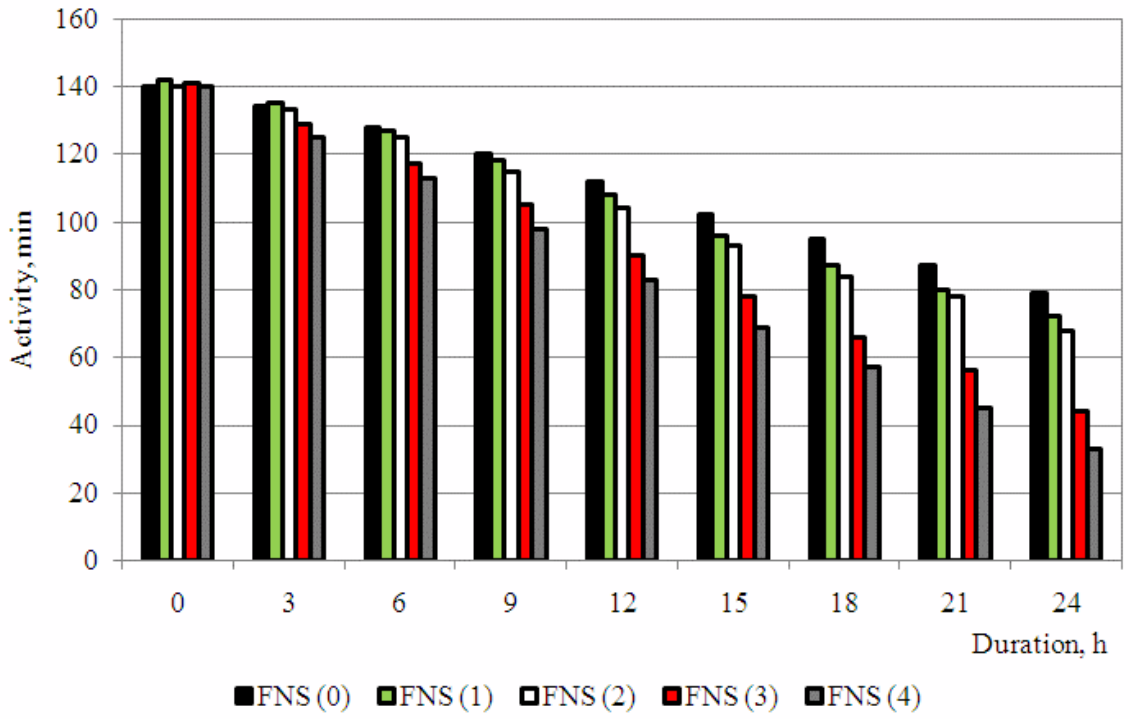

Figure 1. The change in the activity of microorganisms from bacterial concentrate "IM-pro 1" depending on the composition of flour nutrient substrates and the duration of cultivation of microorganisms

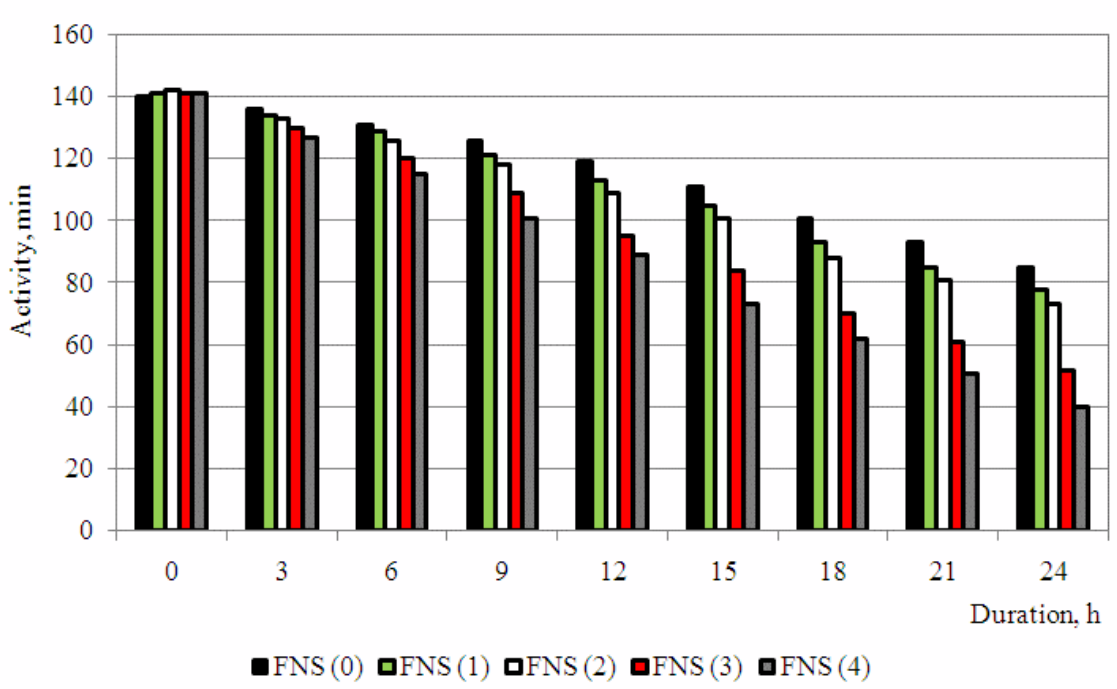

Figure 2. The change in the activity of microorganisms from bacterial concentrate "TV-M" depending on the composition of flour nutrient substrates and the duration of cultivation of microorganisms

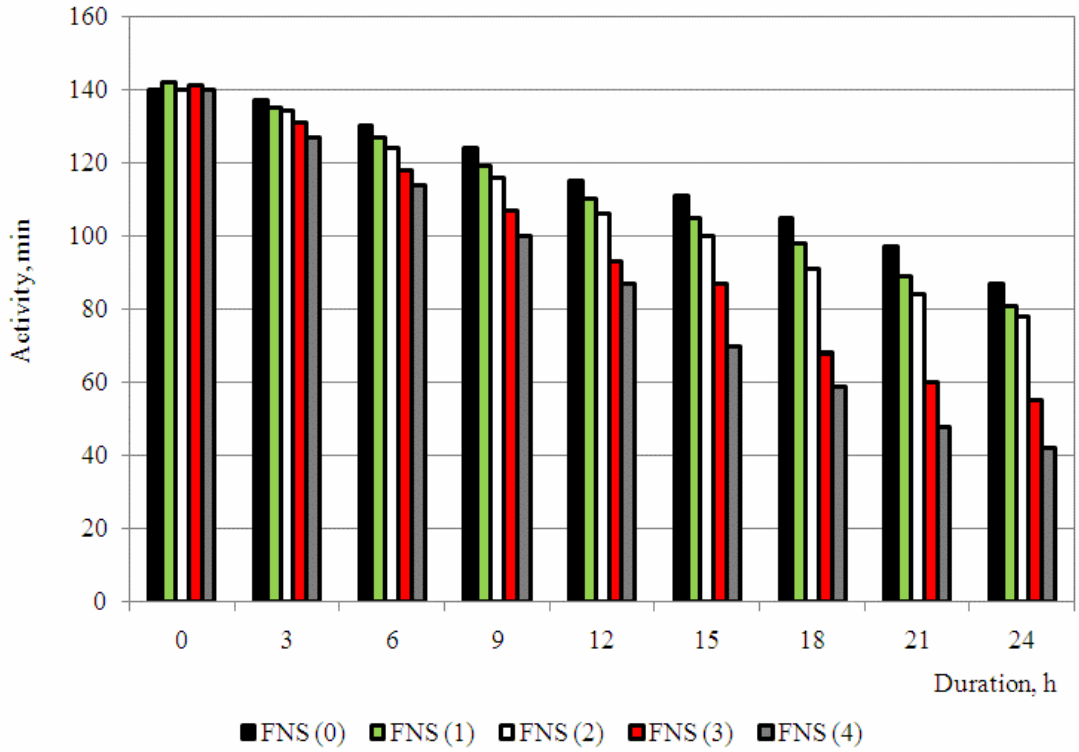

Figure 3. The change in the activity of microorganisms from bacterial concentrate "LBC" depending on the composition of flour nutrient substrates and the duration of cultivation of microorganisms

The activity of microorganisms from the bacterial concentrate "IM-pro 1" was 112 min after 12 h of cultivation and 79 min after 24 hours of cultivation using FNS (0). At the same time, the activity of microorganisms from this bacterial concentrate was 83 min after 12 h of cultivation and 33 min after 24 h of cultivation using FNS (4). It is worth noting that the activity of microorganisms for the traditional liquid acid-forming enzymes used for national types of bread ranges from an average of 75 - 90 min. Thus, the possibility of changing the duration of cultivation of microorganisms from bacterial concentrate "IM-pro 1" exists depending on the composition of flour nutrient substrates. Therefore, the possibility of changing the duration of preparation of liquid acid-forming enzymes using this bacterial concentrate in a wide range exists in a discrete mode of production of national types of bread.

The activity of microorganisms from other bacterial concentrates had a higher rate that is the activity was worse, compared with the activity of microorganisms from the bacterial concentrate "IM-pro 1". The activity of microorganisms from the bacterial concentrate "TV-M" was 119 min after 12 h of cultivation and 85 min after 24 h of cultivation using FNS (0). At the same time, the activity of microorganisms from this bacterial concentrate was 89 min after 12 h of cultivation and 40 min after 24 h of cultivation using FNS (4). The activity of microorganisms from bacterial concentrate "LBC" was 115 min after 12 h of cultivation and 87 min after 24 h of cultivation using FNS (0). At the same time, the activity of microorganisms from this bacterial concentrate was 87 min after 12 h of cultivation and 42 min after 24 h of cultivation using FNS (4). Such a change in the activity of microorganisms from bacterial concentrates "TV-M" and "LBC" also entails a change in the duration of preparation of liquid acid-forming enzymes using these bacterial concentrates, but in a small range. It is not always possible to use the discrete mode of production of national types of bread.

Conclusions

The choice of bacterial concentrates was carried out on the basis of optimal technological parameters (temperature, active acidity, maximum titratable acidity) necessary for the cultivation of microorganisms in liquid acid-forming enzymes. Researches have shown that the possibility of using Belarusian bacterial concentrates for the preparation of liquid acid-forming enzymes in one stage in a discrete mode of production of national types of bread exists. It was found that the possibility of changing the duration of the only stage of preparation of liquid acid-forming enzymes in a wide range in the discrete mode of production of national types of bread exists mainly when using the bacterial concentrate "IM-pro 1".

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